

LE Magazine February 2003

ABSTRACTS

Eye Health

Use of vitamin supplements and cataract: the Blue Mountains Eye Study.

PURPOSE: To investigate relationships between use of vitamin supplements and the three principal cataract types in a population-based sample. **METHODS:** We studied 2873 of the 3654 participants (79%) aged 49 to 97 years attending the cross-sectional Blue Mountains Eye Study who completed a detailed food frequency questionnaire, which included type, dose and duration of vitamin supplement use. Masked grading of nuclear, cortical and posterior subcapsular opacities from lens photographs was performed, using the Wisconsin method. **RESULTS:** Use of multivitamin supplements was associated with reduced prevalence of nuclear cataract, odds ratio 0.6, 95% confidence interval 0.4 to 1.0, $P = .05$. For both nuclear and cortical cataract, longer duration of multivitamin use was associated with reduced cataract prevalence (nuclear cataract, trend $P = .02$; cortical cataract, trend $P = .03$). Use of thiamin supplements was associated with reduced prevalence of nuclear (odds ratio 0.6, confidence interval 0.4 to 1.0, $P = .03$, dose trend $P = .03$) and cortical cataract (odds ratio 0.7, confidence interval 0.5 to 0.9, $P = .01$, dose trend $P = .02$). Riboflavin (odds ratio 0.8, confidence interval 0.6 to 1.0, $P = .05$) and niacin (odds ratio 0.7, confidence interval 0.6 to 1.0, $P = .04$) supplements exerted a weaker protective influence on cortical cataract. Vitamin A supplements were protective against nuclear cataract (odds ratio 0.4, confidence interval 0.2 to 0.8, $P = .01$, dose trend $P = .01$). Folate (odds ratio 0.4, confidence interval 0.2 to 0.9, $P = .03$) appeared protective for nuclear cataract, whereas both folate (odds ratio 0.6, confidence interval 0.3 to 0.9, $P = .01$, dose trend $P = .04$) and vitamin B12 supplements (odds ratio 0.7, confidence interval 0.5 to 1.0, $P = .03$, dose trend $P = .02$) were strongly protective against cortical cataract. **CONCLUSIONS:** Long-term use of multivitamins, B group and vitamin A supplements was associated with reduced prevalence of either nuclear or cortical cataract. A strong protective influence on cortical cataract, from use of folate or vitamin B12 supplements, is a new finding.

Am J Ophthalmol 2001 Jul;132(1):19-26

Serum status of carotenoids and tocopherols in patients with age-related cataracts: a case-control study.

BACKGROUND: Cataract is an important health problem that increase with age, causes decreased visual acuity and constitute a major cause of disability in the elderly. Epidemiological studies have shown that elevated serum levels and/or intake of several antioxidants, such as carotenoids, vitamin E and ascorbic acid, are associated with a diminished risk for cataracts. **OBJECTIVE:** To assess the serum fat-soluble antioxidant status in patients with cataracts and its relationship with visual function. **METHODS:** One hundred thirty eight patients with senile cataracts, classified according to visual acuity, and 110 age and sex-matched controls were studied for individual carotenoids and tocopherols in serum by a quality-controlled HPLC method. One-way ANOVA analysis and logistic regression analysis were applied. **RESULTS:** Higher serum levels of lutein and zeaxanthin were associated as risk factors for cataract while b-cryptoxanthin and g-tocopherol appeared as protective variables. Higher levels of zeaxanthin and lower concentrations of b-cryptoxanthin were associated with cataracts in people < 61y whereas only lower levels of g-tocopherol were shown in subjects > 61y. No significant correlations (adjusted for sex and age) were found between visual acuity and serum concentrations of carotenoids or tocopherols. **CONCLUSION:** Although the relation between carotenoids and cataracts is biologically plausible, serum carotenoid levels are highly dependent on dietary intake and thus may not be clinically relevant biomarkers for cataracts risk.

J Nutr Health Aging 2002;6(1):66-8

Antioxidant systems in rat lens as a function of age: effect of chronic administration of vitamin E and ascorbate.

Oxidative damage occurring in the lenses of patients with senile cataract may be due to partially reduced forms of oxygen. We assayed the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Red), and glucose-6-phosphate dehydrogenase (G6PD) in rat lenses at different ages (1, 4 and 24 months), and also evaluated lens glutathione (GSH) levels and the effects of chronic administration of vitamin E and sodium ascorbate. We observed a significant age-related decrease in GSH-Px, GSH-Red and G6PD activities, but no age-related change in SOD activity. Chronic treatment with both vitamin E and sodium ascorbate failed to restore enzymatic activities to the levels of younger rats. An age-related reduction in GSH content was also observed; however, chronic administration of vitamin E, but not of sodium ascorbate, restored GSH levels to those of younger rats.

Experimental evidence for interactive effects of chronic UV irradiation and nutritional deficiencies in the lens.

The eye lens is subjected to many risk factors over time, which contribute to changes in its transparency, finally leading in combination to cataract development. Ultra violet (UV) radiation is regarded as one of the widespread risk factors contributing to cataract formation, for example in combination with nutritional deficiencies. Both factors possibly contribute to the high number of cataracts in the sunbelt region of the world. In this study, two essential nutritional factors were investigated in Brown Norway rats, zinc and vitamin E deficiencies, alone and in combination with UV-A and UV-B irradiation. Young female Brown Norway rats were put on a special diet for 10 weeks, either highly deficient in zinc or in vitamin E. The diet was otherwise identical to the control diet. Two weeks after putting the animals on the diet, UV irradiation was started in some of the groups with mydriatic pupils with three irradiation sessions per week (UV-A 1 J/cm²; UV-B 0.2 J/cm²). Irradiation was continued until the end of the diet treatment period. Body weight and food consumption were established at weekly intervals, as well as slitlamp microscopy to monitor changes in anterior eye segment morphology. In addition changes in transparency of the cornea and lens have been monitored and evaluated with a Scheimpflug camera (Topcon SL-45) at baseline, and after four and eight weeks of irradiation. After sacrifice of the animals, the lens wet weight as well as the activity of superoxide dismutase (SOD) were determined. Zinc deficiency alone led to an almost complete arrest of body weight increase. In the cornea, UV-A in combination with zinc or vitamin E deficiency did not have any interactive effects. The combination of UV-B and zinc deficiency showed subtractive instead of additive effects on corneal transparency and neovascularization. In the lens both deficiencies positively interacted with UV-A and UV-B by increasing the density of the capsular and cortical layers. The lens fresh weight was significantly lower in zinc-deficient animals additionally irradiated with UV-A or UV-B. The activity of SOD was significantly lower in the lenses of zinc- or vitamin E-deficient animals additionally irradiated with UV-B. The experiments presented clearly demonstrate that dietary zinc and vitamin E deficiencies do interact with UV radiation damage in the cornea and lens of Brown Norway rats.

Dev Ophthalmol 2002;35:113-24

CoQ10/Parkinson's

Ubiquinone (coenzyme q10) and mitochondria in oxidative stress of Parkinson's disease.

Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease affecting approximately 1% of the population older than 50 years. There is a worldwide increase in disease prevalence due to the increasing age of human populations. A definitive neuropathological diagnosis of Parkinson's disease requires loss of dopaminergic neurons in the substantia nigra and related brain stem nuclei, and the presence of Lewy bodies in remaining nerve cells. The contribution of genetic factors to the pathogenesis of Parkinson's disease is increasingly being recognized. A point mutation which is sufficient to cause a rare autosomal dominant form of the disorder has been recently identified in the alpha-synuclein gene on chromosome 4 in the much more common sporadic, or 'idiopathic' form of Parkinson's disease, and a defect of complex I of the mitochondrial respiratory chain was confirmed at the biochemical level. Disease specificity of this defect has been demonstrated for the parkinsonian substantia nigra. These findings and the observation that the neurotoxin 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine (MPTP), which causes a Parkinson-like syndrome in humans, acts via inhibition of complex I have triggered research interest in the mitochondrial genetics of Parkinson's disease. Oxidative phosphorylation consists of five protein-lipid enzyme complexes located in the mitochondrial inner membrane that contain flavins (FMN, FAD), quinoid compounds (coenzyme Q10, CoQ10) and transition metal compounds (iron-sulfur clusters, hemes, protein-bound copper). These enzymes are designated complex I (NADH:ubiquinone oxidoreductase, EC 1.6.5.3), complex II (succinate:ubiquinone oxidoreductase, EC 1.3.5.1), complex III (ubiquinol:ferrocytochrome c oxidoreductase, EC 1.10.2.2), complex IV (ferrocytochrome c: oxygen oxidoreductase or cytochrome c oxidase, EC 1.9.3.1), and complex V (ATP synthase, EC 3.6.1.34). A defect in mitochondrial oxidative phosphorylation, in terms of a reduction in the activity of NADH CoQ reductase (complex I) has been reported in the striatum of patients with Parkinson's disease. The reduction in the activity of complex I is found in the substantia nigra, but not in other areas of the brain, such as globus pallidus or cerebral cortex. Therefore, the specificity of mitochondrial impairment may play a role in the degeneration of nigrostriatal dopaminergic neurons. This view is supported by the fact that MPTP generating 1-methyl-4-phenylpyridine (MPP(+)) destroys dopaminergic neurons in the substantia nigra. Although the serum levels of CoQ10 is normal in patients with Parkinson's disease, CoQ10 is able to attenuate the MPTP-induced loss of striatal dopaminergic neurons.

Biol Signals Recept 2001 May-Aug;10(3-4):224-53

Age-related changes in the lipid compositions of rat and human tissues.

The levels of cholesterol, ubiquinone, dolichol, dolichyl-P, and total phospholipids in human lung, heart, spleen, liver, kidney, pancreas and adrenal from individuals from one-day-old to 81 years of age were investigated and compared with the corresponding organs from two to 300 day-old rats. The amount of cholesterol in human tissues did not change significantly during aging, but the level of this lipid in the rat was moderately elevated in the organs of the oldest animals. In human pancreas and adrenal the ubiquinone content was highest at one year of age, whereas in other organs the corresponding peak value was at 20 years of age,

and was followed by a continuous decrease upon further aging. A similar pattern was observed in the rats, with the highest concentration of ubiquinone being observed at 30 days of age. Dolichol levels in human tissues increase with aging, but they increase to very different extents. In the lungs this increase is seven-fold, and in the pancreas it is 150-fold. The elevation in the dolichol contents of rat tissues ranges from 20 to 30-fold in our material. In contrast, the levels of the phosphorylated derivative of dolichol increased to a more limited extent, i.e., two to six-fold in human tissues and even less in the rat. These results demonstrate that the levels of a number of lipids in human and rat organs are modified in a characteristic manner during the life span. This is in contrast to phospholipids, which constitute the bulk of the cellular lipid mass.

Lipids 1989 Jul;24(7):579-84

Age-related decline in dopamine transporters: analysis of striatal subregions, nonlinear effects and hemispheric asymmetries.

Neuroimaging studies have documented an age-related decline in striatal dopamine transporters (DATs) as a marker of dopaminergic neurodegeneration. The authors further elucidated the effects on this neural system in healthy aging, in contrast to Parkinson disease (PD). The effects of age on striatal DAT availability were examined in a large, healthy subject sample (N=126) with [¹²³I]2beta-carbomethoxy-3beta-(4-iodophenyl)tropane ([¹²³I]beta-CIT) and single photon emission computed tomography (SPECT). Striatal DAT availability (V₃') showed a significant inverse correlation with age, declining in a nearly linear manner by 46% over the age range 18 to 88 years, or 6.6% per decade. Rates of decline were comparable for caudate (48%) and putamen (45%), with only minimal increase in left-right asymmetry with age. Hemispheric asymmetries were unrelated to the handedness of subjects. These results demonstrate that aging is associated with a relatively symmetric loss of DATs in the caudate and putamen in both hemispheres. These findings have implications not only for healthy aging but also for neurodegenerative disorders such as PD.

Am J Geriatr Psychiatry 2002 Jan-Feb;10(1):36-43

Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease.

Parkinson's disease is a widespread condition caused by the loss of midbrain neurons that synthesize the neurotransmitter dopamine. Cells derived from the fetal midbrain can modify the course of the disease, but they are an inadequate source of dopamine-synthesizing neurons because their ability to generate these neurons is unstable. In contrast, embryonic stem (ES) cells proliferate extensively and can generate dopamine neurons. If ES cells are to become the basis for cell therapies, we must develop methods of enriching for the cell of interest and demonstrate that these cells show functions that will assist in treating the disease. Here we show that a highly enriched population of midbrain neural stem cells can be derived from mouse ES cells. The dopamine neurons generated by these stem cells show electrophysiological and behavioural properties expected of neurons from the midbrain. Our results encourage the use of ES cells in cell-replacement therapy for Parkinson's disease.

Nature 2002 Jul 4;418(6893):50-6

Results of chronic subthalamic nucleus stimulation for Parkinson's disease: a 1-year follow-up study.

BACKGROUND: Deep brain stimulation (DBS) has been established as an alternative approach for the treatment of advanced Parkinson's disease (PD). Recently, the subthalamic nucleus (STN) has been identified as the optimal target for DBS. **METHODS:** Thirty-eight patients have undergone surgery for advanced PD since 1996. They include 12 females and 26 males with a mean age of 55.6 years. The mean stage on the Hoehn and Yahr Scale was 3.5 (off condition). Electrodes (Medtronic DBS 31389) were stereotactically implanted into the STN bilaterally. Targeting was performed using computerized tomography (CT) scans and ventriculography (VG). After four days of external stimulation, permanent neurostimulators were implanted. Patients were evaluated preoperatively and 1, 6 and 12 months postoperatively. Evaluations were performed in defined on and off states using the Unified Parkinson's Disease Rating Scale (UPDRS) as well as the Hoehn and Yahr Scale, the dyskinesia scale, and the Activities of Daily Living (ADL) Scale. **RESULTS:** Significant improvement of all motor symptoms was found in all patients (UPDRS motor score 32/48 preoperatively versus 15/30 at 12-month follow-up, $p < 0.001$). Daily off-times were reduced by 35%. Dyskinesias also improved markedly (UPDRS IV: 3.2/3.1 [on/off] vs. 0.9/1.3 at 12 months follow-up). Postoperative L-dopa medication was adjusted (mean reduction: 53%). Complications occurred in two patients (5%) who developed infections, leading to system removal. Systems were replaced after six months. Two patients (5%) had a permanent worsening of a previously known depressive state and developed progressive dementia. **CONCLUSIONS:** TN stimulation is a relatively safe procedure for treating advanced PD. The possibility of readjusting the stimulation parameters postoperatively improves the therapeutic outcome and reduces side effects in comparison to ablative methods.

Surg Neurol 2002 May;57(5):306-11; discussion 311-3

Novel physical treatments for the management of neuropsychiatric disorders.

OBJECTIVE: To briefly describe the novel non-drug physical interventions currently in use in the investigation and treatment of neuropsychiatric disorders regarding their efficacy and potential future applications. **METHODS:** A systematic review of the literature concerning transcranial magnetic stimulation (TMS), deep brain stimulation (DBS), vagus nerve stimulation (VNS) and neurosurgery for mental disorders (NMD) was conducted using Medline and literature known to the authors. **RESULTS:** A summary of each procedure is provided giving a succinct overview of efficacy, current applications and possible future indications. **CONCLUSION:** Novel and innovative physical interventions are currently being used to study brain function in health and disease. In particular, TMS has quickly established itself as a useful investigational tool and is emerging as a possible antidepressant therapy. Similarly, VNS has been applied successfully in the management of intractable epilepsy and is undergoing evaluation in the management of patients with treatment-resistant depression. DBS has shown significant promise in the treatment of Parkinson's disease and may have use in the management of obsessive-compulsive disorder. Finally, neurosurgical procedures for the treatment of mental disorders have been sufficiently refined to stage a comeback, although rigorous scientific study of their efficacy and indications is still necessary.

J Psychosom Res 2002 Aug;53(2):709-19

Subthalamic GAD gene therapy in a Parkinson's disease rat model.

The motor abnormalities of Parkinson's disease (PD) are caused by alterations in basal ganglia network activity, including disinhibition of the subthalamic nucleus (STN), and excessive activity of the major output nuclei. Using adeno-associated viral vector-mediated somatic cell gene transfer, we expressed glutamic acid decarboxylase (GAD), the enzyme that catalyzes synthesis of the neurotransmitter GABA, in excitatory glutamatergic neurons of the STN in rats. The transduced neurons, when driven by electrical stimulation, produced mixed inhibitory responses associated with GABA release. This phenotypic shift resulted in strong neuroprotection of nigral dopamine neurons and rescue of the parkinsonian behavioral phenotype. This strategy suggests that there is plasticity between excitatory and inhibitory neurotransmission in the mammalian brain that could be exploited for therapeutic benefit.

Science 2002 Oct 11;298(5592):425-9

Distribution of coenzyme Q homologues in brain.

Ubiquinone (coenzyme Q10), in addition to its function as an electron and proton carrier in mitochondrial electron transport coupled to ATP synthesis, acts in its reduced form (ubiquinol) as an antioxidant, inhibiting lipid peroxidation in biological membranes and protecting mitochondrial inner-membrane proteins and DNA against oxidative damage accompanying lipid peroxidation. Tissue ubiquinone levels are subject to regulation by physiological factors that are related to the oxidative activity of the organism: they increase under the influence of oxidative stress, e.g. physical exercise, cold adaptation, thyroid hormone treatment, and decrease during aging. In the present study, coenzyme Q homologues were separated and quantified in the brains of mice, rats, rabbits and chickens using high-performance liquid chromatography. In addition, the coenzyme Q homologues were measured in cells such as NG-108, PC-12, rat fetal brain cells and human SHSY-5Y and monocytes. In general, Q1 content was the lowest among the coenzyme homologues quantified in the brain. Q9 was not detectable in the brains of chickens and rabbits, but was present in the brains of rats and mice. Q9 was also not detected in human cell lines SHSY-5Y and monocytes. Q10 was detected in the brains of mice, rats, rabbits, and chickens and in cell lines. Since both coenzyme Q and vitamin E are antioxidants, and coenzyme Q recycles vitamins E and C, vitamin E was also quantified in mice brain using HPLC-electrochemical detector (ECD). The quantity of vitamin E was lowest in the substantia nigra compared with the other brain regions. This finding is crucial in elucidating ubiquinone function in bioenergetics; in preventing free radical generation, lipid peroxidation, and apoptosis in the brain; and as a potential compound in treating various neurodegenerative disorders.

Neurochem Res 2002 May;27(5):359-68

Effects of coenzyme Q10 in early Parkinson disease: evidence of slowing of the functional decline.

BACKGROUND: Parkinson disease (PD) is a degenerative neurological disorder for which no treatment has been shown to slow the progression. **OBJECTIVE:** To determine whether a range of dosages of coenzyme Q10 is safe and well tolerated and could slow the functional decline in PD. **DESIGN:** Multicenter, randomized, parallel-group, placebo-controlled, double-blind, dosage-ranging trial. **SETTING:** Academic movement disorders clinics. **PATIENTS:** Eighty subjects with early PD who did not require treatment for their disability. **INTERVENTIONS:** Random assignment to placebo or coenzyme Q10 at dosages of 300, 600, or 1200 mg/d. **MAIN OUTCOME MEASURE:** The subjects underwent evaluation with the Unified Parkinson Disease Rating Scale (UPDRS) at the screening, baseline, and 1-, 4-, 8-, 12- and 16-month visits. They were followed up for 16 months or until disability requiring treatment with levodopa had developed. The primary response variable was the change in the total score on the UPDRS from baseline to the last visit. **RESULTS:** The adjusted mean total UPDRS changes were +11.99 for the placebo group, +8.81 for the 300-mg/d group, +10.82 for the 600-mg/d group, and +6.69 for the 1200-mg/d group. The P value for the primary analysis, a test for a linear trend between the dosage and the mean change in the total UPDRS score, was .09, which met our prespecified criteria for a positive trend for the trial. A prespecified, secondary analysis was the comparison of each treatment group with the placebo group,

and the difference between the 1200-mg/d and placebo groups was significant ($P = .04$). CONCLUSIONS: Coenzyme Q10 was safe and well tolerated at dosages of up to 1200 mg/d. Less disability developed in subjects assigned to coenzyme Q10 than in those assigned to placebo, and the benefit was greatest in subjects receiving the highest dosage. Coenzyme Q10 appears to slow the progressive deterioration of function in PD, but these results need to be confirmed in a larger study.

Arch Neurol 2002 Oct;59(10):1541-50

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ABSTRACTS

Cancer/vitamins

Inhibition of cigarette smoke-related DNA adducts in rat tissues by indole-3-carbinol.

Indole-3-carbinol (I3C) found in various cruciferous vegetables has been shown to exert anti-carcinogenic activity in several target organs. In this study, we have investigated the effects of I3C on cigarette smoke-related lipophilic DNA adduct formation, potentially a key step in chemical carcinogenesis. Female Sprague-Dawley rats were exposed to sidestream cigarette smoke in a whole-body exposure chamber for six h per day, seven days a week for four weeks. Control animals received only vehicle while the intervention groups received I3C (1.36 or 3.40 mmol/kg, b.wt.) daily by gavage starting from one week prior to smoke initiation until the end of the experiment. Analysis of tissue DNA by nuclease P1-mediated ³²P-postlabeling showed one major and several minor smoke-related adducts in lung, trachea, heart and bladder. The high dose of I3C significantly inhibited the major adducts in lung (#5) and trachea (#3) by 55% each; minor adducts were slightly inhibited (20% to 40%). The low dose of I3C showed lesser degree of inhibition (30% to 40%) in both lung and trachea; however, it was found statistically significant in lung only. The major smoke-related adduct in bladder (#2) was strongly inhibited (>65%) by high dose of I3C approaching adduct levels achieved in sham-exposed rats. A small but statistically significant decrease in the smoke-related DNA adduct (#5) in heart tissue was also observed by intervention with high dose I3C. Low levels (30 to 50 adducts/10(10) nucleotides) of I3C-derived DNA adducts were also found in all the tissues examined although their significance remains unknown. These data show significant inhibition of cigarette smoke-related DNA adducts by I3C, particularly in the lung, trachea and bladder.

Mutat Res 2000 Jul 20;452(1):11-8

Placebo-controlled trial of indole-3-carbinol in the treatment of CIN.

OBJECTIVE: Most precancerous lesions of the cervix are treated with surgery or ablative therapy. Chemoprevention, using natural and synthetic compounds, may intervene in the early precancerous stages of carcinogenesis and prevent the development of invasive disease. Our trial used indole-3-carbinol (I-3-C) administered orally to treat women with CIN as a therapeutic for cervical CIN. **METHODS:** Thirty patients with biopsy proven CIN II-III were randomized to receive placebo or 200, or 400 mg/day I-3-C administered orally for 12 weeks. If persistent CIN was diagnosed by cervical biopsy at the end of the trial, loop electrocautery excision procedure of the transformation zone was performed. HPV status was assessed in all patients. **RESULTS:** None (0 of 10) of the patients in the placebo group had complete regression of CIN. In contrast four of eight patients in the 200 mg/day arm and four of nine patients in the 400 mg/day arm had complete regression based on their 12-week biopsy. This protective effect of I-3-C is shown by a relative risk (RR) of 0.50 ((95% CI, 0.25 to 0.99) P = 0.023) for the 200 mg/day group and a RR of 0.55 ((95% CI, 0.31 to 0.99) P = 0.032) for the 400 mg/day group. HPV was detected in seven of 10 placebo patients, in seven of eight in the 200 mg/day group, and in eight of nine in the 400 mg/day group. **CONCLUSIONS:** There was a statistically significant regression of CIN in patients treated with I-3-C orally compared with placebo. The 2/16 alpha-hydroxyestrone ratio changed in a dose-dependent fashion.

Gynecol Oncol 2000 Aug;78(2):123-9

Fraction of prostate cancer incidence attributed to diet in Athens, Greece.

Diet appears to be a major determinant in the incidence of prostate cancer. In a case-control study conducted in Athens, Greece, we found that dairy products, butter and seed oils were positively associated with risk of prostate cancer, whereas cooked and raw tomatoes were inversely associated. We utilized the data from this study to calculate the population attributable fractions under alternative assumptions of feasible dietary changes. For each subject, a dietary score was calculated and categorized into approximately quintiles, representing increasing levels of prostate cancer risk as a function of the intake of the five discriminatory food groups or items. Population attributable fractions in terms of this dietary score were calculated taking into account multivariate adjustment. We observed that, if all individuals were shifted to the baseline category, the incidence of prostate cancer in this study population would be reduced by 41% (95% confidence interval 23% to 59%). However, if all individuals were shifted to the adjacent lower risk quintile, the expected incidence reduction would be a more modest 19%. The incidence of prostate cancer in Greece could be reduced by about two-fifths if the population increased the consumption of tomatoes and reduced the intake of dairy products, and substituted olive oil for other added lipids.

Eur J Cancer Prev 2000 Apr;9(2):119-23

Flavonoids apigenin and quercetin inhibit melanoma growth and metastatic potential.

Flavonoids are a class of polyphenolic compounds widely distributed in the plant kingdom, which display a variety of biological activities, including chemoprevention and tumor growth inhibition. Our aim was to investigate the effects of several polyphenols on the growth and metastatic potential of B16-BL6 melanoma cells in vivo. Intraperitoneal administration of quercetin, apigenin, (-)-epigallocatechin-3-gallate (EGCG), resveratrol and the anti-estrogen tamoxifen, at the time of i.m. injection of B16-BL6 cells into syngeneic mice, resulted in a significant, dose-dependent delay of tumor growth, without toxicity. The relative descending order of potency was EGCG > apigenin = quercetin = tamoxifen > resveratrol > control. Furthermore, polyphenols significantly potentiated the inhibitory effect of a non-toxic dose of cisplatin. When tested for the ability to inhibit lung colonization, quercetin, apigenin and tamoxifen (but not EGCG or resveratrol) significantly decreased the number of B16-BL6 colonies in the lungs in a dose-dependent manner, with quercetin and apigenin being more effective than tamoxifen. Interestingly, quercetin, apigenin and tamoxifen (but not EGCG or resveratrol) significantly decreased the invasion of B16-BL6 cells in vitro, with quercetin and apigenin being more effective than tamoxifen. This suggests that anti-invasive activity is one of the mechanisms underlying inhibition of lung colonization by quercetin and apigenin. In conclusion, quercetin and apigenin inhibit melanoma growth and invasive and metastatic potential; therefore, they may constitute a valuable tool in the combination therapy of metastatic melanoma.

Int J Cancer 2000 Aug 15;87(4):595-600

Excessive intake of zinc impairs immune responses.

The effect of administration of large amounts of zinc on immune response and serum lipoproteins was examined. Eleven healthy adult men ingested 150 mg of elemental zinc twice a day for six weeks. This was associated with a reduction in lymphocyte stimulation response to phytohemagglutinin as well as chemotaxis and phagocytosis of bacteria by polymorphonuclear leukocytes. Serum high-density lipoprotein concentration decreased significantly and low-density lipoprotein level increased slightly. The common food fad of zinc supplementation with resultant excessive intake could have deleterious effects in healthy persons.

JAMA 1984 Sep 21;252(11):1443-6

Flavonoids (apigenin, tangeretin) counteract tumor promoter-induced inhibition of intercellular communication of rat liver epithelial cells.

We have shown previously that two flavonoids, apigenin and tangeretin, enhance gap junctional intercellular communication (GJIC) in rat liver epithelial cells, named REL cells. Here, we show that these two flavones also antagonize the inhibition of GJIC induced by tumor promoters like 12-O-tetradecanoyl-phorbol-acetate (TPA) and 3,5-di-tert-butyl-4-hydroxytoluene (BHT). Their preventive effect is rapid. It does not seem to involve any change of the amount of the connexin expressed in REL cells, connexin 43 (Cx 43), and in its phosphorylation state. Other flavonoids tested including naringenin, myricetin, catechin and chrysin did not enhance GJIC nor counteract TPA-induced inhibition of GJIC.

Cancer Lett 1997 Mar 19;114(1-2):207-10

Review article: cyclooxygenase? a target for colon cancer prevention.

Use of nonsteroidal anti-inflammatory drugs such as aspirin, which are known to inhibit cyclooxygenase activity, reduces the relative risk of colorectal cancer in humans by 40% to 50%. Animal and human studies have shown a 50% to 80% reduction in tumour multiplicity following treatment with a variety of nonsteroidal anti-inflammatory drugs. Two isoforms of cyclooxygenase have been described, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). In 85% of colorectal adenocarcinomas taken from humans, COX-2 levels are two to 50-fold higher than levels in adjacent normal intestinal mucosa, while COX-1 levels are unchanged. These observations raise the question: Does COX-1 or COX-2 provide a useful target for prevention or treatment of colorectal cancer?

Aliment Pharmacol Ther 2000 Apr;14 Suppl 1:64-7

Chlorophyllin

Chlorophyllin protects cells from the cytostatic and cytotoxic effects of quinacrine mustard but not of nitrogen mustard.

Chlorophyllin (CHL), the sodium and copper salt of chlorophyll, is capable of inhibiting the mutagenic activity of many chemical compounds. Several mechanisms have been advanced to explain the antimutagenic activity of CHL, including its antioxidant properties and its ability to form complexes with mutagens. The present study was designed to reveal whether the heterocyclic aromatic nature of a potential mutagen is essential to its sensitivity to CHL. Toward this end, the inhibitory effect of CHL on two compounds of similar chemical reactivity (mustards), that either embodied an aromatic structure (quinacrine mustard; QM) or did not (nitrogen mustard; NM), were compared. Human leukemic HL-60 and breast carcinoma MCF-7 cells were treated with QM or NM in the absence or presence of various concentrations of CHL. Both QM and NM when administered for one to two h at

micromolar concentrations exerted similar effects; both arrested cells in G2 phase of the cell cycle, induced apoptosis and reduced the clonogenicity of MCF-7 cells. The simultaneous addition of 0.22 M CHL to cultures receiving QM virtually abolished the QM-induced inhibition of cell growth and clonogenicity. In contrast, CHL had no effect on reducing the cytostatic or cytotoxic activity of NM. CHL alone, at a concentration of 0.22 M, had minimal effect on growth of HL-60 cells slightly perturbing their progression through G2. The results are consistent with the model that explains the inhibition of the activity of mutagens or antitumor drugs with aromatic structures by CHL as mediated by its ability to sequester these molecules within heterologous mutagen:CHL complexes that are maintained by stacking interactions. Therefore, excess of chlorophyll in the diet, by sequestering aromatic mutagens (or antitumor drugs with a heterocyclic structure, if taken orally), may inhibit their accessibility to cells, thereby reducing their activity.

Int J Oncol 2001 Apr;18(4):849-53

Chemopreventive effect of chlorophyllin on mutagenicity and cytotoxicity of 6-sulfooxymethylbenzo[a]pyrene.

The chemopreventive activity of chlorophyllin (CHL) was monitored by using 6-sulfooxymethylbenzo[a]pyrene (SMBP) which is an ultimate metabolite of benzo[a]pyrene (B[a]P). CHL was quite effective in reducing both cytotoxicity and mutagenicity for SMBP in dose dependent manner up to 12.5 mM CHL in Chinese hamster V79 cells. The inhibitory patterns of CHL for SMBP were also confirmed in *Salmonella typhimurium* strains TA98 and TA100. Mutation frequency caused by SMBP was diminished almost to a control level at a 50 nmol CHL. A similar but less effective prevention of CHL was indicated in the mammalian and the bacterial mutagenicity assays with 6-hydroxymethylbenzo[a]pyrene (HMBP). The inhibitory effect of CHL against assault of SMBP on V79 cells was found to be related to the reduced cellular uptake of SMBP and further the remarkably lowered DNA adducts.

Cancer Lett 1996 Oct 22;107(2):223-8

Early detection and prevention of colorectal cancer (review).

Colorectal cancer is a leading cause of cancer-related deaths, and the two most important considerations for avoidance of this disease are early detection and prevention. If metastasis has occurred to distant sites, such as the liver and lung, the five-year survival rate for colorectal cancer is below 10%, but this increases to greater than 90% when the cancer is found early. Early detection can be facilitated by use of the digital rectal exam, fecal occult blood test, sigmoidoscopy, and colonoscopy, but these methods might be supplemented in the future by other screening assays using intermediate biomarkers. One interesting biomarker, the aberrant crypt focus (ACF), has been observed in resected human colons, and is the earliest detectable morphological change in the colons of experimental animals treated with carcinogens such as the cooked meat heterocyclic amines. The ACF can also be used as an end-point to screen for potential inhibitors of colorectal cancer; using this approach, we identified conjugated linoleic acids, indole-3-carbinol, chlorophyllin, and tea polyphenols as promising inhibitors in the colon. These compounds can be added to a growing list of natural and synthetic agents that might be effective against colorectal cancer, including selenium, calcium and nonsteroidal anti-inflammatory agents. However, results from human clinical trials with several of these compounds have highlighted the need for detailed mechanism data before recommendations can be made for wide-scale use in humans. In the meantime, the best approach to reducing the risk of colorectal cancer would be to increase the dietary intake of fruits, vegetables and cereals, while reducing the overall intake of fat, particularly from animal sources.

Oncol Rep 1999 Mar-Apr;6(2):277-81

Study of the forces of stabilizing complexes between chlorophylls and heterocyclic amine mutagens.

Chlorophyllin (CHL), a water-soluble derivative of chlorophyll, forms molecular complexes with heterocyclic amine mutagens in vitro. In a previous study [Dashwood and Guo (1993): *Environ Mol Mutagen*, 22:164-171], we observed an inverse correlation between the binding constants of several mutagen-CHL complexes and the antimutagenic potency of CHL in the *Salmonella* assay. The present investigation utilized molecular mechanics methods of energy minimization and spectrophotometric titration to examine structural features of chlorophylls, chlorins, and porphyrins that might be important for complex formation with heterocyclic amines. The exocyclic amine group of the mutagen aligned consistently with acid groups in CHL, suggesting that H-bond or electrostatic interactions facilitate complex formation. Replacement of the exocyclic amine with a nitro group abrogated this specific orientation and raised the minimized energies of the complexes. No relationship was found between complex strength and the specific positions of amine or methyl groups on the mutagen. However, the presence of methyl groups increased the minimized energies and lowered the binding constants of the complexes, perhaps due to partial disruption of pi-pi interaction by steric effects. All of the compounds examined, including chlorophyll a, required the presence of pi-pi interactions to form stable complexes with the heterocyclic amines. In general, the present results were in agreement with the inhibitory potency of each compound in the *Salmonella* assay, and they provide further support for the hypothesis that chlorophylls in the diet might act as interceptor molecules of food-borne carcinogens and mutagens.

Environ Mol Mutagen 1996;27(3):211-8

Chlorophyllin intervention reduces aflatoxin-DNA adducts in individuals at high risk for liver cancer.

Residents of Qidong, People's Republic of China, are at high risk for development of hepatocellular carcinoma, in part from consumption of foods contaminated with aflatoxins. Chlorophyllin, a mixture of semisynthetic, water-soluble derivatives of chlorophyll that is used as a food colorant and over-the-counter medicine, has been shown to be an effective inhibitor of aflatoxin hepatocarcinogenesis in animal models by blocking carcinogen bioavailability. In a randomized, double-blind, placebo-controlled chemoprevention trial, we tested whether chlorophyllin could alter the disposition of aflatoxin. One hundred and eighty healthy adults from Qidong were randomly assigned to ingest 100 mg of chlorophyllin or a placebo three times a day for four months. The primary endpoint was modulation of levels of aflatoxin-N(7)-guanine adducts in urine samples collected three months into the intervention measured by using sequential immunoaffinity chromatography and liquid chromatography-electrospray mass spectrometry. This aflatoxin-DNA adduct excretion product serves as a biomarker of the biologically effective dose of aflatoxin, and elevated levels are associated with increased risk of liver cancer. Adherence to the study protocol was outstanding, and no adverse events were reported. Aflatoxin-N(7)-guanine could be detected in 105 of 169 available samples. Chlorophyllin consumption at each meal led to an overall 55% reduction ($P = 0.036$) in median urinary levels of this aflatoxin biomarker compared with those taking placebo. Thus, prophylactic interventions with chlorophyllin or supplementation of diets with foods rich in chlorophylls may represent practical means to prevent the development of hepatocellular carcinoma or other environmentally induced cancers.

Proc Natl Acad Sci U S A 2001 Dec 4;98(25):14601-6

Protection by chlorophyllin and indole-3-carbinol against 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced DNA adducts and colonic aberrant crypts in the F344 rat.

The most abundant heterocyclic amine in fried ground beef, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), induces colon carcinomas in the male F344 rat. The potential chemopreventive effects of two compounds, namely, the 'interceptor molecule' chlorophyllin (CHL) and a modulator of carcinogen activation, indole-3-carbinol (I3C), were examined in a PhIP colon carcinogenesis model. During weeks three and four of a 16-week study, F344 rats were given PhIP by oral gavage (50 mg/kg body weight, alternating days). Inhibitors were given either before and during PhIP exposure, after PhIP treatment, or continuously for 16 weeks. Treatment of rats with 0.1% CHL in the drinking water inhibited the formation of aberrant crypt foci (ACF) with ≥ 4 crypts/focus, from 1.4 ± 0.9 in controls to 0.7 ± 0.3 following post-initiation CHL treatment, and to 0.3 ± 0.5 in rats given CHL continuously for 16 weeks (mean \pm SD; $P < 0.05$). Potent inhibition of PhIP-induced ACF occurred following initiation, post-initiation and continuous exposure to 0.1% I3C in the diet. Using the initiation protocol, I3C completely inhibited the induction of the ACF with ≥ 4 crypts/focus. In a separate experiment, rats were given 0.1% CHL in the drinking water or 0.1% I3C in the diet for four weeks. At the end of week three, animals received 50 mg PhIP/kg body weight by single oral gavage and PhIP-DNA adducts were quantified in the colon and several other tissues by ^{32}P -postlabeling analysis. In addition, the urine and feces were collected to study the effects of inhibitor treatment on PhIP metabolism and excretion. No significant protection against PhIP-DNA adduct formation was detected in the colon after CHL dosing, nor was a consistent pattern of CHL inhibition observed in several other tissues. In contrast, I3C shifted the time-course of adducts in all tissue; compared with controls, adducts were increased by I3C at six h but decreased at 24 h and seven days following PhIP treatment. Analysis of urine metabolites revealed that I3C and CHL decreased the excretion of unmetabolized PhIP and 4'-hydroxy-PhIP but increased the phase II detoxification products PhIP-4'-O-glucuronide and PhIP-4'-sulfate. In the feces, the elimination of unmetabolized PhIP was increased from 54.5% in controls to approximately 67% in CHL-treated rats and decreased to 28% in rats given I3C ($P < 0.05$). These results support a protective role for CHL and I3C against PhIP-induced colon carcinogenesis through mechanisms which alter the uptake or metabolism of the carcinogen, and by suppression in the post-initiation phase.

Carcinogenesis 1995 Dec;16(12):2931-7

Inhibition of radiation-induced DNA damage in plasmid pBR322 by chlorophyllin and possible mechanism(s) of action.

Naturally occurring compounds capable of protecting DNA against ionizing radiation and chemical mutagens have considerable potential for prevention of mutation-based health impairment including cancer and other degenerative diseases. Chlorophyllin (CHL), a water-soluble derivative of chlorophyll, has been examined for its ability to protect DNA against radiation induced strand breaks using an in vitro plasmid DNA system. Gamma-radiation, up to a dose of 6 Gy (dose rate 1.25 Gy/min), induced a dose-dependent increase in single-strand breaks (ssbs) in plasmid pBR322 DNA. CHL per se did not induce, but inhibited radiation-induced ssbs in a concentration-dependent manner; 500 microM giving about 90% protection. The protection afforded by CHL was comparatively less than that of trolox, a water-soluble analogue of alpha-tocopherol. To elucidate the underlying mechanism(s), reaction of CHL with the radiation-derived hydroxyl radical (OH) and deoxyribose peroxy radical (ROO) was studied by pulse radiolysis. CHL exhibited a rate constant of $6.1 \pm 0.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ with OH and $5.0 \pm 1.3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ with ROO. To our knowledge, this is the first report providing direct evidence of free radical-scavenging properties of CHL. The results showed that CHL, effectively protects plasmid DNA against ionizing radiation, in an in vitro system independent of DNA repair or other cellular defense mechanisms. The ability of CHL to scavenge OH and ROO, may contribute to its protective effects against radiation induced DNA damage in the pBR322 system.

Mutat Res 1999 Mar 10;425(1):71-9

Scavenging of reactive oxygen species by chlorophyllin: an ESR study.

The antioxidant effects of chlorophyllin (CHL), a water-soluble analog of the green plant pigment chlorophyll, on different reactive oxygen species (ROS) were investigated by electron spin resonance (ESR) spectroscopy. As a standard, we have used the ability of CHL to scavenge the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. CHL inhibits the formation of 5,5-dimethyl-1-pyrroline-N-oxide adduct with hydroxyl radical (DMPO-OH adduct) generated by gamma-radiation in a dose-dependent manner. At a concentration of 1 mM, CHL caused more than 90% inhibition of ESR signal intensity of this adduct. However, the results obtained with the Fenton reaction were different. We also found evidence for the inhibition of 1O₂-dependent formation of the 2,2,6,6-tetramethyl-piperidine oxide (TEMPO) radical during photosensitization of methylene blue with visible light. CHL was also able to inhibit hydrogen peroxide induced oxidation of phenol red. The rate constant of the reaction of CHL with H₂O₂ was found to be 2.7 x 10⁶ M⁻¹ s⁻¹. In conclusion, CHL has potent antioxidant ability involving scavenging of various physiologically important ROS.

Free Radic Res 2001 Nov;35(5):563-74

Effect of chemopreventive agents on DNA adduction induced by the potent mammary carcinogen dibenzo[a,l]pyrene in the human breast cells MCF-7.

Over 1500 structurally diverse chemicals have been identified which have potential cancer chemopreventive properties. The efficacy and mechanisms of this growing list of chemoprotective agents may be studied using short-term bioassays that employ relevant end-points of the carcinogenic process. In this study, we have examined the effects of eight potential chemopreventive agents, N-acetylcysteine (NAC), benzylisocyanate (BIC), chlorophyllin, curcumin, 1,2-dithiole-3-thione (D3T), ellagic acid, genistein, and oltipraz, on DNA adduction of the potent mammary carcinogen dibenzo[a,l]pyrene (DBP) using the human breast cell line MCF-7. Bioactivation of DBP by MCF-7 cells resulted in the formation of one predominant (55%) dA-derived and several other dA- or dG-derived DNA adducts. Three test agents, oltipraz, D3T, and chlorophyllin substantially (>65%) inhibited DBP-DNA adduction at the highest dose tested (30 microM). These agents also significantly inhibited DBP adduct levels at a lower dose of 15 microM, while oltipraz was effective even at the lowest dose of 5 microM. Two other agents, genistein and ellagic acid were moderate (45%) DBP-DNA adduct inhibitors at the highest dose tested, while NAC, curcumin, and BIC were ineffective. These studies indicate that the MCF-7 cell line is an applicable model to study the efficacy of cancer chemopreventive agents in a human setting. Moreover, this model may also provide information regarding the effect of the test agents on carcinogen bioactivation and detoxification enzymes.

Mutat Res 2001 Sep 1;480-481:97-108

Nutrients

Progress in cancer chemoprevention: development of diet-derived chemopreventive agents.

Because of their safety and the fact that they are not perceived as "medicine," food-derived products are highly interesting for development as chemopreventive agents that may find widespread, long-term use in populations at normal risk. Numerous diet-derived agents are included among the >40 promising agents and agent combinations that are being evaluated clinically as chemopreventive agents for major cancer targets including breast, prostate, colon and lung. Examples include green and black tea polyphenols, soy isoflavones, Bowman-Birk soy protease inhibitor, curcumin, phenethyl isothiocyanate, sulforaphane, lycopene, indole-3-carbinol, perillyl alcohol, vitamin D, vitamin E, selenium and calcium. Many food-derived agents are extracts, containing multiple compounds or classes of compounds. For developing such agents, the National Cancer Institute (NCI) has advocated codevelopment of a single or a few putative active compounds that are contained in the food-derived agent. The active compounds provide mechanistic and pharmacologic data that may be used to characterize the chemopreventive potential of the extract, and these compounds may find use as chemopreventives in higher risk subjects (patients with precancers or previous cancers). Other critical aspects to developing the food-derived products are careful analysis and definition of the extract to ensure reproducibility (e.g., growth conditions, chromatographic characteristics or composition), and basic science studies to confirm epidemiologic findings associating the food product with cancer prevention.

J Nutr 2000 Feb;130(2S Suppl):467S-471S

Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells.

Sulforaphane is an isothiocyanate that is present naturally in widely consumed vegetables and has a particularly high concentration in broccoli. This compound has been shown to block the formation of tumors initiated by chemicals in the rat. Although sulforaphane has been proposed to modulate the metabolism of carcinogens, its mechanism of action remains poorly understood. We have previously demonstrated that sulforaphane inhibits the reinitiation of growth and decreases the cellular viability of quiescent human colon carcinoma cells (HT29). Moreover, the weak effect observed on differentiated CaCo2 cells suggests a specific anticancer activity for this compound. Here we investigated the effect of sulforaphane on the growth and viability of HT29 cells during their exponentially growing phase. We observed that sulforaphane induced a cell cycle arrest in a dose-dependent manner, followed

by cell death. This sulforaphane-induced cell cycle arrest was correlated with an increased expression of cyclins A and B1. Moreover, we clearly demonstrated that sulforaphane induced cell death via an apoptotic process. Indeed, a large proportion of treated cells display the following: (a) translocation of phosphatidylserine from the inner layer to the outer layer of the plasma membrane; (b) typical chromatin condensation; and (c) ultrastructural modifications related to apoptotic cell death. We also showed that the expression of p53 was not changed in sulforaphane-treated cells. In contrast, whereas bcl-2 was not detected, we observed increased expression of the proapoptotic protein bax, the release of cytochrome c from the mitochondria to the cytosol, and the proteolytic cleavage of poly(ADP-ribose) polymerase. In conclusion, our results strongly suggest that in addition to the activation of detoxifying enzymes, induction of apoptosis is also involved in the sulforaphane-associated chemoprevention of cancer.

Cancer Res 2000 Mar 1;60(5):1426-33

Vitamin D and vitamin D analogs in cancer treatment.

The secosteroid hormone 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃) is a key player in the regulation of bone mineralization and calcium homeostasis. In addition, 1,25-(OH)₂D₃ has antiproliferative and prodifferentiation effects on various cells in vitro and in vivo. The growth-inhibitory properties of 1,25-(OH)₂D₃ could be harnessed in the treatment of cancer. However, its use as an anti-cancer drug is limited because of the calcemic effects of pharmacological doses. In an attempt to dissociate the antiproliferative and calcemic effects, numerous vitamin D₃ analogs were developed. The mechanisms by which 1,25-(OH)₂D₃ and 1,25-(OH)₂D₃ analogs exert their growth-inhibitory effects are not clear but include effects on cell differentiation, apoptosis, cell cycle regulation, metastases, and angiogenesis. In the current review aspects involved in the tumor suppressive activity of 1,25-(OH)₂D₃ and 1,25-(OH)₂D₃ analogs will be addressed. The use of vitamin D₃ compounds, alone or in combination with other drugs, in cancer treatment and the potential drawbacks will also be discussed.

Curr Drug Targets 2002 Feb;3(1):85-94

Se-methylselenocysteine induces apoptosis through caspase activation and Bax cleavage mediated by calpain in SKOV-3 ovarian cancer cells.

Se-methylselenocysteine (Se-MSC) is a potent chemopreventive agent in many test systems and has been shown to inhibit tumor promotion and induce apoptosis, but its mechanism of action is still not well understood. The present study was designed to assess the mechanism of Se-MSC on the induction of apoptosis in SKOV-3 ovarian cancer cells. Se-MSC displayed strong inhibitory effects on cell proliferation and viability of SKOV-3 cells in dose and time dependent manners and induced apoptosis. Investigation of the mechanism of Se-MSC-induced apoptosis revealed that treatment with Se-MSC produced morphological features of apoptosis and DNA fragmentation. This was associated with caspase-3 activation and cleavage of poly(ADP-ribose) polymerase and phospholipase C-gamma1 proteins. However, SKOV-3 cells treated with Se-MSC did not demonstrate cytochrome c accumulation in the cytosol during apoptosis induction. Pretreatment of cells with the caspase inhibitors (z-VAD-fmk and DEVD-CHO) prevented Se-MSC-induced apoptosis. These results suggested that Se-MSC induces apoptosis through cytochrome c-independent caspase-3 activation in SKOV-3 cells. In late stage of apoptosis, p18kDa fragment of Bax was generated with the down-regulation of the expressions of survivin, X-linked inhibitor of apoptosis protein, and human inhibitor of apoptosis protein 1 following Se-MSC treatment, suggesting that the modulation of Bax and IAP (inhibitors of apoptosis) family proteins play some role in Se-MSC-mediated apoptosis. Pre-treatments of z-VAD-fmk and the calpain inhibitor, calpeptin inhibited Bax cleavage. These results suggested that Bax cleavage is mediated by calpain, and calpain activation may be a caspase-dependent one. Taken together, the chemopreventive effects of Se-MSC may be related in part to the caspase-3 activation, the down-regulation of IAP family proteins, and Bax cleavage mediated by caspase-dependent calpain activation.

Cancer Lett 2002 Aug 8;182(1):83-92

Se-methylselenocysteine induces apoptosis mediated by reactive oxygen species in HL-60 cells.

Recent studies have implicated apoptosis as one of the most plausible mechanisms of the chemopreventive effects of selenium compounds, and reactive oxygen species (ROS) as important mediators in apoptosis induced by various stimuli. In the present study, we demonstrate that Se-methylselenocysteine (MSC), one of the most effective selenium compounds at chemoprevention, induced apoptosis in HL-60 cells and that ROS plays a crucial role in MSC-induced apoptosis. The uptake of MSC by HL-60 cells occurred quite early, reaching the maximum within 1 h. The dose-dependent decrease in cell viability was observed by MSC treatment and was coincident with increased DNA fragmentation and sub-G(1) population. 50 microM of MSC was able to induce apoptosis in 48% of cell population at a 24 h time point. Moreover, the release of cytochrome c from mitochondria and the activation of caspase-3 and caspase-9 were also observed. The measurement of ROS by dichlorofluorescein fluorescence revealed that dose- and time-dependent increase in ROS was induced by MSC. N-acetylcysteine, glutathione, and deferoxamine blocked cell death, DNA fragmentation, and ROS generation induced by MSC. Moreover, N-acetylcysteine effectively blocked caspase-3 activation and the increase of the sub-G(1) population induced by MSC. These results imply that ROS is a critical mediator of the MSC-induced apoptosis in HL-60 cells.

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