

**TERZO CONGRESSO MONDIALE
di MEDICINA INTEGRATA
15-16-17 settembre 2006**

“La minaccia OGM sui modelli alimentari di accompagnamento alla terapia immunitaria e disintossicante”

Relatore:

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Dirigente Medico Regionale della Guardia di Finanza del Friuli
Venezia Giulia**

Terapia Breuss: un caso clinico documentato

Paziente diabetico e iperteso con cancro al polmone (lobo superiore di destra) diagnosticato nel febbraio del 2006, di 6 x 4,5 centimetri, con interessamento del mediastino.

Anamnesi ufficiale dell'ospedale: *NSCLC lobi sup. pulm. dex cum infiltratio mediastini; diabetes mellitus (typ II); Hypertensio art.*

NSCLC: *Non-Small-Cells Lung Cancer* (Cancro del Polmone a Cellule-Non-Piccole)

Il paziente rifiutava la Chemio e si affidava a terapia simil-gersoniana (nello specifico Terapia Breuss) accettando una dieta totalmente priva di proteine, priva di vitamina B12, priva di cloruro di Sodio, priva di Glucosio.

Piante impiegate giornalmente, per 42 giorni: 3 etti di *Beta vulgaris cruenta* (Barbabietola rossa), 1 etto di Carote crude biologiche (*Daucus carota*), 1 etto di tubero di *Meum mutellina* (Sedano di Monte, Levistico) o di Sedano selvatico (*Apium graveolens*), 30 grammi di Rafano (*Cochlearia armoracia*), 1 etto di decotto di bucce di Patate (*Solanum tuberosum*); bevute fredde;

Tempi e modalità di somministrazione : METODO BREUSS

In visita medica venerdì 14 luglio 2006, si riscontrava notevole riduzione della massa tumorale, da verosimile “*deproteinatio tumoris*” con quadro flogistico interlobare destro da Risposta Immunitaria (Cascata Immunitaria). Si riscontrava anche scomparsa dell'ipertensione arteriosa.

Foto 1 : X-Ray polmone del febbraio 2006

Foto 2: X-Ray polmone del maggio 2006

DIAP. 3

Il Cancro è una malattia degenerativa dovuta a carenze di vitamine e/o a intossicazione da sostanze chimiche presenti nei cibi.

Le vitamine e le sostanze pro-vitaminiche presenti nelle piante naturali di comune alimentazione umana possono essere stimate in numero superiore a 13.000-15.000.....

DIAP. 4

L'introduzione nell'agricoltura moderna degli Organismi Geneticamente Modificati (O.G.M.) è una ingiustificata e pericolosissima alterazione di ciò che l'Evoluzione ha prodotto nelle piante in centinaia di milioni di anni:

piante sulle quali si è basata la successiva evoluzione biochimica dei complessi organismi animali superiori, culminati con l'avvento dei Mammiferi negli ultimi 65 milioni di anni e quindi con la comparsa dell'Uomo;

pertanto il delicato equilibrio biochimico della specie umana dipende dall'integrità delle specie vegetali così come l'Evoluzione le ha condotte fino a noi, poiché la Salute di ciascuno di noi è basata sulla Biochimica cellulare umana, e questa dipende, nella propria complessità genomica (DNA), dall'utilizzo di migliaia di vitamine e di complessi fitochimici presenti in Natura.

DIAP. 5

La pianta è infatti un organismo complesso, frutto dell'evoluzione biologica avvenuta in centinaia di milioni di anni:

ogni modificazione genetica provocata in essa dall'Uomo

(con radiazioni come a Chernobyl,

o con retro-virus come attualmente compiuto con gli O.G.M.),

produrrà comunque un danno,

un danno che non potrà essere riconosciuto,

poiché l'Uomo conosce bene soltanto poche decine di vitamine e di altre sostanze pro-vitaminiche.

Viceversa, le vitamine e le altre sostanze contenute nelle piante sono decine di migliaia, e sono queste le responsabili del corretto funzionamento della complessa biochimica umana e del genoma umano (DNA).

DIAP. 6

Oggi, per ottenere il vantaggio di una maggiore produzione agricola, si ricorre al metodo di modificare il patrimonio genetico delle piante naturali, allo scopo di:

- 1) modificarne la struttura
- 2) renderle sterili (per obbligare gli agricoltori a comprare nuovi semi ogni anno)
- 3) brevettarne la trasformazione indotta.
- 4) e rivendere in tutto il mondo il prodotto così ottenuto.

DIAP. 7

Si afferma inoltre che esista sostanziale equivalenza tra:

- 1) il prodotto geneticamente modificato (OGM)
- 2) e quello ottenuto con la selezione dei caratteri genetici, cioè tramite incrocio naturale di piante (come da sempre fatto dall'umanità nel corso di migliaia di anni).

Da parte nostra si afferma invece che tale “*sostanziale equivalenza*” è assolutamente insostenibile, perché:

l'incrocio naturale di piante avviene con semi naturali della stessa specie, mentre la manipolazione genetica (OGM) avviene superando le barriere di specie vegetali, mediante introduzione di geni di altre specie vegetali, o addirittura di batteri, virus o animali.

Infatti la maggior parte dei geni usati dall'ingegneria genetica provengono da specie viventi che non hanno mai fatto parte dell'alimentazione umana e, addirittura, sono provenienti da DNA non appartenenti a piante ma ad animali, batteri o virus e/o retrovirus transgenici.

DIAP. 8

Si possono così ravvisare OTTO minacce immediate:

PRIMO: *Perdita dei complessi pro-vitaminici e vitaminici delle piante*

SECONDO: *mutazioni genetiche delle piante e conseguente alterazione della Biochimica umana*

TERZO: *fallimento della dieta-anti-cancro*

QUARTO : *malattie indotte da virus transgenici*

QUINTO : *intossicazione da veleni sintetizzati da piante transgeniche*

SESTO: *possibili carestie a livello mondiale a causa della tecnologia “TERMINATOR”*

SETTIMO: *modificazione transgenica di piante naturali*

OTTAVO: *scomparsa irreversibile del patrimonio genetico naturale delle piante naturali*

DIAP. 9

PRIMO: Perdita dei complessi pro-vitaminici e vitaminici delle piante

E' un danno che, se propagato a piante alimentari di uso comune, potrebbe rendere del tutto impossibile la cura dei tumori e di molte altre malattie tramite quanto considerato in questo lavoro.

Le vitamine naturali, contenute nelle piante, provocano la APOPTOSI (suicidio) nelle cellule tumorali, o forme di morte correlate (PSEUDO-APOPTOSI), come nel caso della vitamina B17.

Apoptosi :

Pseudo-apoptosi (B17):



mevalonati

Cancer Letters 175 (2002) 129–139

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Volatile isoprenoid constituents of fruits, vegetables and herbs cumulatively suppress the proliferation of murine B16 melanoma and human HL-60 leukemia cells

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Received 27 May 2001; received in revised form 15 August 2001; accepted 16 August 2001

Abstract

Substantial evidence from epidemiological studies supports the inverse association between the intake of fruits, vegetables and other plant products and cancer incidence. Cancer-preventive constituents of fruits and vegetables may inhibit carcinogen activation, enhance carcinogen detoxification, prevent carcinogens from interacting with critical target sites, or impede tumor progression. These activities, however, are achievable only when levels of individual bioactive constituents reach beyond those attainable from a normal balanced diet. Isoprenoids, a broad class of mevalonate-derived phytochemicals ubiquitous in the plant kingdom, suppress the proliferation of tumor cells and the growth of implanted tumors. A search for volatile isoprenoid constituents of food products spanning seven plant families identified 179 isoprenoids. Of these, 41 purchased from commercial sources were screened for efficacy in suppressing the proliferation of murine B16 melanoma cells. Individual isoprenoids suppressed the proliferation of B16 and HL-60 promyelocytic leukemia cells with varying degrees of potency. Cell cycle arrest at the G₀-G₁ phase and apoptosis account, at least in part, for the suppression. Blends of isoprenoids suppressed B16 and HL-60 cell proliferation with efficacies equal to the sum of the individual impacts. These findings suggest that the cancer-protective property of fruits, vegetables, and related products is partly conferred by the cumulative impact of volatile isoprenoid constituents. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Isoprenoids; Murine B16 melanoma; Human HL-60 leukemia

1. Introduction

The inverse association between cancer risk and fruit and vegetable consumption, evidenced by more than 250 epidemiological studies with rare disputable exceptions on certain types of cancer [1], has been well documented [2]. Numerous bioactive constituents have been hypothesized to act as cancer-preventing agents by inhi-

biting the activation of procarcinogens, enhancing the detoxification of carcinogens, preventing carcinogens from interacting with critical target sites, or impeding the progression of carcinogenesis [3–5]. These constituents further modulate the promoter-independent growth of tumors by affecting the expression of genes involved in cell signaling, particularly those regulating cell proliferation, the cell cycle, apoptosis and differentiation [3]. Alternatively, bioactive constituents may suppress enzymatic pathways that provide products essential for the post-translational processing and biological activity of proteins critical in cell proliferation [6,7].

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Eugenolo : APOPTOSI : 75-150 μ M
B-ionone : " 70 μ M
Farnesolo : " 30 μ M
Geraniolo : " 70-140 μ M

Terpeneols : 380 μ M
Limonene : 40 μ M (melanoma)
180 μ M (Leukemia)

Table 3

IC₅₀ values reflecting the relative tumor-suppressive potencies of volatile isoprenoids

Isoprenoid	IC ₅₀ (μmol/l) ^a
<i>trans, trans</i> Farnesol	28 ± 16 ^b
Citral ^c	30 ± 10
Nerolidol	65 ± 11
Thymol	120 ± 15
Perillaldehyde	120 ± 17
Carvacrol	120 ± 15
Geraniol	139 ± 27
β-Ionone	140 ± 23
Geranyl butyrate	150
Eugenol	163 ± 27
Geranyl acetone	171 ± 35
Camphene	178
Geranyl acetate	185 ± 35
β-Caryophyllene	190
α-Ionone ^d	190
Cresol	200
Perillyl alcohol	250 ± 28
Ocimene	250
Menthol	250
Carvyl acetate	267 ± 42
Fenchol	300
Fenchone	>300
Menthone	>300
Myrtenol	300
Nerol	332 ± 33
Geranic acid	338
α-Pinene	350
Citronellal	350
Cineole	400
Linalool	400
α-Terpineol	400
Citronellol	415
Citronellyl propionate	450
<i>d</i> -Limonene	450 ± 43
<i>p</i> -Cymene	>500
<i>p</i> -Mentha-2,8-dien-1-ol	>500
Verbenone	>500

Diap. 11

179

Volatile

Isoprenoids

(multi di. etc)

provocano

APOPTOSI

nei CANCRO

e Leucemie

Abscisic acid ^b	α-Cubebene ^d	β-Ionone ^{a,d}	Ocimene ^c
Acorenone ^e	<i>p</i> -Cymene ^{a,g}	γ-Ionone ^a	Ocimenol ^c
Alloaromadendrene ^f	<i>p</i> -Cymen-8-ol ^b	3,4-Didehydro-β-ionone ^b	Perillaldehyde ^d
Aromadendrene ^g	<i>p</i> -Cymen-9-ol ^b	Dihydro-α-ionone ^b	Perillyl alcohol ^d
<i>trans</i> -α-bergamotene ^e	Damascenone ^{a,b,e}	Dihydro-β-ionone ^b	α-Phellandrene ^{a,b,d,f,g}
Bisabolene ^d	3-Hydroxy-β-damascone ^b	Epoxy-β-ionone ^a	β-Phellandrene ^{a,b,d,g}
Borneol ^e	4-Hydroxy-β-damascone ^b	3-Hydroxy-7,8-dihydro-β-ionone ^b	α-Pinene ^{a,g}
Bornyl acetate ^g	β-Elemene ^{a,d,g}	3-Hydroxy-5,6-epoxy-β-ionone ^b	β-Pinene ^{b,d,g}
Isoborneol ^g	γ-Elemene ^a	3-Hydroxy-α-ionone ^b	α-Pinene oxide ^g
8-Cadinene ^d	δ-Elemene ^a	3-Hydroxy-β-ionone ^b	Pinocampione ^b
Cadinene ^g	Estragol ^g	4-Hydroxy-β-ionone ^b	Piperitol ^g
Cadinol ^g	Eugenol ^{a,g}	β-Methyl-β-ionone ^b	Pipertone ^{a,g}
Camphene ^{a,d,g}	Isoeugenol ^{a,c,g}	4-Oxo-β-ionone ^b	Pristane ^a
Camphor ^{h,g}	Methyl eugenol ^g	Pseudoionone ^a	Pulegone ^g
5-Caranol ^g	β-Farnesene ^{a,b,g}	Isopropenaphone ^b	Sabinene ^{a,g}
3-Carene ^{a,d,g}	Farnesol ^a	Isopulegol ^d	Sabinene hydrate ^g
Carvacrol ^{a,c,g}	Farnesol ^{a,c}	Limonene ^{a,g}	Sabinal ^d
Carvacrol methyl ether ^g	Farnesyl acetate ^{a,c}	Limonene-1,2-epoxide ^d	Santalol ^g
Carvone ^{a,d,g}	β-Farnesol ^a	Linalool ^{a,g}	Selinadiene ^d
Pinocarvone ^g	Farnesyl acetone ^a	Dihydrolinalool ^b	β-Selinene ^d
1-Carveol ^{a,d,g}	α-Fenchol ^{b,g}	Linalool oxides ^{a,c}	γ-Selinene ^d
Pinocarveol ^g	Fenchone ^{h,g}	Linalyl acetate ^{a,b,d,g}	α-Sinensal ^d
Carvyl acetate ^b	Fenchyl acetate ^g	Longifolene ^d	β-Sinensal ^d
β-Caryophyllene ^{a,d,g}	Fendyl alcohol ^g	<i>p</i> -Mentha-2,8-dien-1-ol ^d	Spathulenol ^g
Caryophyllene oxide ^g	Geraniol ^{a,b,d,g}	<i>trans</i> - <i>p</i> -menthen-9-ol ^d	Styrene ^b
α-Cedrene ^c	Geranic acid ^{a,c}	Menthyl acetate ^g	<i>cis</i> -1,8-terpin ^e
Cineole ^c	Geraniol ^{a,c,g}	Menthyl acetate ^g	<i>trans</i> -1,8-terpin ^e
1,4-Cineole ^c	Geranyl acetate ^{a,b,d,g}	Neomenthyl ^g	α-Terpineol ^{a,d,g}
1,8-Cineole ^{c,g}	Geranyl acetone ^{a,b}	Neomenthyl ^g	γ-Terpineol ^{a,g}
2-Hydroxy-1,8-cineole ^c	Geranyl butyrate ^a	Isomenthyl ^g	Terpinen-1-ol ^c
Cinnamaldehide ^b	Germacrene A ^a	α-Murolene ^{a,g}	Terpinen-4-ol ^{a,c,g}
Ethyl cinnamate ^b	Germacrene B ^{a,g}	Myrcenol ^c	α-Terpineol ^{a,g}
Methyl cinnamate ^{h,g}	Germacrene C ^a	Myrcen-2-ol ^c	4-Terpineol ^{d,g}
Citral ^d	Germacrene D ^a	Myrcene ^{a,g}	Terpinolene ^{a,c,g}
Cyclocitral ^{a,b}	Bicyclogermacrene ^g	Myrtenol ^b	α-Terpinyol acetate ^{b,g}
Citronellal ^{a,d}	Hottienol ^{h,g}	Nerol ^{a,b,d}	<i>cis</i> -dihydro-α-terpinyl acetate ^g
Citronellol ^{a,c,g}	α-Humulene ^{a,d,g}	Nerol oxide ^c	Thujene ^{c,d,g}
Citronellyl acetate ^{a,d}	α-Ionol ^b	Nerol acetate ^{a,g}	Thujone ^g
Citronellyl butyrate ^a	β-Ionol ^b	Nerolidol ^{a,b}	Thymol ^{c,g}
Citronellyl propionate ^a	3-Hydroxy-7,8-dihydro-β-ionol ^b	α-Nerolidol ^{b,d,g}	Tricyclene ^g
Hydroxycitronellol ^c	3-Hydroxy-β-ionol ^b	β-Nerolidol ^{b,g}	Vanillin ^{b,d}
α-Copaene ^g	3-Oxo-α-ionol ^b	Noodkatone ^a	Valencene ^d
β-Copaene ^d	4-Oxo-β-ionol ^b	Ocimene ^{b,d,g}	Verbenone ^{b,g}
o-Cresol ^c	3-Oxo-7,8-dihydro-α-ionol ^b	Allo-ocimene ^{d,f,g}	Vitispirane ^g
<i>p</i> -Cresol ^{a,c}	α-Ionone ^{a,c}		

^a Solanaceae; ^b Rosaceae; ^c Ericaceae; ^d Rutaceae; ^e Viaceae; ^f Anacardiaceae; ^g Labiaceae; ^h Lamiaceae.



Acacetin inhibits the proliferation of Hep G2 by blocking cell cycle progression and inducing apoptosis

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Abstract

Flavonoids are a broadly distributed class of plant pigments, universally present in vascular plants and responsible for much of the coloring in nature. They are strong antioxidants that occur naturally in foods and can inhibit carcinogenesis in rodents. In this study, we examined acacetin (5,7-dihydroxy-4'-methoxyflavone), a flavonoid compound, for its effect on proliferation in a human liver cancer cell line, Hep G2. The results showed that acacetin inhibited the proliferation of Hep G2 by inducing apoptosis and blocking cell cycle progression in the G1 phase. Enzyme-linked immunosorbent assay showed that acacetin significantly increased the expression of p53 and p21/WAF1 protein, contributing to cell cycle arrest. An enhancement in Fas/APO-1 and its two form ligands, membrane-bound Fas ligand and soluble Fas ligand, as well as Bax protein, was responsible for the apoptotic effect induced by acacetin. Taken together, our study suggests that the induction of p53 and activity of the Fas/Fas ligand apoptotic system may participate in the antiproliferative activity of acacetin in Hep G2 cells.

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Keywords: Acacetin; p53; Cell cycle; Fas/APO-1; FasL; Apoptosis

1. Introduction

Hepatocellular carcinoma (HCC), one of the most common cancers in the world, develops from transformed hepatocytes during the course of chronic liver disease. It is responsible for approximately 1 million deaths annually, mainly in underdeveloped and developing countries [1,2]. Apoptosis has been characterized as a fundamental cellular activity to maintain the physiological balance of the organism. It is also involved in immune defense machinery [3] and plays a necessary role as a protective mechanism against carcinogenesis by eliminating damaged cells or abnormal excess cells proliferated owing to various chemical agents' induction [3,4]. Emerging evidence has demonstrated that the anticancer activities of certain chemotherapeutic agents

are involved in the induction of apoptosis, which is regarded as the preferred way to manage cancer [3,4].

Flavonoids are a broadly distributed class of plant pigments, universally present in vascular plants and responsible for much of the coloring in nature [5]. They are strong antioxidants that occur naturally in foods and can inhibit carcinogenesis in rodents [5,6]. Acacetin (5,7-dihydroxy-4'-methoxyflavone), a flavonoid compound, has been reported to possess antiperoxidative, anti-inflammatory, and antiparasmodial effects [7–9], and to enhance differentiation-inducing activity in HL-60 cells [10]. In addition, acacetin can also inhibit glutathione reductase, cytochrome P450, and topoisomerase I-catalyzed DNA religation [11–13]. In this study, we determined the antiproliferative activity of acacetin, and examined its effect on cell cycle distribution and apoptosis in the human liver cancer cell line, Hep G2. Furthermore, to establish the anticancer mechanism of acacetin, we assayed the levels of p53, p21/WAF1, Fas/APO-1 receptor, Fas ligand (FasL), and Bax, which are strongly associated with the signal transduction pathway of apoptosis and affect the chemosensitivity of tumor cells to anticancer agents.

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Abbreviations: FasL, Fas ligand; mFasL, membrane-bound Fas ligand; sFasL, soluble Fas ligand; DMEM, Dulbecco's modified Eagle's medium; Z-IETD-FMK, N-benzoyloxycarbonyl-fluoromethylketone; PI, propidium iodide; ELISA, enzyme-linked immunosorbent assay.

Fas/APO1 → CASPASE 8 ⇒ CASPASE 3
APPTOSI = 10–20 µg/mL (in 48 hr)



Antioxidant and anticancer activity of extract from *Betula platyphylla* var. *japonica*

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Abstract

The antioxidant and anticancer properties of a medicinal plant, *Betula platyphylla* var. *japonica* were investigated. The total methanol extract of *B. platyphylla* var. *japonica* had protective effects against hydrogen peroxide (H₂O₂) in the Chinese hamster lung fibroblast (V79-4) cell line and induced apoptotic cell death in human promyelocytic leukemia (HL-60) cells, a cancer cell line. *B. platyphylla* var. *japonica* extract significantly increased cell viability against H₂O₂. The extract also showed high 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (IC₅₀ 2.4 µg/ml) and lipid peroxidation inhibitory activity (IC₅₀ below 4.0 µg/ml). Furthermore, *B. platyphylla* var. *japonica* extract reduced the number of V79-4 cells arrested in G₂/M in response to H₂O₂ treatment and increased the activities of several cellular antioxidant enzymes, including superoxide dismutase, catalase and glutathione peroxidase. Treatment with *B. platyphylla* var. *japonica* extract induced cytotoxicity and apoptosis in HL-60 cells, as shown by nucleosomal DNA fragmentation, increases in the subdiploid cell population, and fluorescence microscopy. *B. platyphylla* var. *japonica* extract gradually increased the expression of pro-apoptotic Bax and led to the activation of caspase-3 and cleavage of PARP. These findings suggest that *B. platyphylla* var. *japonica* exhibits potential antioxidant and anticancer properties.

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Keywords: Antioxidant; Anticancer; Apoptosis; *Betula platyphylla* var. *japonica*

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APPTOSI 50% 150 µg/mL



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Toxicology Letters 155 (2005) 343–351

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Betulinic acid induces apoptosis in human chronic myelogenous leukemia (CML) cell line K-562 without altering the levels of Bcr-Abl

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Available online 8 December 2004

Abstract

Betulinic acid (BA), a plant derived triterpenoid, isolated from various sources shows cytotoxicity in cell lines of melanoma, neuroectodermal and malignant brain tumors. Chronic myelogenous leukemia (CML) is characterized by Philadelphia chromosome (Bcr-Abl), a molecular abnormality leading to the intrinsic tyrosine kinase activity that provides growth and survival advantage to the cells. Present study describes the cytotoxicity of BA on human CML cell line K-562, positive for Bcr-Abl. The decrease in the viability of K-562 cells treated with BA at different concentrations and time intervals was assessed using MTT assay. Cell death induced by BA was determined to be apoptotic as measured by FACS analysis of PI stained nuclei. PS externalization by Annexin-V fluorescence and characteristic DNA fragmentation. DiOC₆(3) fluorescent probe determined alterations in the mitochondrial membrane potential (MMP). RT-PCR confirmed the expression levels of Bcr-Abl in controls and K-562 cells treated with BA. The rapid loss of MMP of K-562 cells upon treatment with BA shows the direct activation of apoptosis at the level of mitochondria, overcoming the resistance of the high levels of expression of Bcr-Abl.
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Keywords: BA; CML; K-562; Cytotoxicity and apoptosis

1. Introduction

Many plant products are being evaluated for their chemotherapeutic potential for diverse diseases and

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Melanoma, leucemia, Astrocytoma III-IV grade

Via mitochondria

Apoptosis = 50% 12 µg/mL
in LEUCEMIA

PP% 40 µg/mL

DIAP 15



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Life Sciences 72 (2002) 1–9

Life Sciences

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23-Hydroxybetulinic acid-mediated apoptosis is accompanied by decreases in *bcl-2* expression and telomerase activity in HL-60 Cells

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Abstract

23-Hydroxybetulinic acid, a derivative of betulinic acid, was investigated for its apoptotic effect and the associated telomerase activity in human leukemia HL-60 cells. Apoptosis and *bcl-2* were determined by flow cytometry analysis. A PCR-based telomeric repeat amplification protocol assay was used to detect telomerase activity. Results showed that 23-hydroxybetulinic acid induced growth arrest and apoptotic cell death in HL-60 cells. The apoptotic events were associated with concurrent down-regulation of *bcl-2* and the telomerase activity. Our data suggest that 23-hydroxybetulinic acid may be a potential cytotoxic agent for treatment of cancer.
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Keywords: 23-Hydroxybetulinic acid; Telomerase; Apoptosis; *bcl-2* gene; HL-60 cells

Introduction

Pulsatilla chinensis (Bunge) Regel is a Chinese medicinal herb for “blood-cooling” and detoxification in traditional Chinese medicine, and as such has been used for the treatment of amoebic dysentery and malaria [1]. We have isolated one of the compounds, 23-hydroxybetulinic acid (23-HBA) from the root of the plant [2]. Structurally, it closely resembles betulinic acid (BetA), which has been identified as

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Apoptosis in leukemia: 25% on 10 µM
50% on 100 µM

Melanoma
Neuroblastoma
Medullary-blastoma
fibroblastoma

Sarcoma & Ewing

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Dietary bioflavonoids induce apoptosis in human leukemia cells

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Abstract

Dietary bioflavonoids are secondary metabolites of plants that are known to have a variety of bio-effects, including anti-cancer activity. In this study, we examined the effects of flavonoids on the growth of human leukemia cells and found that certain flavonoids induce apoptosis in a variety of human leukemia cells. The apoptosis induced by bioflavonoids was dose-dependent and was accompanied by a disruption of the mitochondrial transmembrane potential and the activation of caspase. Our data suggests that dietary bioflavonoids may be useful chemotherapeutic reagents for leukemia patients.
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Keywords: Bioflavonoid; Apoptosis; Acute lymphoblastic leukemia; Precursor-B-cell

1. Introduction

Flavonoids are ubiquitously occurring and widely consumed secondary metabolites of plants [1,2]. Flavonoids can be divided into three main groups: Flavones, Flavonones (2,3-dihydroflavones), and isoflavones, which differ in structure and ring substitutions [3]. They have diverse pharmacological properties, including antioxidant, cytoprotective, and anti-inflammatory activities [1,2], and have also been reported to display anti-viral [4] and anti-parasitic [5] activities.

Moreover, some flavonoids are known to act as anti-cancer reagents. For example, Yoshida et al. reported that Quercetin markedly inhibited the growth of human gastric cancer cells [6]. Record et al. also described the inhibition of B16 melanoma cells by Genistein, both in vivo and in vitro [7]. Huang et al. demonstrated that Luteolin and Quercetin

significantly inhibited the proliferation of epidermoid carcinoma A431 cells with an overexpression of epidermal growth factor receptor [8]. Indeed, some bioflavonoids like Quercetin and Genistein have already been used as chemotherapeutic agents in phase trials [9,10].

In an attempt to examine the effects of flavonoids on the growth of human leukemic cells, we challenged cultured human leukemic cell lines with several kinds of flavonoids. In the present study, we demonstrated that certain flavonoids can induce significant apoptosis in a variety of human leukemia cells.

2. Materials and methods

2.1. Cells and reagents

The cell line BV-173 that were established from a patient in an acute relapse who most likely had Ph1-positive chronic myelogenous leukemia [11]; the acute-phase of chronic myelogenous leukemia-derived cell lines K-562 (Japanese Cancer Research Resources Bank, JCRB, Tokyo, Japan)

Abbreviations: ALL, acute lymphoblastic leukemia; CD, cluster of differentiation; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PC-5, PE-Cy-5; PC-7, PE-Cy-7; topo, topoisomerase
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Flavoni
Flavononi
Iso-Flavoni

Boswellic acid acetate induces differentiation and apoptosis in highly metastatic melanoma and fibrosarcoma cells

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Abstract

The aim of the study was to investigate the antitumor and/or preventive effect of BC-4, an isomeric compound isolated from the plant *Boswellia carteri* Birdw. containing alpha- and beta-boswellic acid acetate in 1:1, MW 498.3. We used the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay to study the growth inhibition activity of BC-4. Tumor cells migration within a three-dimensional collagen matrix was recorded by time-lapse videomicroscopy and computer-assisted cell tracking. Topoisomerase II was isolated from mouse melanoma B16F10 cells and its activity was determined by its ability to cut plasmid pBR322 DNA. The secretion and activity of matrix metalloproteinases (MMPs) from human fibrosarcoma HT-1080 cells were determined by gelatin zymography. BC-4 was a of matrix metalloproteinases (MMPs) from human fibrosarcoma HT-1080 cells, blocked the cell population in G1 phase and cytostatic compound and could induce the differentiation of B16F10 mouse melanoma cells, blocked the cell population in G1 phase and inhibited topoisomerase II activity. The G1 phase population of B16F10 cells was increased from 57.4 to 87.7%, while S phase population was reduced from 33.3 to 5.9% after treatment with BC-4 at 25 μM concentration for 48 h. BC-4 also inhibited the migration activity of B16F10. BC-4 could induce apoptosis of HT-1080 cells, as proved by acridine orange fluorescence staining, Wright-Giemsa staining, electrophoresis, DNA fragmentation and flow cytometry. BC-4 inhibited the secretion of MMPs from HT-1080 cells, too. In conclusion, if it turns out that BC-4 is a well tolerated substance, exhibiting no significant toxicity or side effects, being evaluated currently in China, BC-4 is a good candidate for the prevention of primary tumor, invasion and metastasis.
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Keywords: Boswellic acid acetate; Differentiation; Apoptosis; Matrix metalloproteinases; Migration; Chemoprevention

1. Introduction

A difficult and complex area of plant research is devoted to a plant with the general title “frankincense”. It is accepted that frankincense is a member of the family Burseraceae, and the genus *Boswellia*. From all of the sources accessed, a few front runners emerged: *Boswellia sacra*, *Boswellia serrata* and *Boswellia carteri*. Frankincense was commonly used for medicinal purposes. Pliny the Elder (1st century), used frankincense as an antidote to hemlock poisoning. The Iranian physician Avicenna (10th century) thought that it was good for body ailments such as tumors, vomiting, dysentery and fevers. *Boswellia carteri* Birdw. is used in traditional Chinese medicine (TCM) which is a remedy for activat-

ing blood circulation, relieving pain against leprosy, cancer, gonorrhea and carbuncles, and as an astringent. Boswellic acid acetate (BC-4) is one of several active principles isolated from the resin of this herb. There are two isoforms of boswellic acid acetate, α-boswellic acid acetate (BC-4-I) and β-boswellic acid acetate (BC-4-II) and both do have a pentacyclic triterpene structure.

Boswellic acid derivatives show a strong antiinflammation effect *in vivo* and this effect has been proved to be due to the inhibition of 5-lipoxygenase (5-LO) [1]. It has also been reported that 5-LO inhibitors inhibit the growth of non-small cell lung cancer [2]. Furthermore, lipoxygenase inhibitors demonstrate an activity as cancer chemopreventive agents [3]. Based on the screening in HL-60 cells for differentiation inducers, BC-4 was identified as a potent differentiation inducer [4]. We have reported that BC-4 can induce myelocytic leukemia cell differentiation at low concentration and at higher concentrations apoptosis in leukemia cells [5]. In addition to hematologic malignancies, an apoptotic effect

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Melanoma
Fibro-SARCOMA } APOPTOSIS nel 60% con 50 μM / 36 hr

Bupleurum → SAIKO SAPONINA : CANCRO del PANCREAS : 5 μM (19)



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Pharmacological evaluation of several major ingredients of Chinese herbal medicines in human hepatoma Hep3B cells

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Abstract

Long-dan-tan (Chinese name) is one of the most common herbal medicines used by Chinese people with chronic liver disease. Accumulated anecdotal evidence suggests that Long-dan-tan may show a beneficial effect in patients with hepatocellular carcinoma. Long-dan-tan is made from five plants: Gentiana root, Scutellaria root, Gardenia fruit, Alisma rhizome, and Bupleurum root. In this study, we have examined the cytotoxic effects of the five major ingredients isolated from the above plants, i.e. gentiopicroside, baicalin, geniposide, alisol B acetate and saikosaponin-d, respectively, on human hepatoma Hep3B cells. Annexin V immunofluorescence detection, DNA fragmentation assays and FACSscan analysis of propidium iodide-staining cells showed that gentiopicroside, baicalin, and geniposide had little effect, whereas alisol B acetate and saikosaponin-d profoundly induced apoptosis in Hep3B cells. Alisol B acetate, but not saikosaponin-d, induced G2/M arrest of the cell cycle as well as a significant increase in caspase-3 activity. Interestingly, baicalin by itself induced an increase in H₂O₂ generation and the subsequent NF-κB activation; furthermore, it effectively inhibited the transforming growth factor-β₁ (TGF-β₁)-induced caspase-3 activation and cell apoptosis. We suggest that alisol B acetate and saikosaponin-d induced cell apoptosis through the caspase-3-dependent and -independent pathways, respectively. Instead of inducing apoptosis, baicalin inhibits TGF-β₁-induced apoptosis via increase in cellular H₂O₂ formation and NF-κB activation in human hepatoma Hep3B cells.

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Keywords: Alisol-B-monoacetate; Saikosaponin-d; Baicalin; Apoptosis; Hep3B

1. Introduction

Herbal medicines which have been used by Chinese people for thousands of years are now being manufactured in many countries as quality-controlled drugs with standardized quantities of ingredients. Long-dan-tan (Chinese name) is one of the most common herbal medicines used by Chinese people suffering from chronic liver disease. Accumulated anecdotal evidence suggests that Long-dan-tan may show a beneficial effect in patients with hepatocellular carcinoma. However, to date it has not been scientifically evaluated in human hepatoma.

Long-dan-tan is made from five important plants, i.e. Gentiana rhizome, Scutellaria root, Gardenia fruit, Alisma rhizome, and Bupleurum root. In this study we have examined the cytotoxic effects of the five major ingredi-

ents isolated from the above herbs, i.e. gentiopicroside, baicalin, geniposide, alisol B acetate and saikosaponin-d, respectively, on human hepatoma Hep3B cells. It has been suggested that gentiopicroside exhibits a modest hepatoprotective effect on D-galactosamine/lipopolysaccharide-induced liver injury in mice (Hase et al., 1997). Baicalin is a flavonoid, and shows anti-inflammatory and antioxidant activities and inhibits hepatic fibrosis (Chang et al., 1993; Lin and Shieh, 1996; Shimizu, 2000). Furthermore, it also plays a role in growth regulation, influencing apoptosis and anti-proliferation, in several types of cancer cells including human prostate cancers (DU145 and PC-3), pancreatic cancers (PANC-1, MiaPaca2, Capan2 and HPAF), colon cancers (Caco-2) and breast cancers (MDA-MB-435 and MCF-7) (So et al., 1997; Ding et al., 1999; Kuntz et al., 1999; Chan et al., 2000). In addition to these anticancer effects, baicalin also profoundly inhibited ascorbic acid-induced lipid peroxidation in rat liver microsomes (Gao et al., 1995). Therefore, herbal medicines whose major ingredient is baicalin are frequently used in

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Capsaicin inhibits growth of adult T-cell leukemia cells

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Abstract

Human T-cell leukemia virus type 1 (HTLV-1)-associated adult T-cell leukemia (ATL) is resistant to conventional chemotherapy. We examined the in vitro effects of capsaicin, the principal ingredients of red pepper, on three ATL cell lines. Capsaicin treatment inhibited the growth of ATL cells both in dose- and time-dependent manner. The inhibitory effect was mainly due to the induction of cell cycle arrest and apoptosis. Capsaicin treatment also induced the degradation of Tax and up-regulation of IκB-α, resulting in the decrease of nuclear factor (NF)-κB/p65 DNA binding activity. In addition, the Bcl-2 level was found to be decreased. Based on these findings, capsaicin may be considered for chemoprevention of ATL.

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Keywords: Capsaicin; ATL cells; Growth inhibition; Cell cycle arrest; Apoptosis; Tax; IκB-α/NF-κB; Bcl-2

1. Introduction

Adult T-cell leukemia (ATL) is an aggressive form of human T-cell malignancy caused by human T-cell leukemia virus type 1 (HTLV-1) [1–3]. Tax, a HTLV-1 transcriptional transactivator protein, can interact with various cellular proteins, such as nuclear factor (NF)-κB, cAMP response element binding (CREB) protein and serum response factor (SRF), thus activates the transcription of proto-oncogenes (*c-fos*, *c-jun*, *fra-1* and *c-myc*), cytokines (IL-2, IL-6, TGF-β and GM-CSF) and cytokine receptor (IL-2R) [4,5]. Tax can also repress the transcription of cellular genes, such as DNA polymerase β and *bax* [6,7]. So Tax is considered to play a crucial role in several pathways on the transformation of T cells by HTLV-1.

Capsaicin, a homovanillic acid derivative (8-methyl-N-vanillyl-6-nonenamide), is an active component of the red pepper of the genus *Capsicum* and has been found to be protective against experimentally induced mutagenesis and tumorigenesis [8,9]. In vitro, capsaicin has been found to inhibit the growth of various immortalized and malignant cells [10,11] and induce apoptosis in transformed cells [12,13]. In addition, capsaicin is also found to be a potent inhibitor of NF-κB activation [14].

NF-κB is a ubiquitous transcription factor that binds to a specific DNA sequence as a dimeric complex composed of various combinations of members of the Rel/NF-κB family, homodimers or heterodimers of RelA (p65), p50, c-Rel, p52 and RelB [15]. In resting lymphocyte, NF-κB dimers are sequestered in the cytoplasm in an inactive form by association with an inhibitory IκB subunit, mainly IκB-α. Following cellular activation, multiple kinase lead to phosphorylation of IκB-α and proteasome-mediated degradation, resulting in the release of an active NF-κB complex that translocates to the nucleus. In the nucleus, NF-κB binds its response elements and activates various genes involved in the inflammation, immune response and cellular growth control [16,17]. The activation of NF-κB is also essential for the inhibition of apoptosis [18]. As activation of NF-κB by Tax protein plays one of the major roles in the pathogenesis of ATL [19], and capsaicin can inhibit the activation of NF-κB, we hypothesized that capsaicin may have an inhibitory potential against HTLV-1 induced-ATL.

Multiple chemotherapy combinations have been tried for the treatment of ATL, however, the results have been disappointing with a median survival time of 8 months [20]. With an objective to find out newer chemotherapeutic agents for ATL, this study was designed to investigate the growth-inhibitory potentials of capsaicin on several ATL cell lines and the possible mechanisms involved in such in vitro growth-inhibitory effect.

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Alisma plantago aquatica : Alisol B } 50 μM
Fis, Saponina

Apoptosis : 50% con 60 μM
80% 100 μM

80% con 200 μM

Prostate

Catechin, a green tea component, rapidly induces apoptosis of myeloid leukemic cells via modulation of reactive oxygen species production *in vitro* and inhibits tumor growth *in vivo*

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Yoshitaka Miyakawa
Kentaro Kinjo
Taketo Yamada
Nobumichi Hozumi
Yasuo Ikeda
Masahiro Kizaki

Background and Objectives. The aim of this study was to investigate the possibility of green tea polyphenol, (-)-epigallocatechin-3-gallate (EGCG) as a novel therapeutic agent for patients with myeloid leukemia.

Design and Methods. We investigated the effects of EGCG on the induction of apoptosis in leukemic cells *in vitro* and *in vivo*. We further examined the molecular mechanisms of EGCG-induced apoptosis in myeloid leukemic cells.

Results. EGCG rapidly induced apoptotic cell death in retinoic acid (RA)-resistant acute promyelocytic leukemia (APL), U9-1 cells within 3 h. EGCG-induced apoptosis in U9-1 cells was associated with the loss of mitochondrial transmembrane potentials ($\Delta\Psi$) and activation of caspase-3 and -9. Elevation of intracellular reactive oxygen species (ROS) production was also demonstrated during EGCG-induced apoptosis of U9-1 as well as fresh myeloid leukemic cells. In NOD/SCID mice transplanted with U9-1 cells, EGCG effectively inhibited tumor growth *in vivo*, and the number of mitoses among the cells significantly decreased in comparison to the number in control mouse cells.

Interpretation and Conclusions. In summary, EGCG has potential as a novel therapeutic agent for myeloid leukemia via induction of apoptosis mediated by modification of the redox system.

Key words: green tea, catechin, apoptosis, leukemic cells, reactive oxygen species.

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Recently, green tea has attracted much attention because of its beneficial health effects; the polyphenolic compounds present in green tea include (-)-epigallocatechin-3-gallate (EGCG), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and epicatechin (EC), which have been shown to have cancer preventive effects in many animal tumor models.¹ In fact, epidemiologic studies have shown that green tea consumption can reduce the incidence of cancer and metastases.²⁻⁶ Green tea has unique characteristics as an agent and has few adverse effects. In addition, it is inexpensive, can be consumed orally, and has a long history as a generally tolerated beverage among all races. Therefore, green tea appears to have the potential of becoming an ideal agent for chemoprevention.⁷ Moreover, EGCG has been shown to induce G₀/G₁ phase cell cycle arrest in human epidermoid carcinoma cells, thereby inhibiting proliferation and inducing apoptosis in many cancer cells *in vitro*.¹⁴ The therapeutic approach to acute leukemia is basically chemotherapy to achieve complete

remission, based on the concept of *total cell killing*.¹⁰ However, severe side effects and complications such as serious infections and bleeding due to anti-cancer drugs are major problems in the clinical setting. In addition, repeated episodes of relapse of the disease may lead to refractory or chemotherapy-resistant leukemia. The clinical evidence thus suggests the limitations of leukemia chemotherapy; novel effective therapeutic approaches with less toxicity are therefore actively being sought. Differentiation-inducing therapy employing a physiologically active derivative of vitamin A, all-trans retinoic acid (ATRA), brought remarkable advances in the therapeutic outcomes of acute promyelocytic leukemia (APL) at the end of the last century.¹¹ However, the clinical remission due to ATRA is of short duration, and most patients who receive continuous treatment with ATRA develop RA-resistant diseases.¹² Therefore, investigators have actively sought out new agents with the ability to stimulate cellular differentiation and induce apoptosis in the types of cells associated with acute leukemia.



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Anticlastogenic, antigenotoxic and apoptotic activity of epigallocatechin gallate: a green tea polyphenol

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Abstract

Modulation of events characteristic of carcinogenesis or of cancer cells is being emphasized as a rational strategy to control cancer. Green tea polyphenol epigallocatechin gallate (EGCG) has been shown to be highly active as a cancer chemopreventive agent. Certain cellular and molecular events relevant to carcinogenesis are also modified by EGCG. The present investigation was carried out to examine the effects of EGCG on the cytogenetic change and DNA damage induced by toxicant H₂O₂ and carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in Chinese hamster V-79 cells in culture. Cytogenetic change as evident by the formation of micronuclei and DNA damage in the form of comet tail length during single cell gel electrophoresis was found to be significantly suppressed by EGCG in a dose dependent manner. Cells preincubated with EGCG were protected from subsequent damage by the genotoxic agents. Apoptosis, a highly organized physiological mechanism to eliminate injured or abnormal cells, is also implicated in multistage carcinogenesis. Initiated cells, cells at promotional stage or fully transformed cells can be eliminated through apoptosis. It was observed that EGCG suppressed growth and proliferation of K-562 cells derived from human chronic myelogenous leukemia. Morphological features of treated cells and characteristic DNA fragmentation revealed that the cytotoxicity was due to induction of apoptosis. This was mediated by activation of caspase 3 and caspase 8. Results show that EGCG not only protects normal cells against genotoxic hazard but also eliminate cancer cells through induction of apoptosis.

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Keywords: Micronuclei; DNA damage; Comet tail; Apoptosis; EGCG; V-79; K-562

1. Introduction

Modulation of events characteristic of carcinogenesis or of cancer cells is being emphasized as a rational strategy to combat cancer. Changes in ploidy

of cells, non-random chromosome aberration and DNA damage are frequently associated with chemical toxicity and carcinogenesis. Prevention of manifestation of these events may prevent induction of cancer. Apoptosis, a programmed cell death [1,2], also plays an essential role as a protective mechanism against carcinogenesis by eliminating genetically damaged cells, initiated cells or cells progressed to malignancy [3]. Induction of apoptosis thus is a highly desirable mode as a chemotherapeutic as well as a chemopreventive strategy for cancer control. Indeed many

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E-mail addresses: msiddiqi@boseinst.emet.in, maqsoodsiddiqi@hotmail.com (M. Siddiqi).

APPTOSI : 50 100 µM / 3 hr
su Leucemia

Leucemia Mieloide

Via Mitochondria
BAX M

50% 50µM

= 100% 100µM



THE VERDE
Via Mitochondria

Toxicology in Vitro 18 (2004) 555–561



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Prooxidant property of green tea polyphenols epicatechin and epigallocatechin-3-gallate: implications for anticancer properties

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Abstract

It is believed that anticancer and apoptosis inducing properties of green tea are mediated by its polyphenolic constituents particularly catechins. A number of reports have shown that green tea polyphenol (–)-epigallocatechin-3-gallate (EGCG) is among the most effective chemopreventive and apoptosis-inducing agents present in the beverage. Plant polyphenols are naturally occurring antioxidants but they also exhibit prooxidant properties. Over the last several years we have shown that various classes of plant polyphenols including flavonoids, curcuminoids and tannins are capable of catalyzing oxidative DNA cleavage particularly in the presence of transition metal ions such as copper and iron. With a view to understand the chemical basis of various pharmacological properties of green tea, in this paper we have compared the prooxidant properties of green tea polyphenols—EGCG and EC (–)-epicatechin. The rate of oxidative DNA degradation as well as hydroxyl radical and superoxide anion formation was found to be greater in the case of EGCG as compared with EC. It was also shown that copper mediated oxidation of EC and EGCG possibly leads to the formation of polymerized polyphenols. Further, it was indicated that copper oxidized catechins were more efficient prooxidants as compared with their unoxidized forms. These results correlate with the observation by others that EGCG is the most effective apoptosis inducing polyphenol present in green tea. They are also in support of our hypothesis that prooxidant action of plant polyphenols may be an important mechanism of their anticancer properties. A model for binding of Cu(II) to EC has been presented where the formation of quinone and a quinone methide has been proposed.

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Keywords: Green tea catechins; DNA cleavage; Antioxidant; Prooxidant; Cu(II) binding

1. Introduction

There has been increasing realization in recent years that several plant derived polyphenolic compounds may possess anticancer and apoptosis-inducing properties (Mukhtar et al., 1998; Clement et al., 1998). Therefore, the role of plant-derived polyphenols in chemoprevention of cancer has emerged as an interesting area of research. The data in literature points to the possible role of green tea as a chemopreventive agent against different types of cancers (Picard, 1996; Sato, 1999; Sadzuka et al., 1998; Otsuka et al., 1998). Tea (*Camellia sinensis*) is the second most common beverage in the world next to water (Wei et al., 1999). Although both green and black teas are derived from *C. sinensis*, it is the

production process which differentiates the two types of teas. Green tea contains polyphenols which include flavanols, flavandiol, flavonoids and phenolic acids. Most of the green tea polyphenols are flavanols, commonly known as catechins. The primary catechins in green tea are (–)-epicatechin (EC), (–)-epicatechin-3-gallate (ECG), (–)-epigallocatechin (EGC), (–)-epigallocatechin-3-gallate (EGCG), (+)-galocatechin and (+)-catechin. It is believed that much of the anticancer effects of green tea are mediated by its polyphenolic constituents (Ahmad et al., 1998; Katiyar and Mukhtar, 1996). During the manufacture of black tea these polyphenols undergo polyphenol oxidase catalyzed oxidative polymerization giving rise to the formation of theaflavins and thearubigins in the process referred to as 'tea fermentation' (Wei et al., 1999). However, it is considered that black tea is not as effective in its chemopreventive properties. Other studies have shown that black tea polyphenols—theaflavins—exhibit



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Review

Cancer chemopreventive activity and bioavailability of tea and tea polyphenols

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Abstract

Consumption of tea (*Camellia sinensis*) has been associated with many health benefits including the prevention of cancer. Based on in vitro experiments, many mechanisms have been proposed to account for the cancer chemopreventive activity. The importance of some of these mechanisms in vivo remains in question due to an incomplete understanding of the bioavailability of the polyphenolic compounds in tea. In this article, the literature on the cancer chemopreventive activity of tea and the tea polyphenols is discussed as well as some of the possible mechanisms for this activity. Whereas studies in animal models and with cell lines have demonstrated cancer preventive activity, the epidemiological data remain mixed. This discrepancy may arise from several factors including lifestyle, correlation between animal models and humans, and differences in metabolism among individuals. Results on the bioavailability and biotransformation of the tea polyphenols help explain some of the differences. We hope this article will spark research efforts on some of the important questions regarding tea polyphenol bioavailability and cancer chemoprevention.

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Keywords: Tea; Polyphenols; Cancer chemoprevention; Bioavailability; Catechin

1. Introduction

Tea (*Camellia sinensis*, family Theaceae) is consumed worldwide and is second only to water in popularity as a beverage. Many health benefits have been ascribed to consumption of this beverage including prevention of cancer, heart disease, and cataracts.

The three major forms of tea—green tea, black tea, and oolong tea—differ in how they are produced and in their chemistry. A typical brewed green tea beverage contains 30–42% catechins by dry weight. These include (–)-epicatechin (EC), (–)-epicatechin-3-gallate

(ECG), (–)-epigallocatechin (EGC) and (–)-epigallocatechin-3-gallate (EGCG) with EGCG being the major component (Fig. 1). In black tea, catechins, theaflavins (TF) and thearubigins (TR) (Fig. 1) account for 3–10, 2–6, and >20%, respectively, of the water-extractable material by dry weight. Tea leaves also contain flavonols, such as quercetin and myricetin as well as the nitrogenous compounds caffeine and theobromine [1].

Whereas many of the beneficial effects of tea have been attributed to the strong antioxidant activity of the tea polyphenolic compounds, other biological mechanisms may also be important. A comprehensive understanding of these potential mechanisms is hindered by the lack of understanding of the bioavailability of the tea polyphenols. Here, we discuss the

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APPTOSI: 6 50 a 100 μ M (4 3 hr)
su Leucemia

APPTOSI (via Mitochondria) su Leucemia
6 50 a 100 μ M (4 3 hr)

Composti fenolici simili:
 gingerol (Alpinia oxyphylla)
 Zingiber officinale
 Curcumin

anche su altri carcinomi
 e linfoma B

27

FEBS 25320

FEBS Letters 506 (2001) 225-230

28



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Cancer Letters 208 (2004) 163-170

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Induction of apoptosis in human lung cancer cells by curcumin

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Received 14 July 2003; received in revised form 24 September 2003; accepted 12 January 2004

Diferulobimetano
 (Composto
 fenolico)

Abstract

Curcumin, a phenolic compound from the rhizome of the plant *Curcuma longa* has anti-inflammatory, antioxidant and anti-cancer activities. Although the precise mode of action of this compound is not yet elucidated, studies have shown that chemopreventive action of curcumin might be due to its ability to induce apoptosis and to arrest cell cycle. This study investigated the cellular and molecular changes induced by curcumin leading to the induction of apoptosis in human lung cancer cell lines—A549 and H1299. A549 is p53 proficient and H1299 is p53 null mutant. The lung cancer cells were treated with curcumin (0–160 μ M) for 12–72 h. Curcumin inhibited the growth of both the cell lines in a concentration dependent manner. Growth inhibition of H1299 cell lines was both time and concentration dependent. Curcumin induced apoptosis in both the lung cancer cell lines. A decrease in expression of p53, bcl-2, and bcl-X_L was observed after 12 h exposure of 40 μ M curcumin. Bak and Caspase genes remained unchanged up to 60 μ M curcumin but showed decrease in expression levels at 80–160 μ M. The data also suggest a p53 independent induction of apoptosis in lung cancer cells.
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Keywords: Curcumin; Lung cancer; bcl-2; bcl-X_L; bak; bax; p53; c-myc; Caspase; PARP

1. Introduction

Cancer causes significant morbidity and mortality and is a major public health problem worldwide. An effective cancer prevention program, diet, and exercise may decrease the incidence of cancer. Plant-derived compounds are known to have curative potential. Curcumin (diferuloylmethane) is a phenolic compound from the plant *Curcuma longa* (Linn). It is

widely used as a coloring and flavoring agent in food [1]. Its anti-inflammatory activity is well documented. Curcumin is not toxic to mammals at very high doses (5–10% by weight of diet) [2].

Curcumin is found to have inhibitory function towards a broad range of tumors such as mammary adenocarcinoma, fore stomach, duodenal and colon cancer as well as 12-O-tetradecanoyl-13-phorbol ester (TPA) induced skin tumors in mice [2,3]. Curcumin is a potent anti-cancer agent and affects cells in a cell type dependent manner. Its ability to induce apoptosis in different cancer cells indicates the possibility of developing curcumin as a universal cancer prophylactic agent.

in leukemia:
 60 μ g/ml
 Apoptosi 50%.

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¹ Both authors contributed equally to this work.

Apoptosi = 50% 40-50 μ M (24 hr)
 30% 100 μ M (24 hr)
 85% 160 μ M (24 hr)

A plant steroid, diosgenin, induces apoptosis, cell cycle arrest and COX activity in osteosarcoma cells

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Abstract Cyclooxygenases (COXs) are key enzymes in the conversion of arachidonic acid into prostanooids which are involved in apoptosis and inflammation. Two distinct COXs have been identified: COX-1 which is constitutively expressed and COX-2 which is induced by different products such as tumor promoters or growth factors. Previously, we demonstrated that a plant steroid, diosgenin, was a new megakaryocytic differentiation inducer of human erythroleukemia cells. In our study, we investigated the effect of diosgenin on the proliferation rate, cell cycle distribution and apoptosis in the human osteosarcoma 1547 cell line. The effects of this compound were also tested on COX expression and COX activities. Diosgenin treatment caused an inhibition of 1547 cell growth with a cycle arrest in G₁ phase and apoptosis induction. Moreover, we found a correlation between p53, p21 mRNA expression and nuclear factor- κ B activation and we observed a time-dependent increase in PGE₂ synthesis after diosgenin treatment. © 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

Key words: Diosgenin; Apoptosis; Cell cycle; Cyclooxygenase; Osteosarcoma cell line

1. Introduction

Cyclooxygenases (COXs) are key enzymes in the conversion of arachidonic acid into prostanooids which are involved in apoptosis, inflammation, mitogenesis and immunomodulation. Two distinct COX isoforms have been identified: COX-1 which is considered to be the constitutively expressed form and thought to serve housekeeping functions and COX-2 which is expressed at very low basal levels and rapidly induced by different products such as tumor promoters, growth factors or inflammatory cytokines.

Many studies report an increase in COX-2 expression in numerous cancer cell lines especially in colorectal cancer cells [1,2] but also in pancreatic carcinoma cells [3], epidermal cancer cells [4], breast cancer cells [5], glioma cells [6] and osteosarcoma cells [7].

Non-steroidal anti-inflammatory drugs (NSAIDs) have been found to inhibit proliferation and to induce apoptosis in human colorectal cell lines in vitro [8,9]. Recently, we de-

scribed that under apoptotic conditions, there was a link between the effects of NS-398, a selective COX-2 inhibitor, on prostaglandin E₂ (PGE₂) release, cell apoptosis and COX-2 expression in the human osteosarcoma 1547 cell line [7].

Previously, we demonstrated that a plant steroid, diosgenin, was a new megakaryocytic differentiation inducer of human erythroleukemia cells [10].

In this study, we investigated the effect of diosgenin on the proliferation rate, cell cycle distribution and apoptosis in the human osteosarcoma 1547 cell line. Moreover, the effects of this compound were tested on COX expression and activity.

2. Materials and methods

2.1. Cell line, cell culture and treatment

The 1547 human osteosarcoma cell line was kindly provided by Professor M. Rigaud (Laboratoire de Biochimie, Faculté de Médecine de Limoges, France). Freshly trypsinized cells were seeded at 4×10^5 cells/cm² and grown in Eagle's minimum essential medium (Gibco BRL, Cergy-Pontoise, France) supplemented with 10% fetal calf serum (FCS) (Gibco BRL), 100 U/ml penicillin and 100 μ g/ml streptomycin. Cultures were maintained in a humidified atmosphere with 5% CO₂ at 37°C. Cell viability was determined by the trypan blue dye exclusion method. For all experiments cells were allowed to adhere and grow for 3 days in culture medium prior to exposure to diosgenin (So-spirosten-3 β -ol, Sigma). A stock solution of 10^{-2} M diosgenin was prepared in ethanol and diluted in culture medium to give a final concentration of 10–100 μ M. The same amount of ethanol was added to control cells.

2.2. Cell proliferation assay

Measurement of cell proliferation was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, trypsinized cells were plated (1200 cells/well) in 96-well culture plates. 3 days later, the seeding medium was removed and replaced by 10% FCS medium containing diosgenin (0–100 μ M) for 24–96 h. MTT test was carried out daily as previously described [11]. Experiments were performed in sextuplicate assays.

2.3. Lactate dehydrogenase (LDH) test

Cells were seeded in 96-well plates at a density of 1200 cells/well and treated without or with diosgenin (20 and 40 μ M). Cytotoxicity detection kit (Boehringer Mannheim) measured the LDH activity released from the cytosol of damaged cells into the supernatant which evaluated the percentage of cell death according to the manufacturer's protocol.

2.4. Cell cycle analysis

Cells were seeded at 3.6×10^4 cells in 6-well culture plates, cultured in 10% FCS medium without or with diosgenin (40 μ M) for 12–48 h. Adherent and floating cell populations were combined and counted, and cell viability was determined by the trypan blue dye exclusion method. For DNA content analysis, 10^6 cells were fixed in 70% etha-

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Leucemia, Osteosarcoma
 APOPTOSI = 40 μ M

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PII: S0024-3205(02)01900-8

APoptosi : 10-20 $\mu\text{g/mL}$ (6-48 hr)



Genisteina: APPTOSI su Leucemia a 5-80 $\mu\text{g/mL}$
 " su gastrica cancer a 60 $\mu\text{g/mL}$
 " su cancer polmon a 30 $\mu\text{g/mL}$
 su VESICOLA a 50 $\mu\text{g/mL}$

Pharmacology & Therapeutics 90 (2001) 157-177

31

Associate editor: S.T. Mayne

Dietary agents in cancer prevention flavonoids and isoflavonoids

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Abstract

Flavones and isoflavones may play a prominent role in cancer prevention since these compounds are found in numerous plants that are associated with reduced cancer rates. This article reviews recent epidemiological and animal data on isoflavones and flavones and their role in cancer prevention. It covers aspects of the bioavailability of these dietary constituents and explores their mechanism of action. Human epidemiology data comes primarily from studies in which foods rich in isoflavones or flavones are associated with cancer rates. The bioavailability approach has been particularly useful with isoflavones because of their abundance in specific foods, including soy foods. The bioavailability of flavones and isoflavones has been shown to be influenced by their chemical form in foods (generally glycoside conjugates), their hydrophobicity, susceptibility to degradation, the microbial flora of the consumer, and the food matrix. Some information is available on how these factors influence isoflavone bioavailability, but the information on flavones is more limited. Many mechanisms of action have been identified for isoflavone/flavone prevention of cancer, including estrogenic/antiestrogenic activity, antiproliferation, induction of cell-cycle arrest and apoptosis, prevention of oxidation, induction of detoxification enzymes, regulation of the host immune system, and changes in cellular signaling. It is expected that some combination of these mechanisms will be found to be responsible for cancer prevention by these compounds. Compelling data suggest that flavones and isoflavones contribute to cancer prevention; however, further investigations will be required to clarify the nature of the impact and interactions between these bioactive constituents and other dietary components. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Flavones; Isoflavones; Cancer; Epidemiology; Bioavailability; Mechanisms

Abbreviations: ACF, aberrant crypt foci; Apc, adenomatous polyposis coli; AOM, azoxymethane; DMBA, dimethylbenz(a)anthracene; GI, gastrointestinal; GST, glutathione-S-transferase; LDL, low-density lipoprotein; MNU, methylnitrosourea; ODMA, O-desmethylangolensin; QR, NAD(P)H:quinone reductase (EC 1.6.99.2); TPA, 12-O-tetradecanoyl-phorbol-13-acetate.

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Genisteina: apoptosis su MELANOMA
 Quercetin: " su K gastrica a 50 μM .

Silimarin: 75 μM su K PR

Apigenine 80 μM su colon cancer
 Luteoline ---



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Chemico-Biological Interactions

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Induction of apoptosis by penta-acetyl geniposide in rat C6 glioma cells

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Received 22 April 2002; received in revised form 14 June 2002; accepted 26 June 2002

Abstract

Penta-acetyl geniposide, (Ac)₅-GP, was produced by acetylation of a glycoside, isolated from an extract of *Gardenia fructus*. Previously, we have reported that C6 glioma cells could be inhibited in culturing as well as in bearing rats by treating with (Ac)₅-GP. In this study, the effect and action of (Ac)₅-GP on inducing cell death was examined in rat C6 glioma cells. Treatment of C6 glioma cells with (Ac)₅-GP caused cell death, chromatin condensation, and internucleosomal DNA ladder. Also, cell cycle arrest at G₀/G₁ phase revealed that (Ac)₅-GP-induced cell death appears to be mediated by apoptosis. In addition, the results also showed that p53 and c-Myc increased due to treatment of (Ac)₅-GP in a dose-response and time-dependent manner. Concomitant with the expression of p53 and c-Myc, decreased level of Bcl-2 and increased level of Bax protein were observed. These results suggest that cell death caused by (Ac)₅-GP through apoptosis and cell cycle arrest at G₀/G₁ may be associated with the induction of p53, c-Myc and may be mediated with apoptosis-related Bcl-2 family proteins. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Penta-acetyl geniposide; C6 glioma cell apoptosis; Cell cycle arrest; p53; c-Myc; Bcl-2; Bax

Abbreviations: (Ac)₅-GP, penta-acetyl geniposide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PI, propidium iodide; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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↑ BAX
 ↑ p53
 ↓ Bcl2

↑ Bax / Bcl2 ↓



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Mutation Research 523–524 (2003) 55–62



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Inhibition of human breast cancer growth by GCPTM (genistein combined polysaccharide) in xenogeneic athymic mice: involvement of genistein biotransformation by β -glucuronidase from tumor tissues

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Abstract

The role of β -glucuronidase in genistein biotransformation was investigated in a human breast cancer MDA-MB-231 xenogeneic athymic mouse model. Genistein combined polysaccharide (GCPTM), a genistein aglycone rich functional food supplement was used in these experiments. Tumor-bearing mice were subjected to oral administration of GCPTM for 28 days. GCPTM treatment significantly inhibited tumor growth. Induction of apoptosis by GCPTM treatment was related to activation of cleavage of poly(ADP-ribose)polymerase, induction of the p21 protein expression and reduction of cyclin B1 expression in the tumor tissues. Genistein exists as a glucuronide conjugate in normal organ tissues, and the conjugated genistein lacks the physiological activity of the aglycone. Tumor tissues contain large amounts of β -glucuronidase, the enzyme that converts the genistein β -glucuronide conjugate into genistein aglycone. The resulting genistein aglycone exerts its chemopreventive activities, including the induction of apoptosis in tumor tissues, and, finally, leads to tumor growth inhibition.

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Keywords: Genistein; Breast cancer; Athymic mice; β -Glucuronidase; Apoptosis

1. Introduction

Epidemiologic as well as laboratory studies have revealed that soy isoflavones exert chemopreventive effects on several types of human cancer [1,2]. Isoflavones identified in soybeans are mainly glycosides, including genistin, daidzin and glycitin, that are

conjugated with glucose [3]. Their active forms are deglycosylated aglycones, such as genistein, daidzein and glycitein [4]. Genistein is a well-known molecule which exerts multiple biological activities. These include inhibition of tyrosine kinases [5], inactivation of DNA topoisomerase II [6], anti-angiogenesis [7] and cell growth arrest by interfering with signal transduction cascades [8]. Soy isoflavone glycosides are degraded into their aglycones mainly through metabolism by gut microflora [9,10]. Soy isoflavone aglycones are absorbed faster and in larger amounts

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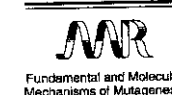
genistein → genistein aglycone ⇒ APOPTOSIS
 β glucuronidase
 Spleen K cells → 70-80 μ g/mg nel K
 20 μ g/mg nel K cells same



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Mutation Research 523–524 (2003) 75–85



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Inhibitory effects of the ginsenoside Rg₃ on phorbol ester-induced cyclooxygenase-2 expression, NF- κ B activation and tumor promotion[☆]

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Abstract

Our previous studies demonstrated the anti-oxidant and anti-tumor promotional properties of the methanol extract of heat-processed *Panax ginseng* C.A. Meyer [Cancer Lett. 150 (2000) 41]. In the present work, we have evaluated anti-inflammatory as well as anti-tumor promoting effects of Rg₃, a major ginsenoside derived from heat-processed ginseng. Pretreatment of dorsal skins of female ICR mice with Rg₃ significantly inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ornithine decarboxylase activity and 7,12-dimethylbenz[*a*]anthracene-initiated papilloma formation. In another experiment, Rg₃ pretreatment abrogated the expression of cyclooxygenase-2 in TPA-stimulated mouse skin. Rg₃ also inhibited the TPA-induced activation of the eukaryotic transcription factor, NF- κ B in both mouse skin and cultured human pro-myelocytic leukemia (HL-60) cells. Moreover, Rg₃ exerted potent inhibitory effects on the activation of another transcription factor, activator protein-1 (AP-1) that is responsible for *c-jun* and *c-fos* oncogenic transactivation. Based on these findings, it is likely that the anti-tumor promoting activity of Rg₃ is mediated possibly through down-regulation of NF- κ B and AP-1 transcription factors. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Anti-tumor promotion; AP-1; Cyclooxygenase-2; Ginseng; Ginsenoside Rg₃; HL-60 cells; Mouse skin carcinogenesis; NF- κ B

1. Introduction

A relatively large number of anti-oxidative and anti-inflammatory substances derived from edible plants have been shown to possess substantial chemopreventive properties [1,2]. For instance, tea polyphenols such as epigallocatechin gallate, resveratrol present in red wine, and curcumin in turmeric possess potent anti-carcinogenic activities (reviewed in [1] and references therein). The role of these phytochemicals in dietary cancer prevention has been extensively investigated and well-documented [1,2]. The roots or rhizome of several varieties of *Panax* plants including *P. ginseng*, *P. notoginseng*, *P. japonicus* and *P. quinquefolium* have been used in traditional oriental medicine for the treatment of many disorders [3]. Among these *Panax* gen., *P. ginseng* C.A. Meyer is one of the most widely used medicinal plants throughout Far East Asian countries including China, Korea

[☆] This manuscript was prepared from a dissertation by Y.-S. Keum in partial fulfillment of the requirement for a Master of Science at Seoul National University.

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Leucemia

Caspases-3 and -7 are activated in goniothalamine-induced apoptosis in human Jurkat T-cells

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Abstract Goniolactone, a plant styrylpyrone derivative isolated from *Goniolactone andersonii*, induced apoptosis in Jurkat T-cells as assessed by the externalisation of phosphatidylserine. Immunoblotting showed processing of caspases-3 and -7 with the appearance of their catalytically active large subunits of 17 and 19 kDa, respectively. Activation of these caspases was further evidenced by detection of poly(ADP-ribose) polymerase cleavage (PARP). Pre-treatment with the caspase inhibitor benzoyloxycarbonyl-Val-Ala-Asp fluoromethyl ketone (Z-VAD.FMK) blocked apoptosis and the resultant cleavage of these caspases and PARP. Our results demonstrate that activation of at least two effector caspases is a key feature of goniolactone-induced apoptosis in Jurkat T-cells.

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Key words: Apoptosis; Caspase-3; Caspase-7; Goniolactone; Jurkat T-cell

1. Introduction

A wealth of information is currently available on the pharmacological activities of many natural products, in particular their potential for use in cancer chemoprevention [1]. The activities of these compounds include anti-proliferative, anti-inflammatory, anti-mutagenic and anti-oxidative mechanisms in addition to effects on drug metabolism, cell differentiation and induction of apoptosis [2]. Dysregulation of apoptosis has now been implicated in the onset or progression of cancer [3]. Consequently, apoptosis represents an innate cellular defense against carcinogen-induced cellular damage by removing and inhibiting survival and growth of altered cells at different stages of carcinogenesis [4].

Overwhelming evidence suggests the involvement of caspases, a family of cysteine proteases which cleave after aspartic acid, in mediating the biochemical events that culminate in apoptosis (reviewed in [5,6]). Currently, 14 different caspases have been identified in humans [7,8]. Caspases exist as inactive pro-forms and require processing to active subunits

either by autoprocesing or via activation by other caspases. It has been proposed that 'initiator' caspases with long pro-domains, such as caspases-8 and -9, either directly or indirectly activate effector 'caspases', such as caspases-3, -6 and -7 [5]. A number of proteins have been shown to be cleaved in cells undergoing apoptosis by the activated caspases [9]. Among these, poly(ADP-ribose) polymerase (PARP, 113–116 kDa), a nuclear enzyme which is activated during DNA damage, is known to be cleaved by caspases, more specifically, caspases-3 and -7. Other cellular proteins that are cleaved by caspases include lamins, U1, 70 kDa, DNA-PK, actin and retinoblastoma (reviewed in [9]). Many potent selective reversible and irreversible peptide-based inhibitors have been developed, which have advanced our understanding of the involvement of proteases during apoptosis [10]. Benzoyloxycarbonyl-Val-Ala-Asp fluoromethyl ketone (Z-VAD.FMK), a general caspase inhibitor, has been shown to block apoptosis in many cell types including rat hepatocytes, thymocytes and human leukemic T-cells [11–16].

In this study, the apoptotic potential of goniolactone, a biologically active plant styrylpyrone derivative isolated from *Goniolactone andersonii*, was investigated in the leukemic T-cell line Jurkat. This plant extract has recently been demonstrated to have anti-proliferative activities in a number of transformed cell lines including MCF-7 and HeLa cells [17,18]. We now demonstrate for the first time that goniolactone induces apoptosis in Jurkat T-cells and that at least two caspases, the effector caspases-3 and -7, are activated as evidenced by cleavage of their intracellular substrate PARP.

2. Materials and methods

2.1. Jurkat T-cell culture and reagents

The leukemic T-cell line Jurkat (Clone E6-1) was obtained from ECACC. The cells were cultured in RPMI 1640 supplemented with 10% foetal bovine serum and 1% Glutamax. Media and serum were obtained from Life Technologies (Paisley, UK). Annexin V/PI was purchased from Bender Medsystems (Vienna, Austria). Z-VAD.FMK was from Enzyme Systems (Dublin, CA, USA).

2.2. Extraction of goniolactone

The dried powdered bark of *G. andersonii* (500 g) was extracted in a Soxhlet apparatus with light petroleum ether for 72 h as described previously [19]. Evaporation of the light petroleum ether afforded a mixture of solid and thick brown oil (10 g). Column chromatography on silica gel using light petroleum and ethylacetate mixtures as the eluent yielded a white solid (1.5 g). Recrystallisation from light petroleum yielded white crystals (1 g), which were identified through spectroscopic data (IR, UV, MS and NMR) as goniolactone (molecular weight 200).

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Altholactone, a novel styryl-lactone induces apoptosis via oxidative stress in human HL-60 leukemia cells

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Abstract

Plant styryl-lactone derivatives isolated from *Goniolactone* sp. are potential compounds for cancer chemotherapy. In this study, we have examined the mechanisms of apoptosis induced by altholactone, a styryl-lactone isolated from the Malaysian plant *G. malayanus* on human HL-60 promyelocytic leukemia cells. Flow cytometric analysis of the externalization of phosphatidylserine (PS) using the annexin V/PI method on altholactone treated HL-60 cells showed a concentration-dependent increase of apoptosis from concentrations ranging from 10.8 (2.5 µg/ml) to 172.4 µM (40 µg/ml). Pre-treatment with the antioxidant *N*-acetylcysteine (1 mM) completely abrogated apoptosis induced by altholactone, suggesting for the involvement of oxidative stress. Further flow cytometric assessment of the level of intracellular peroxides using the fluorescent probe 2',7'-dichlorofluorescein diacetate (DCFH-DA) confirmed that altholactone induced an increase in cellular oxidative stress in HL-60 cells which was suppressed by *N*-acetylcysteine. In summary, our results demonstrate for the first time that altholactone induced apoptosis in HL-60 cells occurs via oxidative stress. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Apoptosis; Altholactone; Styryl-lactone; Oxidative stress; *N*-acetylcysteine

1. Introduction

Low molecular weight compounds especially derived from natural sources such as plants are

currently being investigated for their pharmacological properties in regulating apoptosis, a cell death program which is pivotal in the pathological process of tumor development (Kinloch et al., 1999). In this respect, the styryl-pyrone derivatives found abundantly in the genus *Goniolactone* species have also been investigated for cytotoxic and antitumor properties (Ali et al., 1997; Cao et al., 1998; Hawariah and Stanslas, 1998; Bermejo et al., 1999).

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Apoptosis 50% 57 µM } Leukemia
Apoptosis 80% 170 µM (40 µg/ml)



PERGAMON

Altholactone

Toxicology in Vitro 17 (2003) 433–439



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Loss of mitochondrial transmembrane potential and caspase-9 activation during apoptosis induced by the novel styryl-lactone goniiothalamine in HL-60 leukemia cells

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Abstract

Styryl-lactones such as goniiothalamine represent a new class of compounds with potential anti-cancer properties. In this study, we investigated the mechanisms of goniiothalamine (GTN), a plant styryl-lactone induced apoptosis in human promyelocytic leukemia HL-60 cells. This plant extract resulted in apoptosis in HL-60 cells as assessed by the externalisation of phosphatidylserine. Using the mitochondrial membrane dye (DIOC₆) in conjunction with flow cytometry, we found that GTN treated HL-60 cells demonstrated a loss of mitochondrial transmembrane potential ($\Delta\psi_m$). Further immunoblotting on these cells showed activation of initiator caspase-9 and the executioner caspases-3 and -7. Pretreatment with the pharmacological caspase inhibitor, benzylloxycarbonyl-Val-Ala-Asp fluoromethyl ketone (Z-VAD.FMK) abrogated apoptosis as assessed by all of the apoptotic features in this study. In summary, our results demonstrate that goniiothalamine-induced apoptosis occurs via the mitochondrial pathway in a caspase dependent manner.

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Keywords: Apoptosis; Caspase activation; Goniiothalamine; Mitochondrial transmembrane potential; Styryl-lactone

1. Introduction

It has long been known that natural products are important sources of new bioactive low molecular weight structures with promising chemotherapeutic properties (reviewed in Harvey et al., 2000). A number of low molecular weight compounds are currently being investigated for their pharmacological properties in manipulating apoptosis, a cell death program which is pivotal in the pathological process of tumor development (Kinloch et

al., 1999). In this respect, the styryl-lactones found abundantly in the genus *Goniiothalamus* species such as goniiothalamine (GTN, Fig. 1) have been investigated for cytotoxic and antitumor properties (Ali et al., 1997; Hawariah and Stanslas, 1998; Cao et al., 1998; Bernejo et al., 1999).

Apoptotic cell death involves complex yet well regulated biochemical processes leading to the well-characterized features such as condensation of chromatin and internucleosomal DNA cleavage (reviewed in Hengartner, 2000). Current paradigms of apoptosis suggest that the loss of mitochondrial transmembrane potential (MTP or $\Delta\psi_m$) occurs earlier in the commitment phase of apoptosis which results in the release of mitochondrial apoptogenic proteins including cytochrome-c and the apoptotic inducing factor (Zamzani et al., 1995; Liu et al., 1996; Kluck et al., 1997; Yang et al., 1997).

Abbreviations: GTN, goniiothalamine; MPT or $\Delta\psi_m$, mitochondrial transmembrane potential; PS, phosphatidylserine; Z-VAD.FMK, benzylloxycarbonyl-Val-Ala-Asp fluoromethyl ketone.

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Apoptosis 50% in 14 hr
in Leukemia
con: 10 µg/mL
50 µM

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Pro to CATECHINE

(Bcl-2)

Induction of Apoptosis by *Hibiscus* Protocatechuic Acid in Human Leukemia Cells via Reduction of Retinoblastoma (RB) Phosphorylation and Bcl-2 Expression

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ABSTRACT. *Hibiscus* protocatechuic acid (PCA), a phenolic compound isolated from the dried flower of *Hibiscus sabdariffa* L. (Malvaceae), demonstrated antioxidant and antitumor promotion effects in our previous study. In the present study, *Hibiscus* PCA was found to inhibit the survival of human promyelocytic leukemia HL-60 cells in a concentration- and time-dependent manner. The study revealed that HL-60 cells underwent internucleosomal DNA fragmentation and morphological changes characteristic of apoptosis after a 9-hr treatment with *Hibiscus* PCA (2 mM). Flow cytometric analysis of the DNA content of cells treated with PCA for 12 hr showed that the cells were distributed mainly in the hypodiploid phase (apoptotic peak, 46.7%), less in the G₁ (34.2%) and S phase (14.0%), and few in the G₂/M phase (5.1%). Moreover, PCA treatment caused an increase in the level of hypophosphorylated retinoblastoma (RB; 180% of control at the 6-hr time point) and, on the contrary, a decline in hyperphosphorylated RB. A rapid loss of RB was observed when the treatment period was extended. Further studies showed that *Hibiscus* PCA application reduced Bcl-2 protein expression to 47%, and increased Bax protein expression to 181% after 1.5 hr as compared with time 0. Overexpression of Bcl-2 in HL-60 cells delayed the occurrence of *Hibiscus* PCA-induced apoptosis. These data suggest that *Hibiscus* PCA is an apoptosis inducer in human leukemia cells, and that RB phosphorylation and Bcl-2 protein may play a crucial role in the early stage. *BIOCHEM PHARMACOL.* 60:3:307–315, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. apoptosis; Bcl-2; *Hibiscus* protocatechuic acid; leukemia; RB

Hibiscus PCA (Fig. 1), a phenolic compound, is isolated from the dried flower of *Hibiscus sabdariffa* L. (Malvaceae), which is an ingredient of local beverages and a Chinese herbal medicine used to treat hypertension, pyrexia, and liver damage. Recently, PCA has been demonstrated to be an efficacious agent in inhibiting the carcinogenic action of various chemicals in different tissues, such as diethylnitrosamine in the liver [1], 4-nitroquinoline-1-oxide in the oral cavity [2], azoxymethane in the colon [3], *N*-methyl-*N*-nitrosourea in glandular stomach tissue [4], and *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine in the bladder [5]. In our previous studies, PCA showed strong antioxidant and antitumor promotion effects [6, 7]. Thus, PCA possesses

anticarcinogenic potential and may play a role in chemoprevention.

Recently, considerable attention has focused on the sequence of events referred to as programmed cell death or apoptosis, and the possible role of this process in mediating the lethal effects of diverse antineoplastic agents in leukemia cells [8]. Apoptosis is a highly regulated process that involves activation of a cascade of molecular events leading to cell death as characterized by cell shrinkage, membrane blebbing, chromatin condensation, and formation of a DNA ladder of multiples of 180–200 bp, caused by internucleosomal DNA cleavage [9, 10]. The prototypic regulator of mammalian apoptosis is the proto-oncogene *bcl-2* [11]. Transfection experiments indicated that *bcl-2* could protect many cell types from apoptosis induced by exposure to a wide variety of adverse conditions and stimuli. On the other hand, studies using transgenic mice suggest that loss of RB function is associated with the induction of p53-dependent apoptosis [12]. It also has been reported that anticancer drugs induce RB hypophosphorylation and consequent G₁ arrest and apoptosis in p53-independent cell lines such as human leukemia HL-60 and U937 cells [13].

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[§] Abbreviations: PCA, protocatechuic acid; BrdU, 5-bromo-2'-deoxyuridine; ICE, interleukin 1β-converting enzyme; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; and RB, retinoblastoma (protein).

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APPTOSIS: 2 mM

5% dpo 5 hr
50% dpo 12 hr
60% dpo 24 hr

Mimosine induces apoptosis in the HL60 human tumor cell line

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Mimosine, a plant amino acid not found in proteins, has been widely used as a synchronizing agent, blocking the progression of cell cycle on the G1/S phase border. The mechanism by which this block is achieved is still unclear. We report that in HL60 cells the synchronization is related to an increase in apoptosis. Another human tumor cell line, K562, is insensitive to both phenomena thereby demonstrating that apoptosis observed in HL60 is line-specific. We hypothesize that the mimosine-induced apoptosis and alteration of the cell cycle is due to the inhibition of hypusine generation.

Keywords: Apoptosis; cell cycle synchronization; HL60; hypusine; K562; mimosine.

(Received 29 June 1999; accepted 20 July 1999)

Introduction

Cell cycle progression appears to be regulated by sequential activation and subsequent inactivation of a growing family of serine/threonine protein kinases, the cyclin-dependent protein kinases (cdk).¹ Many physiological substrates of cdk have been identified, including histone H1, lamins, nucleolin, and microtubules. During the cell cycle, the cyclins appear and disappear at specific regulatory points. In mammalian cells, progression through G1 seems to be controlled by the C-, D-, and E-type cyclins, whereas progression through the rest of the cell

cycle seems to be controlled by the A- and B-type cyclins. Another important factor which controls cell cycle progression is the intracellular level of iron, which is required for DNA synthesis.^{2,3} The main cellular requirement for iron occurs late in the G1 and S phases, and is due to the increased activity of the ribonucleotide reductase.⁴ Therefore, by modifying the expression of cyclins or the intracellular iron levels, it is possible to modify cell cycle progression. Mimosine [α -amino- β -(3-hydroxy-4-oxo-1,4-dihydropyridine-1-yl)propanoic acid] is a naturally occurring amino acid found in the seeds and foliage of the legume genera *Mimosa* and *Leucaena*. The compound was shown to cause inhibition of hair growth and loss of hair in mice,⁵ to act as a defleecing agent in sheep,^{6,7} and to induce fetal resorption in rats.⁸ Mimosine was also shown to block the cell cycle earlier than aphidicolin,⁹ an inhibitor of DNA-polymerase activity.

Furthermore, mimosine inhibits various mammalian enzymes *in vitro*, such as tyrosinase,¹⁰ dopamine β -hydroxylase,¹⁰ deoxyhypusyl hydroxylase (DOHH)¹¹ and, H1 kinase.¹²

Recent studies¹³ demonstrated that cyclin D levels are decreased after treatment with mimosine in MDA-MB-435 human breast cancer cells and that this agent also inhibits cyclin-E-associated kinase activity.¹⁴ Moreover, mimosine is able to chelate transition metal ions such as Fe^{2+} and binds to a 50-kDa protein identified as serine hydroxymethyltransferase (SHMT), an enzyme involved in the penultimate step of thymidine biosynthesis.¹⁵ It is not yet clear if the mimosine-induced cell cycle block is due to the alteration of one or more of these metabolic pathways or if an upstream event directly modified by mimosine exists. One of the most investigated pathways altered by mimosine is the activity of DOHH. This enzyme allows the formation of the amino acid hypusine in the eukaryotic initiation factor 5A (eIF-5A).¹⁶ Hypusine is present in eIF-5A only, is essential for its activity

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Su cellule del CANCRO APOPTOTICO-Resistenti alle vie classiche

APOPTOSI = 10% con 100 μ M
50% " 200 (13 hr)
60% " 400 (72 hr)

Zingiberaceae family : - gingerol - yakkuchinone
- PARADOL - CURCUMIN



Fenolo

Cancer Letters 177 (2002) 41-47

CANCER
Letters

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Induction of apoptosis and caspase-3 activation by chemopreventive [6]-paradol and structurally related compounds in KB cells

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Abstract

[6]-paradol, a pungent phenolic substance found in ginger and other Zingiberaceae plants, has been demonstrated to be an effective inhibitor of tumor promotion in mouse skin carcinogenesis. In the present study, we found that [6]-paradol and other structurally related derivatives, [10]-paradol, [3]-dehydroparadol, [6]-dehydroparadol, and [10]-dehydroparadol, with the exception of [3]-paradol induce apoptosis in an oral squamous carcinoma cell line, KB, in a dose-dependent manner. [10]-paradol and [10]-dehydroparadol exhibited a similar extent of cytotoxicity to that of [6]-paradol. [6]-Dehydroparadol and [3]-dehydroparadol appeared to be more potent, with an IC_{50} less than 40 μ M. Treatment of KB cells with an apoptosis-inducing concentration of [6]-dehydroparadol caused induction of proteolytic cleavage of pro-caspase-3. These results suggest that [6]-paradol and structurally related derivatives induce apoptosis through a caspase-3-dependent mechanism. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: [6]-paradol; Apoptosis; KB cells; Caspase-3

1. Introduction

Many natural compounds are known to be effective and versatile chemopreventive agents in a number of animal tumor models. Some chemopreventive agents derived from the dietary condiment, such as [6]-gingerol from ginger and organosulfur compounds

in garlic induce apoptosis in several kinds of tumor cells [1-4]. Thus induction of apoptosis may be a good implication for the mechanism of chemopreventive agents [5-7].

Ginger (*Zingiber officinale*) is one of the most frequently and heavily consumed spices throughout the world. In addition to its extensive use as a seasoning ingredient, the rhizome of ginger is used in traditional oriental herbal medicine for the management of symptoms such as common cold, digestive disorders, rheumatism, neuralgia, colic and motion sickness. The rhizome of ginger contains [6]-gingerol as the

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CARCINOMA Squamoso, Leucemia
APOPTOSI = 40-75 μ M (8 hr)



Methanolic extract of *Pereskia bleo* (Kunth) DC. (Cactaceae) induces apoptosis in breast carcinoma, T47-D cell line

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Available online 30 October 2004

Abstract

Currently, breast cancer is the leading cause of cancer-related death in women. Therefore, there is an urgent need to develop alternative therapeutic measures against this deadly disease. Here, we report the cytotoxicity activity and the mechanism of cell death exhibited by the methanol extract prepared from *Pereskia bleo* (Kunth) DC. (Cactaceae) plant against human breast carcinoma cell line, T-47D. In vitro cytotoxicity screening of methanol extract of *Pereskia bleo* plant indicated the presence of cytotoxicity activity of the extract against T-47D cells with EC₅₀ of 2.0 µg/ml. T-47D cell death elicited by the extract was found to be apoptotic in nature based a clear indication of DNA fragmentation which is a hallmark of apoptosis. In addition, ultrastructural analysis also revealed apoptotic characteristics (the presence of chromatin margination and apoptotic bodies) in the extract-treated cells. RT-PCR analysis showed the mRNA expression levels of c-myc, and caspase 3 were markedly increased in the cells treated with the plant extract. However, p53 expression was only slightly increased as compared to caspase 3 and c-myc. Thus, the results from this study strongly suggest that the methanol extract of *Pereskia bleo* may contain bioactive compound(s) that caused breast carcinoma, T-47D cell death by apoptosis mechanism via the activation of caspase-3 and c-myc pathways. © 2004 Elsevier Ireland Ltd. All rights reserved.

Keywords: *Pereskia bleo*; Cytotoxicity; T-47D cell line; Anticancer; Medicinal plants; Apoptosis; DNA fragmentation

1. Introduction

Breast cancer is the most common cancer in women in most parts of the world today. In the year 2000, there were 1,050,346 cases reported with 372,969 deaths from breast cancer worldwide. The incidence ranged from an average of 95 per 100,000 in more developed countries to 20 per 100,000 in less developed countries (Ferlay et al., 2001). In the USA alone, 184,000 cases of breast cancer are detected annually. The National Cancer Institute (USA) estimates that one in every eight women in the USA will develop breast cancer over their lifetime. Thus, breast cancer is a worldwide disease and needs to be addressed seriously.

For many years, the cytotoxic actions of the chemotherapeutic drugs were ascribed solely to their ability to induce

genotoxic death (Kamesaki, 1998). However, there were accumulating evidences that these agents exert their cytotoxic effects mainly by inducing apoptosis in tumor cells. Impairment of apoptosis is known to be related to cell immortality and carcinogenesis and the induction of apoptosis in neoplastic cells, therefore, is vital in cancer treatment. The chemotherapeutic drugs that have been observed to induce apoptosis in vitro include etoposide, camptothecin, VM26, vincristine, cis-platinum, cyclophosphamide, paclitaxel, 5-fluorouracil and doxorubicin (Kaufman, 1989; Walker et al., 1991; Shinomiya et al., 1994; Havrilesky et al., 1995; Huschtscha et al., 1996). In accordance with these in vitro studies, other studies also provide evidences that chemotherapeutic agents induce apoptotic tumor cell death in vivo. Experimental studies of murine tumors have demonstrated that cis-platinum, cyclophosphamide and other chemotherapeutic agents induced apoptosis in various tumors in vivo (Meyn et al., 1994, 1995). Several clinical studies have also shown

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Breast cancer

Apoptosis = 50%

2 µg/ml



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0024-3205/97 \$17.00 + .00

MOLECULAR MECHANISMS IN THE ANTIPROLIFERATIVE ACTION OF QUERCETIN

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Summary

A single treatment with quercetin (5.5 µM), a plant flavonoid, activated both apoptosis and differentiation programs in K562 human leukemia cells. K562 cells expressed commitment to apoptosis after 1 h exposure, however, at least 12 h of drug exposure was needed to induce differentiation. Early (1 h) down-regulation of the c-myc and Ki-ras oncogenes and rapid reduction of inositol-1,4,5-trisphosphate (IP₃) concentration (IC₅₀ = 9 µM, 1 h incubation) are part of the antiproliferative action of quercetin and appear to relate to induction of differentiation and/or apoptotic program of K562 leukemia cells treated with quercetin.

Key Words: quercetin, K562 leukemia cells, apoptosis, cell differentiation, c-myc, Ki-ras, IP₃

Quercetin (3,3',4',5,7-pentahydroxyflavone) (Fig. 1), a plant flavonoid, is widely distributed in the plant kingdom and occurs naturally in a wide range of fruits and vegetables (1). Quercetin inhibits the growth of malignant cells, arresting them in the late G₁ phase of the cell cycle (2). It blocks signal transduction pathways by inhibiting protein tyrosine kinase, PI- and PIP-kinases (1-phosphatidylinositol 4-kinase and 1-phosphatidylinositol 4-phosphate 5-kinase) resulting in a reduction of IP₃ concentration which should decrease the release of Ca²⁺ from intracellular sources (3). Quercetin is well known to inhibit various tyrosine protein kinases and serine/threonine protein kinases (4, 5). However, the exact mechanisms responsible for the antitumor effect of quercetin are not yet completely understood.

The purpose of this investigation was to gain a deeper insight into the molecular mechanisms of the antiproliferative action of quercetin. Here we report that c-myc and Ki-ras oncogenes, which are overexpressed in the K562 cells, are down-regulated after quercetin treatment, concurrently the drug induced inhibition of the phosphatidylinositol cascade. To further evaluate the mechanisms of drug action we also extended our observations to induction of apoptosis and cell differentiation.

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Flavonoid.
Active on Leucemia

Apoptosis = 50–550 µM



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Life Sciences

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Phyllanthus urinaria triggers the apoptosis and Bcl-2 down-regulation in Lewis lung carcinoma cells

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Abstract

Phyllanthus urinaria (*P. urinaria*), a widely used herb medicine, was tested for the anticancer effect in its water extract for the first time. The water extract of *P. urinaria* significantly decreased the number of Lewis lung carcinoma cells in a dose- and time-dependent manner as determined by MTT assay. However, the water extract of *P. urinaria* did not exert any cytotoxic effect on normal cells such as endothelial cells and liver cells. Result from flow cytometry revealed a dose-dependent increase of dead cells 24 hours after treating Lewis lung carcinoma cells with *P. urinaria* extract. The anticancer activity of *P. urinaria* extract was due to the apoptosis induced in Lewis lung carcinoma cells, which was demonstrated by DNA fragmentation analysis and increased caspase-3 activity. The apoptosis triggered by *P. urinaria* extract in Lewis lung carcinoma cells was associated with the down-regulation of Bcl-2 gene expression, but not with p53, p21 and Bax. Furthermore, the partial inhibition of *P. urinaria*-induced apoptosis in Lewis lung carcinoma cells by pretreatment with cyclosporin A, a mitochondria permeability transition pore inhibitor, suggesting that *P. urinaria* extract induced the apoptosis of Lewis lung carcinoma cells, at least in part, through a mitochondria-associated intrinsic pathway.

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Keywords: *Phyllanthus urinaria*; Apoptosis; Bcl-2; Mitochondria

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Anti
Leucemia
Melanoma

Apoptosis : 50% 2 ug/mL in 24h.



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Cancer Letters 190 (2003) 157–163

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Resveratrol is a potent inducer of apoptosis in human melanoma cells

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Abstract

Resveratrol is a plant polyphenol found in grapes and red wine. It has been found to have beneficial effects on the cardiovascular system. Resveratrol also inhibits the growth of various tumor cell lines in vitro and inhibits carcinogenesis in vivo. In this study we examined the effect of resveratrol on growth of two human melanoma cell lines. We found that this plant polyphenol inhibited growth and induced apoptosis in both cell lines, with the amelanotic cell line A375 being more sensitive. The potential involvement of different MAP kinases in the action of resveratrol was also examined. Although resveratrol did not alter the phosphorylation of p38 or JNK MAP kinases in either cell line, it induced phosphorylation of ERK1/2 in A375, but not in SK-mel28 cells. These results suggest that in vivo studies of the effect of resveratrol on melanoma are warranted and that this plant polyphenol might have effectiveness as either a therapeutic or chemopreventive agent against melanoma.

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Keywords: Resveratrol; Melanoma; Apoptosis; MAP kinase

1. Introduction

Resveratrol is a polyphenol found in high concentration in red grapes, red wine, peanuts and pines [1]. In these plants resveratrol is synthesized in response to stress conditions such as an infection and thus can be considered to be a phytoalexin [2]. Resveratrol has estrogenic activity in mammals [3,4] and therefore is

classified as a phytoestrogen. Resveratrol is a potent inhibitor of tumor promotion [5,6]. It has also been shown to inhibit the growth of colonic tumor cells [7], leukemic cells [8,9], breast and prostate cancer cells [10–15]. The mechanism of resveratrol action is not understood. It has antioxidant activity [16,17] and has been shown to inhibit cyclooxygenase activity [18–20].

Human melanoma is a tumor whose frequency is increasing at an alarming rate. If detected early and surgically excised the 5-year survival rate is favorable. However, later stages of the disease are difficult to treat and long-term survival is low. Interferon-γ

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¹ Both laboratories contributed equally to this study.

Melanoma (breast, leukemia, prostate, colon) }
Apoptosis : 30-100 μM (24 hr) }
Leukemia
p53

44



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Mutation Research 523–524 (2003) 145–150



Fundamental and Molecular
Mechanisms of Mutagenesis

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Review

Molecular mechanism of the chemopreventive effect of resveratrol

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Abstract

Chemoprevention is a promising approach to control human cancer. Resveratrol has been shown to have a potent chemopreventive effect in multiple carcinogenesis models. However, the precise mechanism explaining its anti-carcinogenic effect is not clear. This review summarizes recent studies from our laboratory on the mechanisms of resveratrol's effects. In JB6 cells, resveratrol was found to induce apoptosis and inhibit tumor promoter-induced cell transformation. We also found that resveratrol-induced activation of p53 and resveratrol-induced apoptosis occurred through a p53-dependent pathway. The MAP kinases, ERKs, JNKs, or p38 kinases, are involved in resveratrol-induced activation of p53 and apoptosis.

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Keywords: Resveratrol; Apoptosis; Chemoprevention; Signal transduction

1. Introduction

Interest in the concept and practice of chemoprevention as an approach to the control of cancer has increased greatly in the past few years. Many agents have been shown to be effective for blocking carcinogenesis in certain human cancer and animal models [1–14]. Resveratrol is thought to be a phytoalexin, one of a group of compounds that are produced in plants during times of environmental stress of pathogenic attack [15]. Resveratrol has been found in at least 72 plant species, a number of which are components of the human diet, such as mulberries, peanuts, and grapes [16]. Relatively high quantities are found in grapes, possibly because of the response of *Vitis vinifera* (Vitaceae) to fungal infection [16]. Fresh grape skin contains about 50–100 µg of resveratrol per gram [17].

In red wine, the concentration of resveratrol is in the range of 1.5–3 mg/l [18,19]. Appreciable amounts are also found in white and rosé wine [19]. Commercial grape juice contains about 4 mg/l of *trans*-resveratrol [20]. The results of several epidemiological studies suggested that decreased coronary heart disease mortality is associated with the moderate consumption of alcohol, especially red wine. This biological activity of red wine has been attributed to one of its constituents, resveratrol [20,21]. Resveratrol has been reported to inhibit platelet aggregation and coagulation, alter eicosanoid synthesis, and modulate lipoprotein mechanisms [22–24].

Data from Jang et al. [10] suggested strong anti-carcinogenesis effects of resveratrol. The inhibition may be due to a blocking effect on the carcinogenesis stages of initiation, promotion, or progression [10]. However, the precise mechanisms of the anti-carcinogenesis effect of resveratrol remain largely unknown.

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leucemia, GBM cancer, breast cancer, prostate
Apoptosis: 2–40 µM } rec'd p53



papillomi cutanei
Polifenoli e flavonoidi

Toxicology Letters 122 (2001) 33–44

Toxicology
Letters

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Topical and oral administration of the natural water-soluble antioxidant from spinach reduces the multiplicity of papillomas in the Tg.AC mouse model

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Abstract

The Tg.AC mouse carrying the v-Ha-ras structural gene is a useful model for the study of chemical carcinogens, especially those acting via non-genotoxic mechanisms. This study evaluated the efficacy of the non-toxic, water-soluble antioxidant from spinach, natural antioxidant (NAO), in reducing skin papilloma induction in female hemizygous Tg.AC mice treated dermally five times over 2.5 weeks with 2.5 µg 12-O-tetradecanoylphorbol-13-acetate (TPA). The TPA-only group was considered as a control; the other two groups received, additionally, NAO topically (2 mg) or orally (100 mg/kg), 5 days/week for 5 weeks. Papilloma counts made macroscopically during the clinical observations showed a significant decrease in multiplicity ($P < 0.01$) in the NAO topically treated group. According to histological criteria, papilloma multiplicity were lower in both topical-NAO and oral-NAO groups, but significantly so only in the oral-NAO mice ($P < 0.01$). The beneficial effect of NAO in the Tg.AC mouse is reported. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Natural antioxidant; Papilloma; Skin; Tg.AC mouse; Transgenic

1. Introduction

Studies with many chemical carcinogens have shown that their effects in the initiation, promo-

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Apoptosis → Via Topica 2 mg/day { + 5 pp/week
Via Orale (750 µl) : 100 mg/kg { + 5 weeks



Sutherlandia frutescens extracts can induce apoptosis in cultured carcinoma cells

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Received 22 June 2004; received in revised form 4 January 2005; accepted 14 January 2005

Abstract

Sutherlandia frutescens popularly known as cancer bush is endemic to Southern Africa. Whole plant parts have been used and traditional healers claim that it can treat cancer. In this study it is shown that a crude aqueous *Sutherlandia frutescens* whole plant extract induced cytotoxicity in neoplastic cells (cervical carcinoma) and CHO (Chinese Hamster Ovary cells) cell lines. Morphological observation and monitoring with other biological assays involving chromatin condensation as well as phosphatidyl serine externalisation point to apoptotic responses. Further biochemical assays showed similar DNA fragmentation patterns induced by *Sutherlandia frutescens* extracts compared to other inducers of apoptosis such as staurosporine and ceramide. Furthermore, *Sutherlandia frutescens* extracts induced apoptosis was confirmed by flow cytometric analysis. These findings warrant further research with a view to develop *Sutherlandia frutescens* extracts for use in anti-cancer therapy.

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Keywords: *Sutherlandia frutescens*; Apoptosis; Cytotoxicity; CHO (Chinese Hamster Ovary) cells; Cancer therapy

1. Introduction

In South Africa traditional healers claimed that *Sutherlandia frutescens* commonly known as cancer bush or “kankerbos” treats cancer. The plant was first used by “khoi san” and the “Nama” people and is distributed mostly along the west coast of the Western Cape (Van Wyk, 1997). Extracts of this plant have been used to treat stomach cancer; decoctions consumed as blood tonic and used for other ailments like cough, uterine disease and eye infection (Moshe et al., 1998). Thomson (2002) described *Sutherlandia frutescens* as a poisonous herb and folklore on this plant describe that decoctions are curative against cancer. Based on these controversial claims this study was set out to analyse the cytotoxic effect of crude plant extract to induce apoptosis in cultured cells. In this study *Sutherlandia frutescens* (Fabaceae) extracts were used to evaluate its ability to induce apoptosis in

CHO and neoplastic cells. Previous work done by Zhao et al. (2003) and Nile et al. (2003) showed that plant extracts are able to induce apoptosis in cancer cells. Previous studies have shown that medicinal plant extracts that induce apoptosis can be used for the purpose of generating therapeutic drug.

Induction of apoptosis, programmed cell death is one approach to cancer therapy (Los et al., 2003). Apoptotic cell death is a physiological mechanism that eliminates unwanted cells by triggering the cell's intrinsic suicide program (Kerr et al., 1972). Impairment of the apoptotic mechanism ultimately generates a pathological condition that includes developmental defects like, autoimmune diseases, neurodegeneration or cancerous neoplasia (Reed et al., 2001). Apoptosis is characterized by morphological changes such as membrane blebbing, cell shrinkage, protein fragmentation, chromatin condensation and DNA degradation followed by rapid engulfment of cell debris by neighbouring cells (Christop, 2003). It is therefore possible to take advantage of this intrinsic mechanism by manipulating the apoptotic process for therapeutic gains. The basis of this study was to broaden the under-

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Apoptosis cell 80% 3,5 milligramm/mL

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Mitochondrial dysfunction as an early event in the process of apoptosis induced by woodfordin I in human leukemia K562 cells

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Received 7 June 2003; accepted 25 August 2003

Abstract

Tannins are a group of widely distributed plant polyphenols, some of which are beneficial to health because of their chemopreventive activities. In the present study, we investigated the effects and action mechanisms of woodfordin I, a macrocyclic ellagitannin dimer, on human chronic myelogenous leukemia (CML) K562 cells. The results showed that woodfordin I was able to suppress the proliferation and induce apoptosis in K562 cells. Apoptosis was evaluated by cytomorphology, internucleosomal DNA fragmentation, and externalization of phosphatidylserine. Woodfordin I treatment caused a rapid and sustained loss of mitochondrial transmembrane potential (MMP), transient generation of reactive oxygen species (ROS), transient elevation of intracellular Ca^{2+} concentration, and cytosolic accumulation of cytochrome c. The activation of caspase-9 and 3, but not caspase-8, was also demonstrated, indicating that the apoptotic signaling triggered by woodfordin I was mediated through the intrinsic mitochondria-dependent pathway. Western blot and immunofluorescence analysis revealed that the anti-apoptotic Bcl-2 and Bcl-x_L levels were downregulated, together with the pro-apoptotic Bax protein. Significantly, woodfordin I-induced apoptosis was associated with a decline in the levels of c-Abl, Bcr-Abl, and cellular protein tyrosine phosphorylation. Considering the consequence of all the events in the process of woodfordin I-induced apoptosis, the mitochondrial dysfunction is directly responsible for the pro-apoptotic effects on K562 cells. Furthermore, because CML is a malignancy of pleuripotent hematopoietic cells caused by the dysregulated tyrosine kinase activity of Bcr-Abl, these findings suggest that woodfordin I may be a potential lead compound against CML.

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Keywords: Woodfordin I; Apoptosis; Tannins; Mitochondria; Bcr-Abl; Bcl-x_L; K562 cells

Introduction

Plant tannins represent one of the most ubiquitous groups of natural polyphenols. Researchers' interests in these somewhat structurally diverse secondary metabolites are heightened by their profound health-beneficial properties in certain beverages and by their identification as the principle curative agents in a variety of traditional herbal medicine. Recent studies have determined a lot of pure tannins with significant biological and pharmacological activities, such as antimicrobial (Burapadaja and Bunchoo, 1995), antiviral (Nakashima et al., 1992), antioxidant (Satoh and Sakagami, 1996), and antitumor activities (Gali et al., 1992; Miyamoto et al., 1993a; Mukhar et al., 1988). Classically, tannins are divided into two chemically distinct groups: the condensed tannins, also referred to as proanthocyanidins, and the hydrolyzable tannins. Of special interest is the rigid structure of the

hydrolyzable macrocyclic ellagitannins, such as woodfordin C (Kuramochi-Motegi et al., 1992), oenothin B (Miyamoto et al., 1993b) and camellin B (Yoshida et al., 1989), which exhibit inherently low cytotoxicity and potent antitumor activity. Woodfordin I was isolated from a traditional Chinese medicine, *Chamaenerion angustifolium* (L.) Scop. The unique structure is characterized as a macrocyclic ellagitannin dimer (Yoshida et al., 1992), indicating its potential biological activity (Fig. 1).

Apoptosis is essential for normal development and the maintenance of homeostasis. It is a highly regulated cellular process with characteristic morphological and biochemical features. Caspases are a highly conserved cysteine protease family and involved in both commitment and execution phases of apoptosis, resulting in cleavage of specific substrate proteins (Sakahira et al., 1998; Thornberry et al., 1997). Two major apoptotic signaling pathways have been defined. The mitochondria-dependent pathway is responsible for extracellular cues and internal insults such as DNA damage. Cytotoxic stress causes pro-apoptotic members of the Bcl-2 family, such

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Non oocyte p53
Caspase 9 (Cytosol c → APAF1...)
Caspase 8
Apoptosis:
5-50 μM

48

35S CaMV promoter \Rightarrow -40% (49)
 di ANTOCIANINE



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Biochemical and Biophysical Research Communications 303 (2003) 326–331

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An overexpression of chalcone reductase of *Pueraria montana* var. *lobata* alters biosynthesis of anthocyanin and 5'-deoxyflavonoids in transgenic tobacco[☆]

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Received 17 February 2003

Abstract

We isolated the chalcone reductase (*pl-chr*) gene of *Pueraria montana* var. *lobata* by using a PCR strategy from cDNA pools of storage roots. A high level of expression of RNA was found in both stems and roots. The genomic Southern blot result suggests that *pl-chr* exists as a member of a small gene family. By introducing a *pl-chr* gene under the control of the 35S CaMV promoter into the pink-flowering Xanthi line of *Nicotiana tabacum*, the flower color was changed from pink to white-to-pink. The contents of anthocyanin in the flowers of the transgenic lines were dramatically decreased by 40%, but the total UV absorption compounds remained unchanged. The production of liquiritigenin in *pl-chr* overexpressed transgenic tobacco lines was confirmed by HPLC and MS analysis. The introduction of *pl-chr* gene provides a method to redirect the flavonoid pathway into 5'-deoxyflavonoid production in non-legume crops, in order to manipulate the phenylpropanoid pathway for isoflavonoid production.
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Keywords: Chalcone reductase; Isoflavonoid; *Pueraria montana* var. *lobata*; Anthocyanin; 5'-Deoxyflavonoids; Transgenic tobacco

Isoflavonoids have a very limited distribution to leguminous plants, but have very important functions such as inducers of Rhizobium nodulation genes and antimicrobial phytoalexins [1,2]. Many isoflavonoids also exhibit medicinal properties and are common constituents in human diets [1,3].

The storage root of *Pueraria montana* var. *lobata* (Willd.) Ohwi contains diverse isoflavonoids [4]. In Korea, the extracts of them have been traditionally used as medicinal supplements for fever, pain, myalgia, alcohol poisoning, and abortifacients. Flavonoids are synthesized via the phenylpropanoid pathway. Chalcone reductase (CHR) is an enzyme that co-acts with chalcone synthase (CHS) to produce 4,2',4'-trihydroxychal-

cone (isoliquiritigenin), which is a precursor of 5-deoxy-(iso)flavonoids, a branch in the first step of the flavonoid pathway. This chalcone is the precursor of the 5-deoxy series of flavonoids and isoflavonoids [5], which include nodulation-induction factors as well as pterocarpin phytoalexins of the Leguminosae [6]. A cDNA encoding chalcone reductase has been cloned from soybean [7], alfalfa [8], *Glycyrrhiza echinata* [9], and *Sesbania rostrata* [10]. In recent years, many genes encoding the enzymes involved in isoflavonoid biosynthesis have been cloned [11–14]. There have also been many reports on metabolic engineering for isoflavone production in non-legume dicot and monocot plants [15–18].

We are currently studying the production of isoflavones in non-legume crops by introducing isoflavonoid pathway genes. From the viewpoint of efficient metabolic engineering, it is very important to determine a method to control the overall metabolic flux within the targeted pathway and endogenous competing pathways. In this study, we report on the isolation and

DIAP. 50

Gravissima è poi l'assenza dei semi dai frutti OGM. L'importanza dei semi come fattori anti-cancro risiede sostanzialmente nel fatto che essi contengono la famosa vitamina B17.

Ma è estremamente grave il fatto che le grandi aziende sementiere OGM stiano immettendo sul mercato agricolo mondiale gli stessi frutti privi però di semi, in particolare:

Cucumis melo,
Citrus limonum,
Citrullus vulgaris,
Solanum lycopersicum,
Vitis vinifera.

La B17 reagisce all'enzima *Beta-glucosidasi*: quest'ultimo è caratteristico di molti tumori, ed è praticamente assente nelle cellule sane;

in tale reazione, l'enzima scinde l'innocua vitamina B17 in due potenti veleni: *ioni-Cianuro* e *Benzaldeide*, quest'ultimo un potente analgesico (anti-dolorifico).

Queste due sostanze, prodotte in piccole quantità dalle stesse cellule tumorali, si combinano allora fra loro all'interno stesso delle cellule tumorali, producendo una sostanza estremamente tossica che uccide la cellula stessa in una sorta di pseudo-apoptosi.

Viceversa, le cellule sane contengono un altro enzima, la *Rodanese*, il quale è presente nelle cellule in quantità inversamente proporzionale alla *Beta-glucosidasi*; se la B17 entra in contatto con le cellule sane, la *Rodanese* neutralizza gli *ioni-Cianuro* e ossida la *Benzaldeide*.

[☆] *Pueraria montana* var. *lobata* chalcone reductase sequence has been submitted to GenBank and the Accession No. awarded is AF462632.

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SECONDO: mutazioni genetiche delle piante e conseguente alterazione della Biochimica umana

A causa dell'introduzione di geni estranei (es. di animali, batteri, virus, retrovirus) nel DNA della pianta, si verifica in essa l'alterazione della normale sequenza genomica, con la comparsa di nuove proteine e/o la perdita di altre proteine di sequenza genomica.

Di qui la comparsa di nuove sostanze simili alle vitamine naturali, ma in realtà con caratteristiche di reattività enzimatica e biochimica diverse da quelle naturali, con induzione pertanto di modifica della loro componente di attività biochimica sul genoma umano, una volta introdotte con l'alimentazione.

Di qui la comparsa potenziale di nuove malattie insorte "artificialmente" a causa di manipolazione genetica (OGM) di organismi vegetali, inquinati geneticamente da nuove molecole simil-vitaminiche dagli effetti induttivi sul DNA umano e sulla sua complessa biochimica del tutto sconosciuta, ma probabilmente foriera di gravi danni data l'estrema complessità e quindi vulnerabilità del DNA umano.

Accumulation of Very-Long-Chain Fatty Acids in Membrane Glycerolipids Is Associated with Dramatic Alterations in Plant Morphology

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Transgenic *Arabidopsis* plants overexpressing the *Arabidopsis* *FATTY ACID ELONGATION1* gene under the control of the 35S promoter from cauliflower mosaic virus accumulated very-long-chain fatty acids (VLCFAs) throughout the plant. In some transformants, C20 and C22 VLCFAs accounted for >30% of the total fatty acids, accumulating at the expense of C16 and C18 fatty acids. These C20 and C22 fatty acids were incorporated into all of the major membrane glycerolipid classes. Plants with a high VLCFA content displayed a dramatically altered morphology, which included the failure of flowering shoots to elongate, a modified spatial pattern of siliques, an altered floral phenotype, and a large accumulation of anthocyanins. In addition, these plants also exhibited a unique alteration of the chloroplast membrane structure. We discuss a possible role for VLCFAs in establishing the shape/curvature of the membranes, which in turn may affect the shape of the cell and ultimately that of the whole plant.

INTRODUCTION

The physical properties of membranes are largely determined by chain length, polarity, and the degree of unsaturation of fatty acids that comprise their lipids. The fact that each membrane in the cell consists of a characteristic set of lipid classes and that each class has a distinct fatty acyl composition suggests that the lipid structure/composition is important for membrane function (Ohlrogge and Browse, 1995). This notion is further supported by the observation that the lipid structure/composition of membranes is conserved throughout the plant kingdom. However, the exact relationship between lipid structure and membrane function is not well understood (Ohlrogge and Browse, 1995).

Common plant fatty acids, which are constituents of structural membrane glycerolipids, are C16 and C18 fatty acids with one to three *cis* double bonds. Fatty acids with chemical structures that differ significantly from this common theme are called unusual fatty acids (van de Loo et al., 1993). Examples of unusual fatty acids include very-long-chain fatty acids (VLCFAs; e.g., erucic [22:1]), medium-chain fatty acids (e.g., lauric [12:0]), hydroxylated fatty acids (e.g., ricinoleic [12OH-18:1]), and fatty acids with different posi-

tions of the double bond (e.g., petroselinic acid [18:1, Δ6]) (van de Loo et al., 1993). These fatty acids occur mainly in storage triacylglycerols (TAGs) of certain oilseed species but are excluded from polar glycerolipids and consequently from the membranes of cells. Presumably, the accumulation of unusual fatty acids in membrane lipids would perturb the integrity of the bilayer and have deleterious effects on the cell. Thus, plants have developed a process(es) to screen out unusual fatty acids from membrane lipids. Although the exact mechanism of this process is yet to be elucidated, there is evidence that phospholipases and acyltransferases contribute to the strong fatty acid bias observed between storage and membrane glycerolipids (Bafor et al., 1991, 1993; Frentzen, 1993; Stahl et al., 1995).

The strict censoring of the fatty acyl composition of membrane lipids has been highlighted recently in transgenic plants. Two unusual fatty acids, lauric and ricinoleic, require a single unique enzyme activity for their synthesis from primary lipid metabolism. Lauric acid biosynthesis requires a medium-chain acyl-acyl carrier protein thioesterase, whereas ricinoleic acid biosynthesis requires an 18:1 hydroxylase. Genes encoding these enzymes were isolated from California bay trees (*UcFatB1*; Voelker et al., 1992) and castor bean (*FAH12*; van de Loo et al., 1995), respectively. Expression of either one of these genes in transgenic plants under the control of the cauliflower mosaic virus 35S promoter resulted in the synthesis and incorporation of the unusual fatty acids in

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TERZO: fallimento della dieta-anti-cancro

Come già dimostrato da Gerson, e da altri medici, moltissime sostanze contenute solo in frutta e verdura cruda e biologica sono in grado d'indurre **CASCATA IMMUNITARIA** contro il tumore, senza quindi necessità di laboriose e costosissime ricerche.

Così, nella Dieta anti-cancro del dott. Gerson, applicata a 153 pazienti sofferenti del caso del **MELANOMA**,

si giungeva,

dopo 5 anni di Dieta-Gerson,

a percentuali di guarigione variabili da:

70-90% (se tumore ancora localizzato)

a 40-70% (se tumore già metastatizzato),

purchè in pazienti non sottoposti precedentemente a Chemio-Terapia.

Terapie Gerson e' Efficace

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ORIGINAL PAPER

FIVE-YEAR SURVIVAL RATES OF MELANOMA PATIENTS TREATED BY DIET THERAPY AFTER THE MANNER OF GERSON: A RETROSPECTIVE REVIEW

GL Gar Hildenbrand, L Christeene Hildenbrand, Karen Bradford, and Shirley W Cavin, MS

GL Gar Hildenbrand, L Christeene Hildenbrand, and Karen Bradford are from the Gerson Research Organization in San Diego, Calif, and Shirley W Cavin is from the University of California, San Diego, Cancer Prevention and Control Program.

Objective • Compare 5-year melanoma survival rates to rates in medical literature.

Design • Retrospective.

Setting • Hospital in Tijuana, Mexico.

Patients • White adult patients (N=153) with superficial spreading and nodular melanoma, aged 25-72 years.

Intervention • Gerson's diet therapy: lactovegetarian; low sodium, fat and (temporarily) protein; high potassium, fluid, and nutrients (hourly raw vegetable/fruit juices). Metabolism increased by thyroid; calorie supply limited to 2600-3200 calories per day. Coffee enemas as needed for pain and appetite.

Main Outcome Measure • 5-year survival rates by stage at admission.

Results • Of 14 patients with stages I and II (localized) melanoma, 100% survived for 5 years, compared with 79% of 15,798 reported by Balch. Of 17 with stage IIIA (regionally metastasized) melanoma, 82% were alive at 5 years, in contrast to 39% of 103 from Fachklinik Hornheide. Of 33 with combined stages IIIA + IIIB (regionally metastasized) melanoma, 70% lived 5 years, compared with 41% of 134 from Fachklinik Hornheide. We propose a new stage division: IVA (distant lymph, skin, and subcutaneous tissue metastases), and IVB (visceral metastases). Of 18 with stage IVA melanoma, 39% were alive at 5 years, compared with only 6% of 194 from the Eastern Cooperative Oncology Group. Survival impact was not assessed for stage IVB. Male and female survival rates were identical for stages I-IIIIB, but stage IVA women had a strong survival advantage.

Conclusions • The 5-year survival rates reported here are con-

siderably higher than those reported elsewhere. Stage IIIA/B males had exceptionally high survival rates compared with those reported by other centers. (*Alternative Therapies in Health and Medicine*. 1995;1(4):29-37)

This article summarizes the clinical outcomes of melanoma patients treated with the nutrition-based cancer therapy proposed by the German physician Gerson¹ (who conducted research at the University of Munich in the 1930s) and contrasts them with rates reported in the literature. To our knowledge, this report is the most thorough retrospective analysis to date of the potential survival benefit of this, or any other, well-known alternative method of cancer management.

The genesis of this inquiry occurred during a landmark study by the US Congressional Office of Technology Assessment (OTA),² to which one of us (GH) was an advisor. In its report, OTA put forward a protocol for best-case reviews based on the premise that, no matter how many patients failed, as few as 10 or 12 cases with objective evidence of tumor response would be enough to propel an investigation by the National Cancer Institute (NCI). Because we had proposed the original best-case review protocol to OTA, we were eager to construct such a review. However, we found OTA's (and later NCI's) protocol to have a serious shortcoming when used retrospectively: its focus on tumor regression only. Adequate documentation of tumor regression is unlikely to be collected in most alternative medical practices.

We abandoned the best-case review for the more informative retrospective review. In contrast to the best-case review, the retrospective review describes all patients, including nonresponders, giving a more adequate impression of the outcomes of treatment.

Our efforts to complete a best-case review, however, were not without some rewards. Practitioners at Centro Hospitalario Internacional del Pacifico, SA (CHIPSA) in Playas de Tijuana, Baja California, Mexico suggested cases with different types of

Financial support: Private grants were provided by Mr Laurence S Rockefeller, Arnold and Ann Gamowitz, and Richard Otto. The Gerson Research Organization holds a contract with the Centro Hospitalario Internacional del Pacifico, SA, to perform retrospective and prospective analyses of patient outcomes.

Diap. 55

Viceversa, con la Chemio-Terapia, la percentuale di guarigione da Melanoma a 5 anni è del 6%, secondo vecchi dati di 10 anni fa.

valore che secondo questa recentissima fonte australiana di MORGAN, del 2004, è invece dello zero per cento,

Overview

The Contribution of Cytotoxic Chemotherapy to 5-year Survival in Adult Malignancies

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ABSTRACT:

Aims: The debate on the funding and availability of cytotoxic drugs raises questions about the contribution of curative or adjuvant cytotoxic chemotherapy to survival in adult cancer patients.

Materials and methods: We undertook a literature search for randomised clinical trials reporting a 5-year survival benefit attributable solely to cytotoxic chemotherapy in adult malignancies. The total number of newly diagnosed cancer patients for 22 major adult malignancies was determined from cancer registry data in Australia and from the Surveillance Epidemiology and End Results data in the USA for 1998. For each malignancy, the absolute number to benefit was the product of (a) the total number of persons with that malignancy; (b) the proportion or subgroup(s) of that malignancy showing a benefit; and (c) the percentage increase in 5-year survival due solely to cytotoxic chemotherapy. The overall contribution was the sum total of the absolute numbers showing a 5-year survival benefit expressed as a percentage of the total number for the 22 malignancies.

Results: The overall contribution of curative and adjuvant cytotoxic chemotherapy to 5-year survival in adults was estimated to be 2.3% in Australia and 2.1% in the USA.

Conclusion: As the 5-year relative survival rate for cancer in Australia is now over 60%, it is clear that cytotoxic chemotherapy only makes a minor contribution to cancer survival. To justify the continued funding and availability of drugs used in cytotoxic chemotherapy, a rigorous evaluation of the cost-effectiveness and impact on quality of life is urgently required. Morgan, G. *et al.* (2004). *Clinical Oncology* 16, 549–560

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Key words: Chemotherapy, combined modality treatment, palliation, quality of life, radiotherapy, survival

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Introduction

In adults, cytotoxic chemotherapy became established in the 1970s as a curative treatment in advanced Hodgkin's disease [1], non-Hodgkin's lymphoma [2], teratoma of testis [3] and as an adjuvant treatment for early breast cancer [4].

The initial results suggested the potential use of cytotoxic chemotherapy as a definitive treatment or as an adjuvant therapy in asymptomatic patients with the aim of improving survival. However, as stated by Braverman [5] and others [6–8], the early gains in a few tumour sites have not been seen in the more common cancers. For most patients, the use of cytotoxic chemotherapy is for the palliation of symptoms and to improve quality of life [9], with prolongation of survival being a less important outcome.

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Some practitioners still remain optimistic that cytotoxic chemotherapy will significantly improve cancer survival [10]. However, despite the use of new and expensive single and combination drugs to improve response rates and other agents to allow for dose escalation, there has been no change in some of the regimens used, and there has been little impact from the use of newer regimens. Examples are non-Hodgkin's lymphoma [11] and ovarian cancer [12], in which cyclophosphamide, adriamycin, vincristine and prednisolone (CHOP) and platinum, respectively, (introduced over 20 years ago) are still the 'gold standard' treatment. Similarly, in lung cancer, the median survival has increased by only 2 months during the same time period [13,14], and an overall survival benefit of less than 5% has been achieved in the adjuvant treatment of breast, colon, and head and neck cancers [15–17].

The recent debate on funding of new cytotoxic drugs [18–20] has highlighted the lack of agreement between medical oncologists and funding bodies on the current and

Una sopravvivenza dello ZERO % che, in questo recentissimo studio australiano di MORGAN, eseguito su oltre 270.000 pazienti sottoposti a CHEMIO, è confermato anche nel caso di:

cancro del pancreas,
sarcoma,
cancro dell'utero,
cancro della prostata,
cancro della vescica,
cancro del rene,
mieloma multiplo,

tale percentuale sale poi all'1% nel caso di:
cancro dello stomaco e cancro del colon,

sale al 2% circa nel caso di:
cancro della mammella e del polmone,

sale al 3-5% nel caso del cancro del retto,

sale al 4-5% nel caso dei tumori al cervello,

sale al 5% nel caso del cancro dell'esofago,

sale al 9% nel caso del cancro dell'ovaio,

sale al 10% nel caso del linfoma NON Hodgkin,

sale al 12% nel caso del cancro della cervice uterina,

sale al 40% circa nel caso del seminoma del testicolo e del Linfoma di Hodgkin.

La chiave di spiegazione di tale efficacia curativa di queste particolari diete vegetariane risiede nel fatto di:

non assimilare mai cibi contenenti tutti i potenziali fattori di crescita cellulare,

in particolare,

EVITARE
l'assimilazione contemporanea (1-3 ore) di

TUTTI e 9 gli AMINOACIDI ESSENZIALI:

Valina, Isoleucina, Leucina, Lisina, Metionina, Istidina, Triptofano, Fenilalanina, Treonina.

perché solo con essi le cellule del cancro possono costruire PROTEINE, e cioè altre cellule malate.

Bisogna anche evitare anche l'assimilazione di:

**acidi nucleici,
vitamina B 12,
acido folico**

(perché determinano la replicazione del DNA della cellula del cancro)

Una volta,
...prima dell'ERA dei cibi O.G.M.,
questa regola era semplicissima da mettere in pratica:

i cibi che contenevano tutto ciò erano unicamente i cibi di origine animale (carne, pesce, uova, latte, formaggio, burro..),
che sia Gerson, sia altri Autori (compresa anche la medicina cinese e indiana) proibivano di assumere per almeno 1 anno.

Risultava così vincente la sola alimentazione vegetariana, cioè a base di sola frutta e di verdura, compresi i cereali e i legumi.

Questi ultimi cibi (cereali e legumi) sono però ricchi di AMINOACIDI ESSENZIALI,
e ciò può stupire che venissero *comunque* impiegati nella terapia del Cancro da molte altre scuole di medicina naturale occidentale, indiana e cinese.

Il successo di queste terapie così lontane fra loro come TEORIA, ma così simili come efficacia PRATICA contro il CANCRO, potrebbe essere spiegato dalla moderna BIOCHIMICA in base al fatto che:

NESSUN CEREALE e NESSUN LEGUME
conteneva da solo TUTTI e 9 gli Aminoacidi Essenziali.

Questi alimenti, se MANGIATI insieme nello stesso pasto (1-3 ORE), determinano l'assimilazione di tutti e 9 gli aminoacidi.

E il corpo umano può così sintetizzare PROTEINE, e costruire quindi cellule (...del cancro).

CONFRONTANDO queste vecchie terapie, quindi, emerge

il DIVIETO ASSOLUTO
di mangiare assieme CERALI + LEGUMI,

cioè Pasta (o Polenta, o Pane o Riso) + Legumi,

poiché, con la moderna BIOCHIMICA,
oggi sappiamo che si provoca
l'integrazione dei 9 aminoacidi essenziali

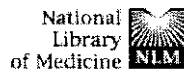
(solo 8 sono contenuti nei cereali,
ma quello mancante è nei legumi).

(solo 8 sono contenuti nei legumi,
ma quello mancante è nei cereali)

con effetto nutrizionale, quindi, simile a quello ottenuto dalla Carne

(in fondo, una volta, un piatto di Pasta e Fagioli era anche chiamatola carne dei poveri....)

Oggi però, tramite l'introduzione in commercio di cereali, legumi e altri vegetali modificati geneticamente (O.G.M.) in molti di questi alimenti sono contenuti TUTTI gli aminoacidi essenziali, rendendo in tal modo effettivamente NON più curabile il Cancro secondo quanto descritto nella terapia Gerson, e da molti altri autori.



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Genetic modification of plants: significant issues and hurdles success.

Day PR.

Center for Agricultural Molecular Biology, Rutgers, The State University
New Jersey, New Brunswick, 08902, U.S.A.

Transformation and regeneration is routine for many crop plants. A genetically engineered tomato with a longer shelf life at full ripeness was introduced in the United States in 1994, and other soon-to-be-released products, both foods and fibers, incorporate genes for resistance to pests, diseases, and environmentally benign herbicides. Other possibilities are altered plant fats and oils, methionine, and lysine-enhanced grain and leg proteins, plant foods that can deliver immunizing antigens, and other way controlling fruit ripening. Food safety concerns include the inadvertent production of toxicants and allergens. Foreign DNA can be introduced in plants by bacterial vectors, direct uptake by protoplasts, and mechanical introduction on metal particles or other materials. Limitations include little control of copy number or site of integration of the introduced DNA, dependence on selectable markers for recovery of traits, and inadequate knowledge of how to control key metabolic steps to maximize desirable traits. Directed genetic change still requires conventional crop breeding to deliver benefits to farmers and consumers.

Publication Types:

- Review
- Review, Tutorial

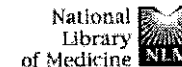
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Expression of the Brazil nut methionine-rich protein and mutants with increased methionine in transgenic potato.

Tu HM, Godfrey LW, Sun SS.

Department of Plant Molecular Physiology, University of Hawaii, Honolulu
96822, USA.

A cDNA encoding the methionine-rich (19 mol% Met) protein in Brazil nut was placed under the regulation of CaMV 35S promoter and nopaline synthase terminator and introduced into the potato cultivar Russet Burbank via Agrobacterium-mediated transformation. To further enhance the Met content in the transgenic plants, chimeric genes containing four mutant constructs, BoxIa (with 5 additional Met), BoxIIa (2 additional Met), BoxIIa1 (7 additional Met), and BoxIIa2 (7 additional Met), were also generated by sequence modifications of the cDNA and transferred into potato. Analysis of the microtubers and leaves of the transgenic potato plants revealed, in general, with the exception of the BoxIIa2, the presence of mRNA transcripts of the expected size and the correctly processed Met-rich kDa subunit polypeptides. The expression levels in the leaves among the various constructs and individual transgenic plants varied between <0.01% and 0.2% of total protein. The corresponding expression in the tubers was usually 2- to 4-fold lower than in leaves. In the case of BoxIIa2, which contains two tandem repeats of the BoxIIa mutant sequence, a larger (10 kDa) polypeptide was detected. These findings demonstrated that it is feasible to exploit the variable region of the Brazil Nut 2S protein for enhanced Met contents and perhaps for other desirable properties.

PMID: 9678578 [PubMed - indexed for MEDLINE]

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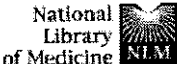
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methionine
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Li wophono
INTRODURE
nelle PIANTE

dalle
Nose del
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Methionine in
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PATATA + Methionine

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*transgenic
 potato
 methionine*

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 Honolulu 96822, USA.

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May 20 2003 11:14

DIAP. 64

QUARTO : malattie indotte da virus transgenici

I virus transgenici con cui oggi si fanno gli Organismi Geneticamente Modificati (O.G.M.) entrano nel DNA della pianta, modificandola in maniera a noi sconosciuta.

Questi virus dovrebbero restare latenti, ma nulla può escludere che possano anche riattivarsi in maniera analoga ai ben noti virus tumorali a RNA (Oncornavirus) o come i virus tumorali a DNA (entrambi induttori di leucemie, sarcomi, carcinomi, gliomi...).

Questi virus possono anche essere portatori di malattie nuove o di malattie abbastanza simili a ben note sindromi purtroppo ancora poco comprese nella loro dinamica (AIDS, Mucca Pazza, etc...), e di cui è ancora molto vaga l'origine (forse virus transgenici?).

In merito a virus impiegati per costruire O.G.M. vi è un'ampia bibliografia (DIAP. 61-64).

Ciò che fa paura è soprattutto il fatto che RETRO-VIRUS sono DELIBERATAMENTE usati introdotti per fare le piante OGM .

Piante che poi mangiamo...con gli eventuali RETRO-VIRUS ancora presenti.....

Ma che cosa sono i RETRO-VIRUS ?

VIRUS tumorali a RNA (Oncorna-virus)

Gli Oncornavirus sono tutti simili fra loro per:

struttura,
composizione chimica,
reazione agli agenti chimici e fisici,
modo di replicazione.

Sono suddivisi nei tipi A, B, C in base a differenze morfologiche, antigeniche ed enzimatiche.

Essi sono una sotto-famiglia (chiamata *Onco-viridae*) nell'ambito della famiglia dei *Retro-viridae*, in quanto che:

Tutti i membri possiedono una *trascriptasi inversa* (DNA polimerasi RNA-dipendente), con la quale possono trascrivere il loro RNA virale nel DNA della cellula ospite.

Successivamente sintetizzano molecole di DNA a doppio filamento, dopo che le molecole di DNA a singolo filamento sono state liberate dall'ibrido RNA-DNA da un altro enzima (RNAasi H).

Questi reperti avvalorano l'ipotesi che l'RNA degli Oncorna-virus si replichi in vivo nell'uomo attraverso un DNA intermediario.

La *trascriptasi inversa* degli Oncornavirus è stata purificata e si è visto che è una proteina della parte interna dei virus, con un peso molecolare di circa 60.000-80.000 Dalton, separabile dagli antigeni gs degli Oncorna-virus.

La *trascriptasi inversa* non è presente solo negli Oncornavirus qui di seguito descritti.

Diversi altri tipi di virus a RNA, che causano infezioni latenti nel loro ospite d'origine, possiedono questo enzima.

Ciò è certo per virus antigenicamente correlati che inducono infezioni “lente” delle pecore (Visna-virus [simile clinicamente alla “sindrome della Mucca Pazza”]), e virus che formano sincizi (foamy) e hanno origine dai primati, dai bovini e dai felini.

Famoso è pure l'HIV, che sembrerebbe correlato con l'insorgenza dell'AIDS (Sindrome da Immuno-Deficienza Acquisita), sindrome comunque non ancora chiarita nella sua origine, essendo stata ipotizzata anche altra origine virale (il ben noto virus oncogenico a DNA “SV40”).

Molte piante OGM (e anche alcuni animali da allevamento, come polli e salmoni) sono anche modificate proprio immettendo al loro interno dei virus di questo tipo (*Retroviridae*), cioè muniti di *trascriptasi inversa* per modificare il DNA della pianta stessa (o per indurre la produzione dell'ormone della crescita o di altri ormoni in animali da allevamento).

Molti di questi virus vengono classificati nella sottofamiglia degli *Oncorna-viridae* (famiglia dei *Retroviridae*), poiché oltre alla presenza della *trascriptasi inversa* (caratteristica dei *Retro-viridae*) hanno in comune altre proprietà biologiche e biofisiche, come quella di provocare tumori.

Sarebbe quindi opportuno indagare meglio i *Retroviridae* impiegati dalle Multinazionali GMO per fare piante OGM (o per indurre la produzione dell'ormone della crescita o di altro tipo in animali da allevamento, come polli e salmoni).

Replicazione degli Oncorna-virus e trasformazione cellulare

Una proprietà comune degli Oncorna-virus è che essi non sono citocidi per le cellule nelle quali si replicano.

Come altri virus, gli Oncorna-virus dopo aver infettato una cellula, attraversano una fase di eclisse.

La cellula infetta produce nuovo virus, continua a moltiplicarsi e può subire o non subire la trasformazione maligna.

Il virus infettivo e le particelle virali sono facilmente messe in evidenza nella maggior parte delle cellule tumorali o delle cellule trasformate in vitro.

I virus maturano sulla membrana cellulare e vengono continuamente liberati dalla cellula per gemmazione della membrana cellulare.

L'RNA virale, penetrato nella cellula, viene trascritto a DNA subito dopo l'infezione: l'ibrido RNA-DNA viene poi ulteriormente trascritto a un DNA a doppio filamento il quale, durante la divisione cellulare, si integra nel DNA della cellula ospite.

Il DNA specifico del virus (Provirus) integrato serve come stampo permanente per la trascrizione delle molecole di RNA della progenie virale, sia come gene trasmissibile ereditariamente per la trasformazione.

Induzione di tumori da Oncorna-virus

Questi virus possono causare tumori, in condizioni naturali, generalmente soltanto nei loro ospiti d'origine, raramente in altri tipi di animali, compreso quindi l'uomo.

Non è noto se il relativo “rispetto delle altre specie”, comune a questi *Retro-viridae* presenti in natura, e qui sottoelencati (Complessi A, B, C, D, E), si sia mantenuto anche nei *Retroviridae* manipolati per produrre piante OGM, o mangimi per animali, o per modificare il DNA stesso di alcuni animali ad uso alimentare per la specie umana (salmoni, polli).

Sappiamo invece che questo “rispetto delle altre specie”, non è applicato nel caso dei virus tumorali a DNA.

Complesso A [Complesso della Leucemia-sarcoma aviario] :*Leucemie*

le affezioni leucemiche sono comuni nei polli, e i virus che inducono leucemia sono ampiamente diffusi in questi animali.

I tipi principali di leucemia virale sono:

la leucemia linfoide (virus della linfomatosi aviaria),
la leucemia mieloide (virus della mieloblastosi aviaria),
la leucemia eritroide (virus della eritroblastosi aviaria).

Il virus infettivo e le particelle fisiche del virus si possono trovare in alte concentrazioni nelle cellule tumorali, nel sangue periferico e in altri organi degli animali infetti, un fenomeno che non si osserva con i virus tumorali a DNA.

I mieloblasti o gli eritroblasti ottenuti da uccelli ammalati e fatti crescere in coltura di tessuto, continuano a liberare virus che, a sua volta, può indurre la malattia per inoculazione nei polli.

Quasi tutti gli allevamenti di polli sono infetti con vari tipi di questi virus, ma specialmente con quello della linfomatosi.

Il virus viene trasmesso *orizzontalmente* mediante la saliva e le feci producendo negli animali adulti un'infezione caratterizzata da viremia transitoria e anticorpi persistenti.

Relativamente pochi volatili adulti sviluppano la malattia clinica. La trasmissione *verticale* è stata dimostrata nella gallina viremica, ma non nel gallo viremico.

Sarcomi

Il virus del sarcoma di Rous ha subito sperimentalmente innumerevoli passaggi da quando fu isolato per la prima volta nel 1911, ed è probabile che attualmente sia diverso dal virus che si trova in natura.

Questi virus aviari, differenti fra loro per oncogenicità, struttura antigenica e spettro d'ospite, causano comunque sarcomi nei volatili di tutte le età e negli embrioni di pollo da laboratorio, ma contrariamente ai virus della linfomatosi, *non* vengono trasmessi naturalmente.

Essi inducono, inoltre, tumori nelle anatre, nei tacchini, nei piccioni e in altri uccelli.

I virus del sottogruppo Schmidt-Ruppin possono infettare le cellule dei mammiferi e indurre così tumori, come già dimostrato quando vengono inoculati nei neonati di ratti, hamster siriani e cinesi, conigli, topi, cavie e *scimmie*.

Questi tumori aviari di solito contengono ancora virus infettivo, mentre quelli dei mammiferi, stranamente, spesso sono privi di questi virus.

Nota 1: Questi tipi di Retrovirus producono particelle di tipo C. Particelle simili agli Oncornavirus di tipo C sono state messe in evidenza con la microscopia elettronica in cellule o nel plasma di pazienti con tumori solidi dell'uomo, come il Linfoma di Hodgkin e NON-Hodgkin, e i sarcomi.

Nota: Attualmente si stanno compiendo ricerche bibliografiche per sapere se virus aviari del sottogruppo D siano stati impiegati per creare piante OGM.

E' comunque noto che Retrovirus di questo tipo (induttori di leucemia nei polli) sono stati usati come vettori per veicolare geni umani nel DNA di questi volatili, allo scopo di aumentarne la produzione.

Questi stessi Retrovirus sono stati anche usati come vettori per impiantare il gene dell'ormone della crescita in alcune specie di pesci di allevamento (Salmoni), allo scopo di farli crescere più in fretta.

Complesso B [Complesso della *Leucemia-sarcoma murino*] :

Leucemie

Sono stati isolati numerosi virus leucemogeni murini che inducono diversi tipi di leucemia.

Per esempio, il virus *Graffi* causa forme mieloidi di leucemia in alcuni ceppi di topo, mentre in altri ceppi di topi si verifica leucemia linfatica in un'alta percentuale di casi.

Il virus *Gross* causa quasi tutti i tipi noti di malattia leucemica: è stato dimostrato che la maggior parte dei virus della leucemia sono murino-patogena nei ratti e che il virus *Moloney* è patogeno pure negli Hamster.

Gli animali neonati sono i più suscettibili agli effetti dei virus leucemogeni, ma la malattia può essere prodotta anche negli animali giovani e adulti.

Fattori genetici hanno un ruolo importante nel determinare la suscettibilità dei topi ai virus, nella natura della malattia causata e nella trasmissione del virus.

Grandi quantità di virus infettivi e di particelle virali sono presenti nel sangue e nei tessuti tumorali degli animali infetti.

I virus leucemogeni murini sono diffusi in natura e il virus di *Gross* è il prototipo di questi agenti responsabili di leucemie naturali.

Sarcomi

Sono stati isolati numerosi ceppi di questi virus.

Essi inducono sarcoma in hamster, ratti e topi neonati.

Il passaggio di alcuni ceppi in cellule di ratto ha dato luogo all'acquisizione di sequenze di acido nucleico di ratto da parte dell'RNA del menoma di questi virus.

Nota 1: Questi tipi di Retrovirus producono particelle di tipo C.

Particelle simili agli Oncornavirus di tipo C sono state messe in evidenza con la microscopia elettronica in cellule o nel plasma di pazienti con tumori solidi dell'uomo, come il Linfoma di Hodgkin e NON-Hodgkin, e i sarcomi.

Complesso C

[Complesso del *tumore (carcinoma) mammario murino*]:

L'oncogenesi da parte di diversi ceppi virali di questo tipo è molto complesso, poiché dovuto all'interazione tra il virus, la costituzione genetica dell'ospite e fattori ormonali.

Il ceppo virale più virulento conosciuto (MuMTV) determina adenocarcinomi mammari nelle femmine di topo, con grandi quantità di *virus infettivo* e di *particelle B* nel tumore, nel latte e nel sangue.

In questi animali il *virus* è *trasmesso dalla madre alla prole attraverso il latte*.

Il virus induce adenocarcinomi della sola ghiandola mammaria e soltanto in topi delle linee suscettibili.

Gli animali che non sviluppano tumori rimangono infetti in modo subclinico e trasmettono il virus alla progenie.

Questi tipi di Retrovirus producono particelle di tipo B.

Particelle simili agli Oncorna-virus di tipo B sono state messe in evidenza nel cancro mammario umano e nel latte sia di donne Parsi (popolazione indiana con incidenza molto elevata di cancro mammario) e sia di donne americane con anamnesi familiare di cancro mammario. Queste particelle contengono RNA ad alto peso molecolare (70S), e l'attività enzimatica della trascrittasi inversa: tutte caratteristiche dei Retrovirus.

Fu riferito che l'RNA presente nelle cellule di varie leucemie umane è un RNA 70S unito ad una trascrittasi inversa.

Nota : *carcinomi mammari nella specie umana.*

Non sappiamo se questi virus possano attecchire anche nelle mucche da latte e passare quindi alla specie umana.

Tutto ciò è comunque estremamente preoccupante alla luce dell'impiego odierno dei mangimi OGM, creati in laboratorio spesso con impiego di Retro-virus, mangimi che da circa 10 anni vengono dati alle mucche da latte, con rischio quindi, a lungo andare, di modificazioni transgeniche spontanee e quindi di possibili "epidemie" di tumori mammari nella specie umana.

Complesso D [Complesso della leucemia-sarcoma felino]:

Il virus della leucemia felina e del sarcoma felino sono stati isolati da *gatti domestici* affetti da leucemia e fibro-sarcoma.

Leucemia

Il virus della leucemia è un agente infettivo comune nelle popolazioni di gatti randagi. La maggior parte delle infezioni sono lievi e transitorie, e solo una piccola percentuale dei gatti presenta leucemie o linfomi nella tarda età. Il 70% dei gatti con leucemia liberano virus infettivo che viene trasmesso facilmente agli animali vicini. I gattini neonati sono i più sensibili allo sviluppo di una viremia persistente e dell'insorgenza di tumori.

Sarcoma

Anche il virus del sarcoma è spesso presente.

Può colpire anche altre specie, fra cui cani, conigli, e *scimmie*.

Nota 1: Questi tipi di Retrovirus producono particelle di tipo C.

Particelle simili agli Oncornavirus di tipo C sono state messe in evidenza con la microscopia elettronica in cellule o nel plasma di pazienti con tumori solidi dell'uomo, come il Linfoma di Hodgkin e NON-Hodgkin, e i Sarcomi.

Passaggio all'uomo: Non sappiamo se questi virus possano attecchire anche specie umana, essendo comunque dimostrato che il virus del sarcoma felino *naturale* (cioè non-OGM) colpisce anche i *primati*.

Tutto ciò è comunque estremamente preoccupante alla luce dell'impiego odierno di mangimi OGM per cani e gatti domestici, creati in laboratorio spesso con impiego di Retro-virus simili, mangimi che da circa 10 anni vengono venduti anche in Europa come cibo per cani e gatti domestici, con rischio quindi, a lungo andare, di possibili "epidemie" di leucemie e di sarcomi prima nei cani domestici e nei gatti randagi e/o domestici, e quindi con possibile successivo passaggio nella specie umana, data la presenza di questi animali domestici nelle case, ed essendo comunque dimostrato che il virus del sarcoma felino *naturale* (cioè non-OGM) colpisce anche i *primati*.

Complesso E [Oncornavirus dei primati]:

Il *virus del sarcoma della scimmia lanosa* (SSV-1) induce sarcomi nelle scimmie apaline neonate;

Il *virus della leucemia del gibbono* (GALV) provoca leucemia in questa specie.

Nota 1: Questi tipi di Retro-virus producono particelle di tipo C.

Particelle simili agli Oncorna-virus di tipo C sono state messe in evidenza con la microscopia elettronica in cellule o nel plasma di pazienti con tumori solidi dell'uomo, come il Linfoma di Hodgkin e NON-Hodgkin, e i sarcomi.

Nota 2: non è noto se Retro-virus simili sono stati impiegati per produrre piante OGM, o mangimi per animali da allevamento.

Altri Retrovirus

Virus Visna

E' un virus che determina demielinizzazione del Sistema Nervoso Centrale, con quadro clinico compatibile a quello della "*sindrome della mucca pazza*".

Il periodo d'incubazione varia da pochi mesi a molti anni. Colpisce le pecore dell'Islanda.

A causa delle notevoli somiglianze tra questo virus e i virus tumorali a RNA, è stato assegnato alla famiglia dei *Retroviridae*. Le somiglianze comprendono: montaggio e maturazione del virione per gemmazione, il diametro del virione (70-100 nm), la presenza di una DNA-polimerasi RNA-dipendente (trascrittasi inversa), di RNA 40S e 70S e di un corredo polipeptidico simile. Inoltre contiene proiezioni e aculei sulla membrana esterna, e le particelle colorate negativamente somigliano a quelle del virus del sarcoma di Rous.

Sono state anche notate strutture interne filamentose (particelle C) simili a quelle descritte per i Retrovirus del complesso aviario, murino e felino.

HIV

E' sospettato di essere l'agente causale dell'AIDS, ma attualmente il dibattito scientifico è ancora aperto. Di recente si è prospettato per l'AIDS un altro agente causale: l'SV40 (Virus tumorale a DNA).

Personalmente, si suggerisce quindi la ricerca,
in pazienti malati di tumore

della verifica di eventuale ibridazione tra RNA polisomiale

(di sospetta origine virale OGM, cioè da Oncorna-virus modificato per produrre piante OGM ad uso alimentare)

ottenuto da tumori umani di pazienti alimentatisi con cibo OGM, e DNA sintetizzato in laboratorio per *trascriptasi inversa* dagli stessi Oncorna-virus modificati per produrre OGM.

Nota: tutto ciò richiede però l'accesso ad informazioni riservate, forse coperte da brevetto, in merito ai modelli di retrovirus impiegati dalle multinazionali OGM, e alle modifiche apportate loro dalle stesse aziende prima della immissione in commercio delle stesse piante OGM.

Molto più difficile rintracciare virus tumorigeni a DNA impiegati dalle multinazionali OGM per modificare il DNA delle piante ad uso alimentare, poiché questi virus (Pox-virus, Herpes-virus, Papova-virus, Adeno-virus), a differenza degli Oncorna-virus, non sono rilevabili nel siero o nelle urine del paziente.

E' però dimostrato che nel citoplasma di cellule tumorali di mammifero infettate e modificate da questi virus a DNA permane una piccola frazione, altamente specifica, di RNA messaggero, che non si trova né in cellule normali, né in cellule tumorali infettate da altri tipi di virus oncogenici a DNA.



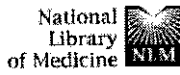
Si tratta quindi di verificare l'eventuale ibridazione tra questo RNA messaggero (di sospetta origine virale OGM, cioè da virus a DNA modificato per produrre piante OGM ad uso alimentare) ottenuto dal citoplasma di cellule tumorali di pazienti alimentatisi con cibo OGM, e DNA sintetizzato in laboratorio dagli stessi virus a DNA modificati per produrre OGM.

Anche qui si richiede però l'accesso ad informazioni riservate, forse coperte da brevetto, in merito ai modelli di virus a DNA impiegati dalle multinazionali OGM, e alle modifiche apportate loro dalle stesse aziende prima della immissione in commercio delle stesse piante OGM.

Un'ibridazione positiva, rivelata dalla formazione di DNA ibrido radioattivo (^{32}P) indica la presenza di sequenze di DNA virale nelle cellule trasformate (Green, Perspect Biol. Med., 1978).

QUINTO : intossicazione da veleni sintetizzati da piante transgeniche

Intossicazione cronica di cibi a causa di sostanze tossiche insetticide contenute nelle piante per renderle resistenti ai parassiti come il *Bacillus thuringiensis*, con conseguente possibile incremento di Cancro, Aborti spontanei, Mutazioni genetiche sulla discendenza, Sindromi da Immunodeficienze acquisite, malattie degenerative e da sostanze tossiche, etc....

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Bacillus thuringiensis subsp. konkukian (serotype H34) superinfection: case report and experimental evidence of pathogenicity in immunosuppressed mice.

Hernandez E, Ramisse F, Ducoureaux JP, Cruel T, Cavallo JD.

Laboratoire de Biologie, Hopital des Armees Begin, Saint Mande, France
haz.eric@mailexcite.com

We present a case of severe war wounds infected by *Bacillus thuringiensis* serotype H34 and describe the experimental protocol used to demonstrate ability to infect mice after cutaneous inoculation. This case is interesting because *B. thuringiensis* is considered to be a contaminant in laboratories receives inadequate attention.

PMID: 9650985 [PubMed - indexed for MEDLINE]

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Bt è patogeno (specie letale in animali immunosoppressi)

SESTO: *pericolo di carestie a livello mondiale a causa della tecnologia "TERMINATOR"*

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☐ 1: FEMS Immunol Med Microbiol. 1999 May;24(1):43-7. Related Articles, Full-Text Article

Bacillus thuringiensis serotype H34 isolated from human and insecticidal strains serotypes 3a3b and H14 can lead to death immunocompetent mice after pulmonary infection.

Hernandez E, Ramisse F, Cruel T, le Vagueresse R, Cavallo JD.

Laboratoire de Biologie, HIA Percy, Clamart, France.

In 1995, we isolated a strain of *Bacillus thuringiensis* serotype H34 from severe human tissue necrosis. This bacterium was able to induce myonecrosis in immunosuppressed mice after cutaneous infection. Its potential pathogenicity for immunocompetent hosts was investigated in a mouse model of pulmonary infection. Mice infected intranasally by a suspension containing 10(8) spores died within 8 h in a clinical toxic-shock syndrome. In the same conditions, infection with a mutant without crystalline toxin, with the supernatant from a culture containing 10(8) bacteria ml(-1) and by the insecticidal strain serotypes 3a3b or H14 led to identical results. Lower inocula simply induced a local inflammatory reaction with bacterial persistence observed during the course of 10 days.

PMID: 10340711 [PubMed - indexed for MEDLINE]

Passaggio a specie "indigene" naturali di grano, riso, mais, patate, legumi, della incapacità da parte delle piante stesse di riprodursi normalmente a causa della tecnologia "TERMINATOR", provocata da impollinazione incrociata, con perdita irreversibile anche per le piante naturali ad uso alimentare, oggi impiegate nell'alimentazione umana, poiché queste ultime saranno state inquinate dai geni transgenici provenienti dalle zone agricole a coltura transgenica (OGM) di tipo "TERMINATOR".

Di qui la potenziale minaccia di future carestie a livello globale, di tipo incontrollato, non essendo più disponibili nel mondo quantità sufficienti di grano, riso, mais, legumi, di tipo "naturale", o comunque NON-TERMINATOR.

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Spore

*pulmoniti
sindrome da
shock tossico*

BT è tossico

è un altro, di altro

in polmonare

SETTIMO: *modificazione transgenica di piante naturali*

Passaggio a specie “indigene” naturali delle sostanze tossiche artificiali, come ad esempio il “*Bacillus thuringiensis*” o di altro tipo, tramite impollinazione incrociata, con potenziale minaccia anche per le piante e le erbe mediche oggi impiegate in Fito-Terapia poiché queste ultime saranno inquinate dai geni transgenici provenienti dalle zone agricole a coltura transgenica (OGM).

OTTAVO : *scomparsa irreversibile del patrimonio genetico delle piante naturali*

Graduale ed irreversibile scomparsa delle diversità biologiche, cioè della normale flora naturale: fenomeno che si sta già evidenziando in U.S.A. a causa delle moderne pratiche di coltivazione che enfatizzano la monocultura transgenica (OGM) rispetto ai metodi di coltivazione differenziati.

Le coltivazioni transgeniche arrecheranno infatti una gravissima minaccia alle zone ricche di bio-diversità (genomi naturali): il flusso transgenico che andrà dalle piante modificate alle piante naturali sarà inevitabile quando il rapporto numerico fra aree coltivate con piante artificiali supererà le superfici delle piante naturali, determinando così la perdita irreversibile di gran parte del patrimonio genetico naturale di tutte le piante esistenti al mondo, attualmente pari a circa 442.000 specie già classificate, su un totale stimato di circa 600.000-800.000 specie.

In sostanza:

Numerose piante sono già scomparse nel corso di questi ultimi anni perché gli agricoltori hanno abbandonato le piante naturali, per adottare invece varietà di piante artificiali, cioè geneticamente modificate, poiché rese uniformi nel proprio genoma, ad alto rendimento di produzione (ma povere di vitamine), intrinsecamente malate (poiché incapaci di sopravvivere in assenza di pesticidi), rese sterili per ragioni di mercato, e infine manipolate geneticamente per essere rese resistenti agli insetti e ad altri animali poiché capaci di produrre esse stesse dei veleni, cioè delle sostanze tossiche che verranno infine mangiate dagli animali di allevamento e dall'uomo stesso.

Persino nelle foreste la varietà genetica è oggi minacciata dalle perdite di habitat, non solo da pratiche di deforestazione scorrette, ma persino dalla contaminazione del patrimonio genetico adattatosi a situazioni locali da parte di ibridi creati dalle grandi ditte sementiere produttrici degli OGM.

I prodotti transgenici rappresentano quindi, proprio per come sono concepiti, una formidabile spinta per accentuare le caratteristiche di unilateralità delle monoculture, e quindi di scomparsa del patrimonio genetico naturale esistente da centinaia di milioni di anni.

Non avremo quindi più, nel futuro più o meno prossimo, tutte quelle varietà di piante (alimentari e non) caratteristiche di ogni particolare regione nazionale o locale.

La contaminazione genetica ambientale indotta da parte di ibridi creati dalle grandi ditte sementiere degli OGM, che inevitabilmente s'incroceranno con le varietà presenti in natura, porterà ad una perdita del patrimonio genetico naturale (non recuperabile in alcun modo), di tutte quelle particolari caratteristiche che sono entrate nel genoma delle piante nel corso dei lunghi processi di adattamento alle varie situazioni ambientali. Tale perdita è oggi gravissima persino per gli ambienti naturali come le foreste.

Sostanzialmente, la base stessa della Biochimica umana è oggi minacciata nella sua più intima essenza (DNA umano) dall'impiego sconsiderato di queste piante artificiali, senza alcuna possibilità di recuperare un patrimonio genetico di oltre 440.000 specie di piante classificate (su un totale 600.000- 800.000 stimate), di cui una buona parte scompariranno nel giro di poche centinaia di anni, minate alla base dai danni genetici introdotti dall'Uomo.

Multinazionali agro-alimentari (Biotech, OGM)

Da alcuni anni si sta verificando la nascita di multinazionali che si definiscono "*multinazionali di scienze della vita*" attive sul mercato farmaceutico, dell'agro business (sementiero e pesticidi) e veterinario.

Sono settori tra loro diversi, ma che sono legati insieme dall'utilizzo delle biotecnologie (OGM) per la realizzazione dei loro prodotti.

Queste multinazionali stanno utilizzando delle strategie economiche molto spregiudicate ed aggressive: dai primi anni '90 stanno operando per acquistare aziende anche di grande dimensioni.

Una di queste, la *Monsanto*, ha acquisito nel termine di pochi anni *Asgrov*, *Agracetus*, *De Calb*, *Cargil*, con un investimento di 10 miliardi di Euro attuali.

La *Dupont*, altro grande gruppo, ha acquistato la *Pioneer* con un investimento di circa 8 miliardi di Euro attuali.

Questi investimenti sembrano avere una logica anti-economica: esse pagano le aziende che rilevano molto più del loro reale valore, come se cercassero di eliminare un potenziale concorrente piuttosto che ottenere un risultato economico a breve termine.

Accanto alle acquisizioni abbiamo anche le fusioni:

Ciba Geigy e *Sandoz* creano *Novartis* (fatturato di 20 miliardi di Euro attuali nel 1997-98).

Dalla fusione della francese *Rhone Poulenc* e della tedesca *Hoechst* nasce *Aventis*.

È sempre in questo contesto che nasce, nell'ottobre 2000, il primo gruppo mondiale di agrochimica, *Syngenta*, - risultato della fusione della svizzera *Novartis* (Azienda ben nota come produttrice di farmaci per Chemioterapia) con l'anglo-svedese *Astra-Zeneca* (anch'essa azienda ben nota come produttrice di farmaci per Chemioterapia) che realizzerà un giro d'affari di circa otto miliardi di euro.

Monsanto, dopo la fusione con *Pharmacia & Upjohn*, una grande ditta farmaceutica (anch'essa azienda ben nota come produttrice di farmaci per Chemioterapia), si occupa ormai solo di agricoltura, con un giro d'affari che nel 2000 ha raggiunto i 5,5 miliardi di dollari.

La situazione attuale è la seguente: pochissime multinazionali (*Syngenta*, *Monsanto*, *Novartis*, *Dupont*, *Aventis*) detengono il 25-30% del mercato sementiero (ma oltre il 90% del mercato delle sementi transgeniche) e dietro questi grandi gruppi si nota una tale polverizzazione da indurre a pensare che questo andamento non potrà che rafforzarsi in futuro non potendo delle aziende di medie dimensioni contrastare la concorrenza di grandi gruppi economici, e l'obiettivo sembra chiaro: riconvertire il settore sementiero tradizionale in biotecnologico (cioè OGM).

Ma il dato impressionante è che ritroviamo gli stessi nomi nel settore farmaceutico, dove le *stesse* multinazionali hanno una posizione dominante.

Multinazionali chimico-farmaceutiche (Big-Farma)

La storia delle multinazionali chimico-farmaceutiche è incredibile per il loro sviluppo vertiginoso, oggi saldatosi in maniera estremamente pericolosa con il mondo agro-alimentare:

L'industria chimico-farmaceutica nacque in Europa nella seconda metà dell'Ottocento: in molti casi si trattava dell'industria dei coloranti che, staccatasi dalla chimica di base si indirizzava verso quei nuovi e più promettenti settori della Chimica specializzata in settori chiave dell'economia.

Negli anni precedenti la Seconda Guerra Mondiale, si formò un cartello internazionale dei farmaci, con sede in Germania, che dominava le industrie chimiche e farmaceutiche di tutto il mondo. Esso aveva diffuso le sue attività in 93 paesi, in ognuno dei quali rappresentava una potente forza economica e politica.

Era conosciuta come IG. Farben..

Essa sarebbe divenuta il pilastro di sostegno della produzione chimica di Hitler durante gli anni della guerra, fornendo prodotti che comprendevano potenti esplosivi, gas tossici e l'ignominioso *Zyklon-B*, la sostanza mortale usata dai nazisti nei campi di sterminio.

Tuttavia, prima della guerra, nel 1928, l'industriale monopolista americano John D. Rockefeller aveva stabilito una concentrazione industriale tra il suo impero internazionale con sede in America e la IG Farben, dando così origine al più grande e più potente cartello farmaceutico che il mondo avesse mai conosciuto.

Il Tribunale militare di Norimberga nel 1946/47 stabilì che la Seconda Guerra Mondiale non sarebbe stata possibile senza questo cartello petrolchimico chiamato *I.G. Farben*.

In conseguenza della sentenza emessa dal tribunale, la *I.G. Farben* fu divisa in *Bayer*, *BASF* e *Hoechst* e alcuni dei suoi dirigenti furono condannati per aver iniziato una guerra contraria al diritto internazionale, genocidio, sfruttamento e saccheggio di proprietà pubblica e privata in paesi stranieri e altri crimini contro l'umanità.

La storia degli antefatti aziendali dietro la seconda guerra mondiale è documentata da un libro di Joseph Borkin "*The Crime and Punishment of IG Farben*" (*Delitto e castigo della I.G. Farben*).

Dopo la guerra, la Germania, con i suoi tre giganti *Bayer*, *Hoechst*, *BASF* ebbe comunque un ruolo importante assieme anche alla Svizzera che, a Basilea, vide nascere e svilupparsi *Ciba*, *Sandoz*, *Roche*: tutte aziende che si sono poi affermate nel mondo.

Ma è negli anni Novanta che sono cominciate le grandi fusioni: nel Regno Unito, nel 1989 due grosse aziende farmaceutiche si fondono nella *Smith Kline-Beecham*: in seguito si fonderanno anche con la *American Home* (circa 25 miliardi di Euro di fatturato annuale).

Nel 1993 la svedese *Pharmacia* compra l'italiana *Farmitalia-Carlo Erba*, poi si fonde con l'americana *Upjohn* nel 1995, e poi ancora con la *Monsanto*, prima di venir comprata dalla *Pfizer*, che in precedenza aveva acquistato l'americana *Parke Davis*.

Nel 1995 avviene la fusione *Glaxo- Wellcome* (circa 14 miliardi di Euro di fatturato annuale).

Nel 1998 la *Smith Kline-Beecham* (circa 62 miliardi di Euro di fatturato annuale) si fonde con *Glaxo-Wellcome*, per un capitale risultante di oltre 90 miliardi di Euro di fatturato annuale.

Nel frattempo, l'inglese *Imperial Chemical Industries* si è fusa con la svedese *Astra*, dando origine alla *Astra-Zeneca*.

Le fusioni sono continuate ad avvenire tra le stesse aziende farmaceutiche presenti sullo stesso tipo di mercato: *Sandoz* e *Ciba Geigy* (*Novartis*, 1996), *Astra- Zeneca* (1998).

Questi colossi non nascono dall'esigenza dei pazienti, ma dall'esigenza di creare monopolio e quindi profitti sempre maggiori.

Ultimi dati :

Giugno 2002 : acquisto della *Aventis* da parte della *Bayer*; l'accordo ha così permesso alla *Bayer* di fare il proprio ingresso nel campo delle sementi OGM.

Giugno 2005: acquisto della *Sementis* da parte della *Monsanto*.

Il Connubio

Si può pertanto affermare che i due cardini dell'economia e della vita di ciascun individuo, l'agricoltura e la farmaceutica, sono controllate in una situazione di sostanziale oligopolio da pochissimi gruppi multinazionali.

Grazie per l'attenzione

CONCLUSIONI:

Siamo di fronte al bivio fra l'accettazione delle modifiche biochimiche delle piante,

con danni immensi alla salute dell'umanità,

oppure la ferma presa di posizione delle Istituzioni democratiche della nostra società

contro le Multinazionali OGM e Chemio-farmaceutiche

che,

nel loro connubio,

stanno dietro all'invasione irresponsabile del mondo tramite OGM.

La soluzione è semplice:

1) divieto assoluto di permettere la coltivazione di piante OGM

2) rivalutazione dell'Agricoltura Biologica

3) Difesa della bio-diversità, in particolare con ripristino della libertà di scambio dei semi contadini

La possibile soluzione: il Ritorno dell'AGRICOLTURA BIOLOGICA

Se si avvierà un ritorno all'Agricoltura Biologica su larga scala, si potrà pensare ad una distribuzione capillare dei prodotti orto-frutticoli, fondata sulla fiducia intercorrente fra produttori di Frutta e Verdura, gli esercenti dei negozi, piccoli o grandi, comprese le grandi catene di supermarket, e gli acquirenti abituali, che dovrebbe essere la migliore garanzia in fatto di prodotti "biologici", al di là di certificazioni più o meno valide sulla bontà del prodotto inteso come "biologico".

Ciò potrebbe riaprire il mercato ad una sana e consapevole competizione concorrenziale fra piccole e grandi aziende italiane, interessate alla ri-valorizzazione dei terreni agricoli ancora sottoposti a tecniche di sfruttamento massivo del suolo che non possono più essere considerate "moderne" nel senso scientifico delle attuali conoscenze di biochimica umana e di biologia ambientale della flora e della fauna.

E' necessario quindi conservare i semi della nostra tradizione agricola, tramandati per migliaia di anni nelle nostre campagne.

Ma se le campagne italiane si spopolano, se le piccole aziende agricole familiari segnano il passo e cedono il posto a poche grandi aziende che coltivano in regime di mono-cultura (leggi: OGM), se l'unico sbocco di mercato è quello della grande distribuzione organizzata, allora non ci sarà speranza per la bio-diversità dell'agricoltura biologica italiana, perché è stata proprio la grande distribuzione dei prodotti alimentari la principale causa della sua scomparsa.

Affinché la biodiversità possa ritornare, affinché le antiche varietà di frutta, verdura, ortaggi, cereali, legumi possano nuovamente essere coltivate, è necessario creare le basi un nuovo *Rinascimento Italiano* della cultura contadina pluri-millenaria della nostra antica terra.

Questa nuova base potrà dare un aiuto economico immenso all'Agricoltura Biologica attraverso la vendita diretta, senza intermediazioni alcune, dei prodotti delle fattorie, provenienti direttamente dalle mani dell'agricoltore alle mani del paziente e dei suoi familiari.

Dovranno essere costruiti piccoli mercati coperti nei paesi del Sud-Italia, dove la LEGGE potrà verificare il rispetto di un giusto prezzo per i prodotti biologici, che potranno essere così decisi nel rispetto dei prodotti simili venduti in altre località vicine, evitando speculazioni, ma venduti sempre al disopra di un certo costo, allo scopo di incentivare l'agricoltore a proseguire con la produzione del biologico, perché questo significherà il rispetto di un "giusto prezzo" per l'agricoltore.

Questo modello che rappresenta l'immediato futuro, per molte aziende è già presente, e genera una serie di effetti positivi sull'economia delle campagne.

Sarà quindi importante ricollegare le persone della campagna alle persone delle città, riscoprendo e rivalorizzando servizi di elenchi gratuiti di aziende agricole del biologico, capaci di praticare anche la vendita diretta dei loro prodotti, cioè del "cibo locale", o per meglio dire, di una "mappa del cibo locale".

SALVIAMO I SEMI CONTADINI :

PETIZIONE PER LA SALVAGUARDIA DELLA BIODIVERSITÀ NATURALE

"Civiltà Contadina" (www.biodiversita.info) si è fatta promotrice in Italia di PETIZIONE PER LA SALVAGUARDIA DELLA BIODIVERSITÀ NATURALE "SALVIAMO I SEMI CONTADINI", per il diritto ai contadini italiani di potersi scambiare i semi e le piante, cosa che attualmente è considerata ILLEGALE.

"Civiltà Contadina" sta raccogliendo firme virtuali, e reali, per sostenere la creazione delle condizioni per rendere legale lo scambio di semi contadini, antichi e del territorio.

Il decreto del Presidente della Repubblica no. 322 del 9 maggio 2001 rende in effetti impossibile ogni cessione o movimento di semi non registrati; mentre il trattato UPOV91 intacca il diritto di risemina dell'agricoltore, ovvero il privilegio che l'azienda agricola ha di riseminare tracciando seme da una parte dei propri raccolti.

D'altra parte, con l'introduzione in coltivazione delle piante OGM si apre il rischio dell'impollinazione spontanea da parte di queste piante artificiali sulle varietà contadine che, a quel punto, ibridandosi con le OGM che sono brevettate, diventerebbero automaticamente di proprietà della ditta sementiera che detiene il brevetto e quindi i loro semi non potrebbero essere più riseminati.

Intanto, le piante di pubblico dominio, cioè quelle che sono frutto di selezioni fatte più di trentacinque anni fa e che non pagano royalties a nessuno, perché sono patrimonio collettivo in quanto "antiche varietà", vanno gradualmente a perdersi, cancellate dai registri europei e sono destinate alla probabile estinzione, e a essere completamente sostituite da "IBRIDI F1" i cui semi non si possono riseminare se non penalizzando fortemente la possibilità di raccolto.

Oggi, oltre il 90% delle sementi delle varietà commerciali di cetrioli, cocomeri, pomodori, melanzane, zucchine, meloni e peperoni sono IBRIDI, e meno del 3% sono le varietà più vecchie di trentacinque anni.

In alcune nazioni europee si è riconosciuta l'esistenza e la possibilità di vendita di alcune varietà storiche, recependo una parte della direttiva CEE su cui si fonda il DPR 322/2001, tuttavia è stata proibita la vendita dei prodotti di quelle varietà.

Inoltre si è chiesto una tassa annua di registrazione che penalizza i piccoli produttori e distributori di sementi.

In Italia non è stata fatta neppure questa applicazione, esponendo il nostro ricco patrimonio storico varietale di semi alla biopirateria OGM e alla copiatura per IBRIDI.

Le varietà moderne, sia ortive sia agrarie, sono commercializzate con l'unico scopo di favorire un'agricoltura industriale e la grande distribuzione organizzata.

Gli ortaggi devono essere capaci di superare raccolte meccaniche, imballaggi meccanizzati, lunghi viaggi refrigerati. Devono avere la maturazione uniforme per favorire la raccolta simultanea, dipendono dalla chimica sia per le concimazioni sia per i trattamenti fito-sanitari.

Devono avere un bell'aspetto, ma il sapore, ovviamente, non c'è più (perdita delle migliaia di vitamine naturali contenute).

Purtroppo, questo "progresso" di varietà di piante sempre più tecnologiche sembra inarrestabile. Perché autorizzare OGM e piante IBRIDE e invece proibire, ostacolare in tutti i modi la libera circolazione dei semi non registrati ?

Dobbiamo forse ritenere che vi sia la volontà di eliminare ogni possibile alternativa all'industria sementiera dell'Agro-bunissess ?

Un ritorno alla biodiversità rurale nei campi è invece auspicabile, non solo per un recupero di sapori e aromi di cui le piante moderne sono povere (non hanno più vitamine), ma anche di colori e forme che rendono piacevole mangiare, e per favorire il movimento del cibo locale, ovvero della vendita diretta di prodotti di fattoria.

L'assurdo è invece che anche un semplice seme di pomodoro, come anche di insalata o di qualsiasi altro ortaggio comune, tradizionale e contadino, solo perché non registrato, diventa un seme proibito.

L'iscrizione nei registri di una varietà è una pratica amministrativa lunga e costosa, inaccessibile agli agricoltori, una via impraticabile per le varietà contadine.

E' quindi urgente togliere queste regolamentazioni, e lasciare piena libertà di scambio e di diffusione gratuita delle varietà storiche italiane.

Quindi:

Per preservare la biodiversità rurale
 Per una agricoltura ricca e variegata
 Per il diritto all'alimentazione libera e sana
 Per riconoscere il valore della nostra civiltà contadina

Si chiede:

L'applicazione della direttiva CEE (98/95) finora disattesa dai governi e la creazione di una lista nazionale che raccolga le varietà locali o dei territori o contadine

L'iscrizione libera e gratuita su queste liste per le varietà di coloro che conservano, selezionano e diffondono queste biodiversità

Che i criteri d'iscrizione siano adattati alle particolarità di queste varietà locali, spesso non uniformi o stabili come quelle selezionate.

Uno spazio di libertà totale per scambi liberi di piante e sementi contadine (in quantità corrispondenti ai bisogni di una piccola fattoria), nel rispetto delle precauzioni fito-sanitarie essenziali.

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