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**JOURNAL
ABSTRACTS****Theaflavin****MECHANISMS OF CANCER PREVENTION BY GREEN AND BLACK TEA POLYPHENOLS.**

Drinking green tea is associated with decreased frequency of cancer development. This review outlines the wide range of mechanisms by which epigallocatechin gallate (EGCG) and other green and black tea polyphenols inhibit cancer cell survival. EGCG suppressed androgen receptor expression and signalling via several growth factor receptors. Cell cycle arrest or apoptosis involved caspase activation and altered Bcl-2 family member expression. EGCG inhibited telomerase activity and led to telomere fragmentation. While at high concentrations polyphenols had pro-oxidative activities, at much lower levels, anti-oxidative effects occurred. Nitric oxide production was reduced by EGCG and black tea theaflavins by suppressing inducible nitric oxide synthase via blocking nuclear translocation of the transcription factor nuclear factor-kappaB as a result of decreased I kappa B kinase activity. Polyphenols up- or down-regulated activity of a number of key enzymes, including mitogen-activated protein kinases and protein kinase C, and increased or decreased protein/mRNA levels, including that of cyclins, oncogenes, and tumor suppressor genes. Metastasis was inhibited via effects on urokinase and matrix metalloproteinases. Polyphenols reduced angiogenesis, in part by decreasing vascular endothelial growth factor production and receptor phosphorylation. Recent work demonstrated that EGCG reduced dihydrofolate reductase activity, which would affect nucleic acid and protein synthesis. It also acted as an aryl hydrocarbon receptor antagonist by directly binding the receptor's molecular chaperone, heat shock protein 90. In conclusion, green and black tea polyphenols act at numerous points regulating cancer cell growth, survival, and metastasis, including effects at the DNA, RNA, and protein levels.

Anticancer Agents Med Chem. 2006 Sep;6(5):389-406

BLACK TEA POLYPHENOLS MIMIC INSULIN/INSULIN-LIKE GROWTH FACTOR-1 SIGNALLING TO THE LONGEVITY FACTOR FOXO1A.

In vertebrates and invertebrates, relationships between diet and health are controlled by a conserved signalling pathway responsive to insulin-like ligands. In invertebrate models for example, forkhead transcription factor family O (FOXO) transcription factors in this pathway regulate the rate of aging in response to dietary cues, and in vertebrates, obesity and age-induced deficits in the same pathway are thought to contribute to dysregulation of hepatic gluconeogenesis through genes such as phosphoenolpyruvate carboxykinase (PEPCK). Recently, we have begun to screen for dietary constituents capable of regulating this pathway in our cell culture model. Here, we identify three black tea theaflavins, theaflavin 3-O-gallate, theaflavin 3'-O-gallate, theaflavin 3,3'-di-O-gallate and thearubigins as novel mimics of insulin/IGF-1 action on mammalian FOXO1a, PEPCK and moreover we provide evidence that the effects on this pathway of the green tea constituent (-)-epigallocatechin gallate depend on its ability to be converted into these larger structures. With the exception of water, tea is the most popular drink globally, but despite this, little is known about the biological availability of black tea polyphenols in vivo or the molecular target(s) mediating the effects presented here. Further investigation in these two areas might provide insight into how age-related metabolic disease may be deferred.

Aging Cell. 2008 Jan;7(1):69-77

TEA POLYPHENOLS BENEFIT VASCULAR FUNCTION.

Tea, the most popular beverage worldwide, is consumed in three basic forms; green tea, black tea and oolong tea. Tea contains over 4,000 chemicals some of which are bioactive. In recent years there has been a mounting interest in understanding the cardiovascular and metabolic benefits of polyphenolic flavonoids in tea, which can be used as a supplement among patients. Diverse cardioprotective effects of consuming tea or tea polyphenols have been described on pathological conditions, e. g. hypertension, atherosclerosis, diabetics, hypercholesterolemia, obesity, and are attributed to antioxidative, anti-thrombogenic, anti-inflammatory, hypotensive and hypocholesterolemic properties of tea polyphenols. This review focuses on cardiovascular benefits of tea polyphenols based on in vitro and in vivo studies on experimental animal models and on studies of human subjects in four areas: (1) vasorelaxant effect; (2) protective effect against endothelial dysfunction; (3) antioxidant effect and (4) hypolipidemic effect. We will briefly discuss the effects of tea on atherosclerosis and hypertension.

ANTI-INVASIVE EFFECTS OF GREEN TEA POLYPHENOL EPIGALLOCATECHIN-3-GALLATE (EGCG), A NATURAL INHIBITOR OF METALLO AND SERINE PROTEASES.

Several reports have attributed to green tea chemopreventive and therapeutic properties. Epidemiological studies have linked the regular use of green tea to a reduced incidence of breast and colon carcinomas. Tea contains several antioxidants, including polyphenols of the catechin (green tea) and theaflavin (black tea) groups. Green tea derivatives have been shown to act in vitro and in vivo as anti-inflammatory, anti-viral and anti-tumor drugs. Despite the extensive body of data only few studies have investigated the molecular mechanisms underlying these effects. In this brief review we focus on the inhibitory activity of catechins derived from green tea toward proteases involved in tumor invasion.

Biol Chem. 2002 Jan;383(1):101-5

THEAFLAVIN, A BLACK TEA EXTRACT, IS A NOVEL ANTI-INFLAMMATORY COMPOUND.

OBJECTIVE: Tea has been around for centuries, and its medicinal properties have been purported in the literature but never fully confirmed. Interleukin-8 is a principle neutrophil chemoattractant and activator in humans. We determined the effects of theaflavin, a black tea-derived polyphenol, on tumor necrosis factor-alpha-mediated expression of the interleukin-8 gene in A549 cells. **DESIGN:** Prospective laboratory study. **SETTING:** University laboratory. **SUBJECTS:** A549 cells. **INTERVENTIONS:** A549 cells were exposed to varying concentrations of theaflavin and analyzed for tumor necrosis factor-alpha-mediated interleukin-8 gene expression. **MEASUREMENTS AND MAIN RESULTS:** Theaflavin inhibited tumor necrosis factor-alpha-mediated interleukin-8 gene expression, as measured by luciferase assay and Northern blot analysis, at concentrations of 10 and 30 microg/mL. This effect appears to primarily involve inhibition of interleukin-8 transcription because theaflavin inhibited tumor necrosis factor-alpha-mediated activation of the interleukin-8 promoter in cells transiently transfected with an interleukin-8 promoter-luciferase reporter plasmid. In addition, theaflavin inhibited tumor necrosis factor-alpha-mediated activation of I κ B kinase and subsequent activation of the I κ B- α /nuclear factor- κ B pathway. Theaflavin also significantly reduced tumor necrosis factor-alpha-mediated DNA binding of activator protein-1. **CONCLUSIONS:** We conclude that theaflavin is a potent inhibitor of interleukin-8 gene expression in vitro. The proximal mechanism of this effect involves, in part, inhibition of I κ B kinase activation and activator protein-1 pathway.

Crit Care Med. 2004 Oct;32(10):2097-103

THEAFLAVIN AMELIORATES CEREBRAL ISCHEMIA-REPERFUSION INJURY IN RATS THROUGH ITS ANTI-INFLAMMATORY EFFECT AND MODULATION OF STAT-1.

Theaflavin, a major constituent of black tea, possesses biological functions such as the antioxidative, antiviral, and anti-inflammatory ones. The purpose of this study was to verify whether theaflavin reduces focal cerebral ischemia injury in a rat model of middle cerebral artery occlusion (MCAO). Male Sprague-Dawley rats were anesthetized and subjected to 2 hours of MCAO followed 24 hours reperfusion. Theaflavin administration (5, 10, and 20 mg/kg, i.v.) ameliorated infarct and edema volume. Theaflavin inhibited leukocyte infiltration and expression of ICAM-1, COX-2, and iNOS in injured brain. Phosphorylation of STAT-1, a protein which mediates intracellular signaling to the nucleus, was enhanced 2-fold over that of sham group and was inhibited by theaflavin. Our study demonstrated that theaflavin significantly protected neurons from cerebral ischemia-reperfusion injury by limiting leukocyte infiltration and expression of ICAM-1, and suppressing upregulation of inflammatory-related prooxidative enzymes (iNOS and COX-2) in ischemic brain via, at least in part, reducing the phosphorylation of STAT-1.

Mediators Inflamm. 2006;2006(5):30490

MODULATION OF THE OXIDATIVE STRESS AND NUCLEAR FACTOR KAPPA B ACTIVATION BY THEAFLAVIN 3,3'-GALLATE IN THE RATS EXPOSED TO CEREBRAL ISCHEMIA-REPERFUSION.

The major pathobiological mechanisms of IR injury include excitotoxicity, oxidative stress, and inflammation. TF3, a major constituent of black tea, possesses biological functions such as anti-oxidative and anti-inflammatory activities. The purpose of this study was to verify the neuronal protective potential of TF3 and its mechanisms against cerebral IR injury in rats. TF3 administration (10 and 20 mg.kg⁻¹) ameliorated the infarct volume. TF3 also decreased the content of MDA and NO. TF3 significantly increased the activity of SOD and GSH-Px, which were reduced by IR injury. Administration of TF3 decreased mRNA and protein expression of COX-2 and iNOS. DNA binding and Western blotting revealed an increase in NF- κ B activation and I κ B depletion in IR brain tissue. Pretreatment with TF3 markedly inhibited IR-induced increase in nuclear localization of NF- κ B, and preserved I κ B in the cytoplasm. The results show that TF3 exerts protective effects against cerebral IR injury by reducing oxidative stress and modulating the NF- κ B activation.

THEAFLAVINS INDUCED APOPTOSIS OF LNCAP CELLS IS MEDIATED THROUGH INDUCTION OF P53, DOWN-REGULATION OF NF-KAPPA B AND MITOGEN-ACTIVATED PROTEIN KINASES PATHWAYS.

Prostate cancer (PCA), the most frequently diagnosed malignancy in men, represents an excellent candidate disease for chemoprevention studies because of its particularly long latency period, high rate of mortality and morbidity. Infusion of black tea and its polyphenolic constituents have been shown to possess antineoplastic effects in androgen dependent PCA in both in vivo and in vitro models including transgenic animals. In the present study, we report that black tea polyphenol, Theaflavins (TF)-induced apoptosis in human prostate carcinoma, LNCaP cells is mediated via modulation of two related pathways: up-regulation of p53 and down-regulation of NF-kappa B activity, causing a change in the ratio of pro-and antiapoptotic proteins leading to apoptosis. The altered expression of Bcl-2 family member proteins triggered the release of cytochrome-C and activation of initiator caspase 9 followed by activation of effector caspase 3. Furthermore, TF also affected the protein expression of mitogen activated protein kinases (MAPK) pathways. Our results demonstrated that TF treatment resulted in down-regulation of phospho-extracellular signal-regulated protein kinase (Erk1/2) and phospho-p38 MAPK expressions. We conclude that TF induces apoptosis in LNCaP cells by shifting the balance between pro-and antiapoptotic proteins and down-regulation of cell survival pathways leading to apoptosis. Further extending this work, we also showed that TF induces apoptosis in androgen independent PCA cell line, PC-3 through caspases and MAPKs mediated pathways. Thus, effect of TF on PCA cell lines seems to be irrespective of their androgen status.

Life Sci. 2007 May 16;80(23):2137-46

THEAFLAVINS ATTENUATE HEPATIC LIPID ACCUMULATION THROUGH ACTIVATING AMPK IN HUMAN HEPG2 CELLS.

Black tea is one of the world's most popular beverages, and its health-promoting effects have been intensively investigated. The antiobesity and hypolipidemic effects of black tea have attracted increasing interest, but the mechanisms underlying these phenomena remain unclear. In the present study, the black tea major component theaflavins were assessed for their hepatic lipid-lowering potential when administered in fatty acid overload conditions both in cell culture and in an animal experimental model. We found that theaflavins significantly reduced lipid accumulation, suppressed fatty acid synthesis, and stimulated fatty acid oxidation. Furthermore, theaflavins also inhibited acetyl-coenzyme A carboxylase activities by stimulating AMP-activated protein kinase (AMPK) through the LKB1 and reactive oxygen species pathways. These observations support the idea that AMPK is a critical component of decreased hepatic lipid accumulation by theaflavin treatments. Our results show that theaflavins are bioavailable both in vitro and in vivo and may be active in the prevention of fatty liver and obesity.

J Lipid Res. 2007 Nov;48(11):2334-43

CHOLESTEROL-LOWERING EFFECT OF A THEAFLAVIN-ENRICHED GREEN TEA EXTRACT: A RANDOMIZED CONTROLLED TRIAL.

BACKGROUND: Tea consumption has been associated with decreased cardiovascular risk, but potential mechanisms of benefit are ill-defined. While epidemiologic studies suggest that drinking multiple cups of tea per day lowers low-density lipoprotein cholesterol (LDL-C), previous trials of tea drinking and administration of green tea extract have failed to show any impact on lipids and lipoproteins in humans. Our objective was to study the impact of a theaflavin-enriched green tea extract on the lipids and lipoproteins of subjects with mild to moderate hypercholesterolemia. **METHODS:** Double-blind, randomized, placebo-controlled, parallel-group trial set in outpatient clinics in 6 urban hospitals in China. A total of 240 men and women 18 years or older on a low-fat diet with mild to moderate hypercholesterolemia were randomly assigned to receive a daily capsule containing theaflavin-enriched green tea extract (375 mg) or placebo for 12 weeks. Main outcome measures were mean percentage changes in total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglyceride levels compared with baseline. **RESULTS:** After 12 weeks, the mean +/- SEM changes from baseline in total cholesterol, LDL-C, HDL-C, and triglyceride levels were -11.3% +/- 0.9% (P =.01), -16.4% +/- 1.1% (P =.01), 2.3% +/- 2.1% (P =.27), and 2.6% +/- 3.5% (P =.47), respectively, in the tea extract group. The mean levels of total cholesterol, LDL-C, HDL-C, and triglycerides did not change significantly in the placebo group. No significant adverse events were observed. **CONCLUSION:** The theaflavin-enriched green tea extract we studied is an effective adjunct to a low-saturated-fat diet to reduce LDL-C in hypercholesterolemic adults and is well tolerated.

Arch Intern Med. 2003 Jun 23;163(12):1448-53

Astaxanthin

EFFECTS OF ASTAXANTHIN ON HUMAN BLOOD RHEOLOGY.

Effects of astaxanthin (AX) derived from *H. pluvialis* on human blood rheology were investigated in 20 adult men with a single-blind method. The experimental group was 57.5 +/- 9.8 years of age and the placebo group was 50.8 +/- 13.1 years of age. A blood rheology test that measures whole blood transit time was conducted using heparinized blood of the volunteers by a MC-FAN apparatus (microchannel array flow analyzer). After administration of AX 6 mg/day for 10 days, the values of the experimental group were decreased from 52.8 +/- 4.9 s to 47.6 +/- 4.2 s ($p < 0.01$) and a comparison of the values between the experimental (47.6 +/- 4.2 s) and the placebo (54.2 +/- 6.7 s) groups showed a significant difference ($p < 0.05$). There were no adverse effects resulting from the administration of AX 6 mg/day for 10 days. Informed consent was obtained from each subject.

J Clin Biochem Nutr. 2008 Sep;43(2):69-74

ASTAXANTHIN, A CAROTENOID WITH POTENTIAL IN HUMAN HEALTH AND NUTRITION.

Astaxanthin (1), a red-orange carotenoid pigment, is a powerful biological antioxidant that occurs naturally in a wide variety of living organisms. The potent antioxidant property of 1 has been implicated in its various biological activities demonstrated in both experimental animals and clinical studies. Compound 1 has considerable potential and promising applications in human health and nutrition. In this review, the recent scientific literature (from 2002 to 2005) is covered on the most significant activities of 1, including its antioxidative and anti-inflammatory properties, its effects on cancer, diabetes, the immune system, and ocular health, and other related aspects. We also discuss the green microalga *Haematococcus pluvialis*, the richest source of natural 1, and its utilization in the promotion of human health, including the antihypertensive and neuroprotective potentials of 1, emphasizing our experimental data on the effects of dietary astaxanthin on blood pressure, stroke, and vascular dementia in animal models, is described.

J Nat Prod. 2006 Mar;69(3):443-9

ASTAXANTHIN: A NOVEL POTENTIAL TREATMENT FOR OXIDATIVE STRESS AND INFLAMMATION IN CARDIOVASCULAR DISEASE.

Oxidative stress and inflammation are implicated in several different manifestations of cardiovascular disease (CVD). They are generated, in part, from the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that activate transcriptional messengers, such as nuclear factor-kappaB, tangibly contributing to endothelial dysfunction, the initiation and progression of atherosclerosis, irreversible damage after ischemic reperfusion, and even arrhythmia, such as atrial fibrillation. Despite this connection between oxidative stress and CVD, there are currently no recognized therapeutic interventions to address this important unmet need. Antioxidants that provide a broad, "upstream" approach via ROS/RNS quenching or free radical chain breaking seem an appropriate therapeutic option based on epidemiologic, dietary, and in vivo animal model data. However, human clinical trials with several different well-known agents, such as vitamin E and beta-carotene, have been disappointing. Does this mean antioxidants as a class are ineffective, or rather that the "right" compound(s) have yet to be found, their mechanisms of action understood, and their appropriate targeting and dosages determined? A large class of potent naturally-occurring antioxidants exploited by nature—the oxygenated carotenoids (xanthophylls)—have demonstrated utility in their natural form but have eluded development as successful targeted therapeutic agents up to the present time. This article characterizes the mechanism by which this novel group of antioxidants function and reviews their preclinical development. Results from multiple species support the antioxidant/anti-inflammatory properties of the prototype compound, astaxanthin, establishing it as an appropriate candidate for development as a therapeutic agent for cardiovascular oxidative stress and inflammation.

Am J Cardiol. 2008 May 22;101(10A):58D-68D

INHIBITION OF CHOROIDAL NEOVASCULARIZATION WITH AN ANTI-INFLAMMATORY CAROTENOID ASTAXANTHIN.

PURPOSE: Astaxanthin (AST) is a carotenoid found in marine animals and vegetables. The purpose of the present study was to investigate the effect of AST on the development of experimental choroidal neovascularization (CNV) with underlying cellular and

molecular mechanisms. METHODS: Laser photocoagulation was used to induce CNV in C57BL/6J mice. Mice were pretreated with intraperitoneal injections of AST daily for 3 days before photocoagulation, and treatments were continued daily until the end of the study. CNV response was analyzed by volumetric measurements 1 week after laser injury. Retinal pigment epithelium-choroid levels of IkappaB-alpha, intercellular adhesion molecule (ICAM)-1, monocyte chemoattractant protein (MCP)-1, interleukin (IL)-6, vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR)-1, and VEGFR-2 were examined by Western blotting or ELISA. AST was applied to capillary endothelial (b-End3) cells, macrophages, and RPE cells to analyze the activation of NF-kappaB and the expression of inflammatory molecules. RESULTS: The index of CNV volume was significantly suppressed by treatment with AST compared with that in vehicle-treated animals. AST treatment led to significant inhibition of macrophage infiltration into CNV and of the in vivo and in vitro expression of inflammation-related molecules, including VEGF, IL-6, ICAM-1, MCP-1, VEGFR-1, and VEGFR-2. Importantly, AST suppressed the activation of the NF-kappaB pathway, including IkappaB-alpha degradation and p65 nuclear translocation. CONCLUSIONS: AST treatment, together with inflammatory processes including NF-kappaB activation, subsequent upregulation of inflammatory molecules, and macrophage infiltration, led to significant suppression of CNV development. The present study suggests the possibility of AST supplementation as a therapeutic strategy to suppress CNV associated with AMD.

Invest Ophthalmol Vis Sci. 2008 Apr;49(4):1679-85

SUPPRESSIVE EFFECTS OF ASTAXANTHIN AGAINST RAT ENDOTOXIN-INDUCED UVEITIS BY INHIBITING THE NF-KAPPAB SIGNALING PATHWAY.

We investigated the effects of astaxanthin (AST), a carotenoid, on endotoxin-induced uveitis (EIU), and over the course of the disease measured the expression of inflammatory cytokines and chemokines in the presence or absence of AST. EIU was induced in male Lewis rats by footpad injection of lipopolysaccharide (LPS). The animals were randomly divided to 12 groups with eight animals in each. Immediately after the inoculation, AST (1, 10, or 100 mg kg⁻¹) was injected intravenously. Aqueous humour was collected at 6, 12 and 24 hr after LPS inoculation and the number of infiltrating cells in the anterior chamber was counted. In addition, we assayed the concentration of protein, nitric oxide (NO), tumour necrosis factor-alpha (TNF-alpha) and prostaglandin E2 (PGE2). Immunohistochemical staining with a monoclonal antibody against activated NF-kappaB was performed in order to evaluate the effects of AST on NF-kappaB activation. Rats injected with AST showed a significant decrease in the number of infiltrating cells in the anterior chamber and additionally there was a significantly lower concentration of protein, NO, TNF-alpha and PGE2 in the aqueous humour. Moreover, even early stages of EIU were suppressed by injection of AST. The number of activated NF-kappaB-positive cells was lower in iris-ciliary bodies treated with 10 or 100 mg kg⁻¹ AST at 3 hr after LPS injection. These results suggest that AST reduces ocular inflammation in eyes with EIU by downregulating proinflammatory factors and by inhibiting the NF-kappaB-dependent signaling pathway.

Exp Eye Res. 2006 Feb;82(2):275-81

EFFECTS OF ASTAXANTHIN ON LIPOPOLYSACCHARIDE-INDUCED INFLAMMATION IN VITRO AND IN VIVO.

PURPOSE: Astaxanthin (AST) is a carotenoid that is found in marine animals and vegetables. Several previous studies have demonstrated that AST exhibits a wide variety of biological activities including antioxidant, antitumor, and anti-Helicobacter pylori effects. In this study, attention was focused on the antioxidant effect of AST. The object of the present study was to investigate the efficacy of AST in endotoxin-induced uveitis (EIU) in rats. In addition, the effect of AST on endotoxin-induced nitric oxide (NO), prostaglandin E2 (PGE2), and tumor necrosis factor (TNF)-alpha production in a mouse macrophage cell line (RAW 264.7) was studied in vitro. METHODS: EIU was induced in male Lewis rats by a footpad injection of lipopolysaccharide (LPS). AST or prednisolone was administered intravenously at 30 minutes before, at the same time as, or at 30 minutes after LPS treatment. The number of infiltrating cells and protein concentration in the aqueous humor collected at 24 hours after LPS treatment was determined. RAW 264.7 cells were pretreated with various concentrations of AST for 24 hours and subsequently stimulated with 10 microg/mL of LPS for 24 hours. The levels of PGE2, TNF-alpha, and NO production were determined in vivo and in vitro. RESULTS: AST suppressed the development of EIU in a dose-dependent fashion. The anti-inflammatory effect of 100 mg/kg AST was as strong as that of 10 mg/kg prednisolone. AST also decreased production of NO, activity of inducible nitric oxide synthase (NOS), and production of PGE2 and TNF-alpha in RAW264.7 cells in vitro in a dose-dependent manner. CONCLUSIONS: This study suggests that AST has a dose-dependent ocular anti-inflammatory effect, by the suppression of NO, PGE2, and TNF-alpha production, through directly blocking NOS enzyme activity.

Invest Ophthalmol Vis Sci. 2003 Jun;44(6):2694-701

ANTIHYPERTENSIVE POTENTIAL AND MECHANISM OF ACTION OF ASTAXANTHIN: II. VASCULAR REACTIVITY AND HEMORHEOLOGY IN SPONTANEOUSLY HYPERTENSIVE RATS.

The current study was designed to determine the effects of a dietary astaxanthin (ASX-O) on vascular reactivity in spontaneously hypertensive rats (SHR), in order to verify its antihypertensive action mechanism. We evaluated contractions induced by phenylephrine (Phe), angiotensin II (Ang II) and the xanthine/xanthine oxidase (Xan/XOD) system, and relaxations induced by

sodium nitroprusside (SNP) as well as endothelium-dependent relaxations mediated by acetylcholine (ACh) in thoracic aorta of the SHR, with and without ASX-O intervention. We also investigated the effects of ASX-O on blood rheology using a microchannel array system. In this study, ASX-O showed a significant modulatory effect on nitric oxide (NO)-induced vasorelaxation by the NO-donor SNP ($p < 0.05$). However, it did not show significant effects in restoring the impaired endothelium-dependent relaxation to ACh in the SHR. On the other hand, the constrictive effects by Phe, Ang II and Xan/XOD were ameliorated by ASX-O ($p < 0.05$). ASX-O also demonstrated significant hemorheological effect by decreasing the microchannel transit time of whole blood. In conclusion, the results suggest that ASX-O may act in modulating the blood fluidity in hypertension, and that the antihypertensive effects of ASX-O may be exerted through mechanisms including normalization of the sensitivity of the adrenoceptor sympathetic pathway, particularly $[\alpha]$ -adrenoceptors, and by restoration of the vascular tone through attenuation of the Ang II- and reactive oxygen species (ROS)-induced vasoconstriction.

Biol Pharm Bull. 2005 Jun;28(6):967-71

ANTIHYPERTENSIVE POTENTIAL AND MECHANISM OF ACTION OF ASTAXANTHIN: III. ANTIOXIDANT AND HISTOPATHOLOGICAL EFFECTS IN SPONTANEOUSLY HYPERTENSIVE RATS.

We investigated the effects of a dietary astaxanthin (ASX-O) on oxidative parameters in spontaneously hypertensive rats (SHR), by determination of the level of nitric oxide (NO) end products nitrite/nitrate ($\text{NO}_2/\text{NO}_3^-$) and lipid peroxidation in ASX-O-treated SHR. Oral administration of the ASX-O significantly reduced the plasma level of $\text{NO}_2/\text{NO}_3^-$ compared to the control vehicle ($p < 0.05$). The lipid peroxidation level, however, was reduced in both ASX-O- and olive oil-treated groups. We also analyzed the post-treatment effects of ASX-O on the vascular tissues by examining the changes in the aorta and coronary arteries and arterioles. The dietary ASX-O showed significant reduction in the elastin bands in the rat aorta ($p < 0.05$). It also significantly decreased the [wall : lumen] aerial ratio of the coronary arteries. These results suggest that ASX-O can modulate the oxidative condition and may improve vascular elastin and arterial wall thickness in hypertension.

Biol Pharm Bull. 2006 Apr;29(4):684-8

ANTIHYPERTENSIVE AND NEUROPROTECTIVE EFFECTS OF ASTAXANTHIN IN EXPERIMENTAL ANIMALS.

Astaxanthin is a natural antioxidant carotenoid that occurs in a wide variety of living organisms. We investigated, for the first time, antihypertensive effects of astaxanthin (ASX-O) in spontaneously hypertensive rats (SHR). Oral administration of ASX-O for 14 d induced a significant reduction in the arterial blood pressure (BP) in SHR but not in normotensive Wistar Kyoto (WKY) strain. The long-term administration of ASX-O (50 mg/kg) for 5 weeks in stroke prone SHR (SHR-SP) induced a significant reduction in the BP. It also delayed the incidence of stroke in the SHR-SP. To investigate the action mechanism of ASX-O, the effects on PGF(2 α)-induced contractions of rat aorta treated with NG-nitro-L-arginine methyl ester (L-NAME) were studied in vitro. ASX-O (1 to 10 μM) induced vasorelaxation mediated by nitric oxide (NO). The results suggest that the antihypertensive effect of ASX-O may be due to a NO-related mechanism. ASX-O also showed significant neuroprotective effects in ischemic mice, presumably due to its antioxidant potential. Pretreatment of the mice with ASX-O significantly shortened the latency of escaping onto the platform in the Morris water maze learning performance test. In conclusion, these results indicate that astaxanthin can exert beneficial effects in protection against hypertension and stroke and in improving memory in vascular dementia.

Biol Pharm Bull. 2005 Jan;28(1):47-52

ASTAXANTHIN LIMITS EXERCISE-INDUCED SKELETAL AND CARDIAC MUSCLE DAMAGE IN MICE.

Dietary antioxidants may attenuate oxidative damage from strenuous exercise in various tissues. Beneficial effects of the antioxidant astaxanthin have been demonstrated in vitro, but not yet in vivo. We investigated the effect of dietary supplementation with astaxanthin on oxidative damage induced by strenuous exercise in mouse gastrocnemius and heart. C57BL/6 mice (7 weeks old) were divided into groups: rested control, intense exercise, and exercise with astaxanthin supplementation. After 3 weeks of exercise acclimation, both exercise groups ran on a treadmill at 28 m/min until exhaustion. Exercise-increased 4-hydroxy-2-nonenal-modified protein and 8-hydroxy-2'-deoxyguanosine in gastrocnemius and heart were blunted in the astaxanthin group. Increases in plasma creatine kinase activity, and in myeloperoxidase activity in gastrocnemius and heart, also were lessened by astaxanthin. Astaxanthin showed accumulation in gastrocnemius and heart from the 3 week supplementation. Astaxanthin can attenuate exercise-induced damage in mouse skeletal muscle and heart, including an associated neutrophil infiltration that induces further damage.

Antioxid Redox Signal. 2003 Feb;5(1):139-44

EFFECTS OF ASTAXANTHIN SUPPLEMENTATION ON EXERCISE-INDUCED FATIGUE IN MICE.

The present study was designed to determine the effect of astaxanthin on endurance capacity in male mice aged 4 weeks. Mice

were given orally either vehicle or astaxanthin (1.2, 6, or 30 mg/kg body weight) by stomach intubation for 5 weeks. The astaxanthin group showed a significant increase in swimming time to exhaustion as compared to the control group. Blood lactate concentration in the astaxanthin groups was significantly lower than in the control group. In the control group, plasma non-esterified fatty acid (NEFA) and plasma glucose were decreased by swimming exercise, but in the astaxanthin group, NEFA and plasma glucose were significantly higher than in the control group. Astaxanthin treatment also significantly decreased fat accumulation. These results suggest that improvement in swimming endurance by the administration of astaxanthin is caused by an increase in utilization of fatty acids as an energy source.

Biol Pharm Bull. 2006 Oct;29(10):2106-10

Vitamin K

MENATETRENONE, A VITAMIN K2 ANALOGUE, INHIBITS HEPATOCELLULAR CARCINOMA CELL GROWTH BY SUPPRESSING CYCLIN D1 EXPRESSION THROUGH INHIBITION OF NUCLEAR FACTOR KAPPA B ACTIVATION.

PURPOSE: Menatetrenone, a vitamin K2 analogue, plays an important role in the production of blood coagulation factors. Menatetrenone has also been shown to have antineoplastic effects against several cancer cell lines including hepatocellular carcinoma (HCC) cells. However, the mechanisms by which vitamin K2 inhibits HCC cell growth have not been fully clarified, and we therefore investigated the molecular basis of vitamin K2-induced growth inhibition of HCC cells. **EXPERIMENTAL DESIGN:** HCC cells were treated with vitamin K2 and the expression of several growth-related genes including cyclin-dependent kinase inhibitors and cyclin D1 was examined at the mRNA and protein levels. A reporter gene assay of the cyclin D1 promoter was done under vitamin K2 treatment. The regulation of nuclear factor kappaB (NF-kappaB) activation was investigated by a NF-kappaB reporter gene assay, an electrophoretic mobility shift assay, a Western blot for phosphorylated I kappa B, and an in vitro kinase assay for I kappa B kinase (IKK). We also examined the effect of vitamin K2 on the growth of HCC cells transfected with p65 or cyclin D1. **RESULTS:** Vitamin K2 inhibited cyclin D1 mRNA and protein expression in a dose-dependent manner in the HCC cells. Vitamin K2 also suppressed the NF-kappaB binding site-dependent cyclin D1 promoter activity and suppressed the basal, 12-O-tetradecanoylphorbol-13-acetate (TPA)-, TNF-alpha-, and interleukin (IL)-1-induced activation of NF-kappaB binding and transactivation. Concomitant with the suppression of NF-kappaB activation, vitamin K2 also inhibited the phosphorylation and degradation of I kappa Balpha and suppressed IKK kinase activity. Moreover, HCC cells overexpressing cyclin D1 and p65 became resistant to vitamin K2 treatment. **CONCLUSION:** Vitamin K2 inhibits the growth of HCC cells via suppression of cyclin D1 expression through the IKK/I kappa B/NF-kappaB pathway and might therefore be useful for treatment of HCC.

Clin Cancer Res. 2007 Apr 1;13(7):2236-45

THE EFFECT OF MENATETRENONE, A VITAMIN K2 ANALOG, ON DISEASE RECURRENCE AND SURVIVAL IN PATIENTS WITH HEPATOCELLULAR CARCINOMA AFTER CURATIVE TREATMENT: A PILOT STUDY.

BACKGROUND: The high recurrence rate of hepatocellular carcinoma (HCC) determines the long-term prognosis for patients with HCC. In the current study, the authors tested the effects of menatetrenone, a vitamin K2 analog, on recurrent HCC and survival after curative treatment. **METHODS:** Sixty-one patients who were diagnosed as free of HCC after surgical resection or percutaneous local ablation were assigned randomly to either a menatetrenone group (n = 32 patients) or a control group (n = 29 patients). Patients in the menatetrenone group received a daily oral dose of 45 mg of menatetrenone. Disease recurrence and survival rates were analyzed in patients with HCC. **RESULTS:** The cumulative recurrence rates in the menatetrenone group were 12.5% at 12 months, 39.0% at 24 months, and 64.3% at 36 months; and the corresponding recurrence rates in the control group were 55.2%, 83.2%, and 91.6%, respectively (P = 0.0002). Similar results were obtained even for patients who had low baseline levels of serum des-gamma-carboxy-prothrombin. Univariate and multivariate Cox proportional hazard analyses showed that the administration of menatetrenone was the only factor related to the recurrence rate of HCC. The cumulative survival rates for the patients who received menatetrenone were 100% at 12 months, 96.6% at 24 months, and 87.0% at 36 months; and the corresponding survival rates for patients in the control group were 96.4%, 80.9%, and 64.0%, respectively (P = 0.051). **CONCLUSIONS:** The current study findings suggested that menatetrenone may have a suppressive effect on recurrence of HCC and a beneficial effect on survival, although a larger, placebo-controlled trial will be required to prove these effects.

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DIETARY INTAKE OF VITAMIN K AND RISK OF PROSTATE CANCER IN THE HEIDELBERG COHORT OF THE EUROPEAN PROSPECTIVE INVESTIGATION INTO CANCER AND NUTRITION (EPIC-HEIDELBERG).

BACKGROUND: Anticarcinogenic activities of vitamin K have been observed in various cancer cell lines, including prostate cancer cells. Epidemiologic studies linking dietary intake of vitamin K with the development of prostate cancer have not yet been conducted. **OBJECTIVE:** We evaluated the association between dietary intake of phyloquinone (vitamin K1) and menaquinones (vitamin K2) and total and advanced prostate cancer in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition. **DESIGN:** At baseline, habitual dietary intake was assessed by means of a food-frequency questionnaire. Dietary intake of phyloquinone and menaquinones (MK-4-14) was estimated by using previously published HPLC-based food-content data. Multivariate-adjusted relative risks of total and advanced prostate cancer in relation to intakes of phyloquinone and

menaquinones were calculated in 11 319 men by means of Cox proportional hazards regression. RESULTS: During a mean follow-up time of 8.6 y, 268 incident cases of prostate cancer, including 113 advanced cases, were identified. We observed a nonsignificant inverse association between total prostate cancer and total menaquinone intake [multivariate relative risk (highest compared with lowest quartile): 0.65; 95% CI: 0.39, 1.06]. The association was stronger for advanced prostate cancer (0.37; 0.16, 0.88; P for trend = 0.03). Menaquinones from dairy products had a stronger inverse association with advanced prostate cancer than did menaquinones from meat. Phylloquinone intake was unrelated to prostate cancer incidence (1.02; 0.70, 1.48). CONCLUSIONS: Our results suggest an inverse association between the intake of menaquinones, but not that of phylloquinone, and prostate cancer. Further studies of dietary vitamin K and prostate cancer are warranted.

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TUMOR-SPECIFICITY AND TYPE OF CELL DEATH INDUCED BY VITAMIN K2 DERIVATIVES AND PRENYLALCOHOLS.

Fourteen vitamin K2 (menaquinone (MK)-n, n = 1-14) and ten prenyl alcohol derivatives (n = 1-10) with different numbers (n) of isoprenyl groups in the side chains were investigated for their cytotoxicity against nine human tumor cell lines and three human normal oral cells. Among the vitamin K2 derivatives, MK-2 (n = 2) showed the greatest cytotoxicity, followed by MK-1 (n = 1) and MK-3 (n = 3). MK-1, MK-2 and MK-3 showed the highest tumor-specific index (TS = > 2.0, 2.0 and > 1.7, respectively). Among the prenyl alcohols, geranylgeraniol (GG) (n = 4) showed the highest cytotoxicity, followed by farnesol (n = 3) and geranyl farnesol (GF) (n = 3). GG showed the highest tumor-specificity (TS = 1.8), followed by farnesol (TS = > 1.4), GF (TS = > 1.3). However, the tumor-specificity of MK-2 and GG was much lower than that of conventional chemotherapeutic agents. The human leukemic cell lines were the most sensitive, whereas the human glioblastoma cell lines were the most resistant to MK-2 and GG. MK-2 did not induce internucleosomal DNA fragmentation in either the human promyelocytic leukemia HL-60 or the human squamous cell carcinoma HSC-4 cell lines. GG induced marginal internucleosomal DNA fragmentation in the HL-60 cells, but not in the HSC-4 cells. Both MK-2 and GG did not induce the formation of autophagosomes, nor did they clearly change the intracellular concentration of three polyamines. Electron spin resonance (ESR) spectroscopy showed that only MK-1 (n = 1), as well as GGF (n = 7) and GFF (n = 8) which had lower cytotoxicity, produced radicals, suggesting the lack of connection between cytotoxicity and radical production. The present study demonstrates that the presence of 1,4-naphthoquinone structure (including alpha,beta-unsaturated ketones) in vitamin K2 derivatives confers on them the ability to induce non-apoptotic cell death.

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THE UTILITY OF VITAMIN K3 (MENADIONE) AGAINST PANCREATIC CANCER.

BACKGROUND: To evaluate the efficacy of vitamin K3 (VK3) against pancreatic cancer, the molecular mechanism of VK3 or gemcitabine (GEM)-induced inhibition of proliferation was characterized. MATERIALS AND METHODS: The cell viability was determined using the 3-[4,5-dimethylthiazol]-2,5-diphenyl tetrazolium bromide (MTT) test method. The expressions of cellular proteins were evaluated by Western blot analysis. For morphological studies of the in vivo transplanted cancer cells, the tissues were stained with hematoxylin and eosin. RESULTS: The IC50 of VK3 for pancreatic cancer cells was calculated for 42.1 +/- 3.5 microM. Western blot analysis showed that VK3 induced rapid phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun NH2-terminal kinase (JNK) 30 minutes after application. ERK but not JNK phosphorylation was maintained for at least 12 hours. Activation of apoptosis by VK3, as shown by molecular weight shifts of the pro-activated 32-kDa form of caspase-3 and poly(ADP-ribose)polymerase (PARP) cleavage of the 112-kDa form, was found. Treatment with the thiol antioxidant, L-cysteine (>0.2 mM), completely abrogated the VK3-induced phosphorylation of ERK, but not the JNK, and inhibition of proliferation. A caspase-3 inhibitor antagonized caspase-3 activation, but had no inhibitory effect on the proliferative activity of VK3. GEM at concentrations >0.1 microg/ml was found to inhibit cell proliferation after 24 hours. GEM also induced phosphorylation of JNK, activation of caspase-3 and accumulation of cyclin B1. Local application of VK3 was found to induce extensive tumor tissue necrosis, but slight hematemesis without necrosis was observed 48 hours after GEM injection. In Western blot, ERK but not JNK phosphorylation, was clearly detected in response to VK3 injection into the tumor tissue. CONCLUSION: The action of VK3 may lead to a favorable outcome against pancreatic cancer, and the detection of ERK phosphorylation in the tissue is important for predicting this effect.

Anticancer Res. 2008 Jan-Feb;28(1A):45-50

VITAMIN K2 INDUCES AUTOPHAGY AND APOPTOSIS SIMULTANEOUSLY IN LEUKEMIA CELLS.

Vitamin K2 (menaquinone-4: VK2) is a potent inducer for apoptosis in leukemia cells in vitro. HL-60bcl-2 cells, which are derived from a stable transfectant clone of the human bcl-2 gene into the HL-60 leukemia cell line, show 5-fold greater expression of the Bcl-2 protein compared with HL-60neo cells, a control clone transfected with vector alone. VK2 induces apoptosis in HL-60neo cells, whereas HL-60bcl-2 cells are resistant to apoptosis induction by VK2 but show inhibition of cell growth along with an increase of cytoplasmic vacuoles during exposure to VK2. Electron microscopy revealed formation of autophagosomes and autolysosomes in HL-60bcl-2 cells after exposure to VK2. An increase of acid vesicular organelles (AVOs) detected by acridine

orange staining for lysosomes as well as conversion of LC3B-I into LC3B-II by immunoblotting and an increased punctuated pattern of cytoplasmic LC3B by fluorescent immunostaining all supported induction of enhanced autophagy in response to VK2 in HL-60bcl-2 cells. However, during shorter exposure to VK2, the formation of autophagosomes was also prominent in HL-60neo cells although nuclear chromatin condensations and nuclear fragments were also observed at the same time. These findings indicated the mixed morphologic features of apoptosis and autophagy. Inhibition of autophagy by either addition of 3-methyladenine, siRNA for Atg7, or Tet-off Atg5 system all resulted in attenuation of VK2-included cell death, indicating autophagy-mediated cell death in response to VK2. These data demonstrate that autophagy and apoptosis can be simultaneously induced by VK2. However, autophagy becomes prominent when the cells are protected from rapid apoptotic death by a high expression level of Bcl-2.

Autophagy. 2008 Jul 1;4(5):629-40

VITAMIN K2-MEDIATED APOPTOSIS IN CANCER CELLS: ROLE OF MITOCHONDRIAL TRANSMEMBRANE POTENTIAL.

Vitamin K2 induces differentiation and apoptosis in a wide array of human cancer cell lines. Vitamin K2-mediated apoptosis proceeds much more slowly than the apoptosis induced by conventional anticancer agents. Thus, it is possible to analyze the underlying mechanism in detail. In this chapter, we focus on the pro-apoptotic effects of vitamin K2 on mitochondrial physiology with particular emphasis on changes in mitochondrial membrane potential ($\Delta\psi_m$). Upon treatment of ovarian cancer TYK-nu cells with vitamin K2, superoxide is produced after two to three days, followed shortly thereafter by release of mitochondrial cytochrome c. This is accompanied by other apoptotic features such as characteristic morphological changes and DNA fragmentation by day four. Data suggest that superoxide production might cause damage to mitochondrial membranes, open permeability transition pores, and result in disruption of $\Delta\psi_m$ with subsequent release of cytochrome c. Both vitamin K2-induced production of superoxide and reduction of $\Delta\psi_m$ are completely inhibited by alpha-tocopherol such that cell viability is retained. Thus, we propose that the loss of $\Delta\psi_m$ caused by superoxide might be the major cause of apoptosis following exposure to vitamin K2. However, other pathways may be involved since cyclosporin A failed to completely inhibit vitamin K2-induced apoptosis.

Vitam Horm. 2008;78:211-26

VITAMIN K-DEPENDENT ACTIONS OF GAS6.

Gas6 (growth arrest-specific gene 6) is the last addition to the family of plasma vitamin K-dependent proteins. Gas6 was cloned and characterized in 1993 and found to be similar to the plasma anticoagulant protein S. Soon after it was recognized as a growth factor-like molecule, as it interacted with receptor tyrosine kinases (RTKs) of the TAM family; Tyro3, Axl, and MerTK. Since then, the role of Gas6, protein S, and the TAM receptors has been found to be important in inflammation, hemostasis, and cancer, making this system an interesting target in biomedicine. Gas6 employs a unique mechanism of action, interacting through its vitamin K-dependent Gla module with phosphatidylserine-containing membranes and through its carboxy-terminal LG domains with the TAM membrane receptors. The fact that these proteins are affected by anti-vitamin K therapy is discussed in detail.

Vitam Horm. 2008;78:185-209

VITAMIN K2 SUPPRESSES MALIGNANCY OF HUH7 HEPATOMA CELLS VIA INHIBITION OF CONNEXIN 43.

The anti-cancer potential of vitamin K(2) (VK(2)) in hepatoma has gained considerable attention but the underlying mechanisms are unclear. Treatment of HuH7 hepatoma cells with VK(2) produced a normal liver phenotype. Following treatment of cells with VK(2), there was an increase in gap junctional intercellular communication activity, accompanied by up-regulation of connexin 32 (Cx32), dominantly expressed in normal hepatocyte. In contrast, Cx43 expression was inhibited. Moreover, the effect of VK(2) on Cx32 was abolished by over-expression of Cx43. Taken together, we propose that the anti-tumor effect of VK(2) is at least partly due to a decrease in Cx43 promoter activity.

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JAPANESE FERMENTED SOYBEAN FOOD AS THE MAJOR DETERMINANT OF THE LARGE GEOGRAPHIC DIFFERENCE IN CIRCULATING LEVELS OF VITAMIN K2: POSSIBLE IMPLICATIONS FOR HIP-FRACTURE RISK.

Increasing evidence indicates a significant role for vitamin K in bone metabolism and osteoporosis. In this study, we found a large geographic difference in serum vitamin K2 (menaquinone-7; MK-7) levels in postmenopausal women. Serum MK-7 concentrations were 5.26 +/- 6.13 ng/mL (mean +/- SD) in Japanese women in Tokyo, 1.22 +/- 1.85 in Japanese women in Hiroshima, and 0.37 +/- 0.20 in British women. We investigated the effect of Japanese fermented soybean food, natto, on serum vitamin K levels. Natto contains a large amount of MK-7 and is eaten frequently in eastern (Tokyo) but seldom in western (Hiroshima) Japan.

Serum concentrations of MK-7 were significantly higher in frequent natto eaters, and natto intake resulted in a marked, sustained increase in serum MK-7 concentration. We analyzed the relation between the regional difference in natto intake and fracture incidence. A statistically significant inverse correlation was found between incidence of hip fractures in women and natto consumption in each prefecture throughout Japan. These findings indicate that the large geographic difference in MK-7 levels may be ascribed, at least in part, to natto intake and suggest the possibility that higher MK-7 level resulting from natto consumption may contribute to the relatively lower fracture risk in Japanese women.

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