

Journal ABSTRACTS

Zinc-Carnosine

NOVEL THERAPEUTIC APPROACHES TO GASTRIC AND DUODENAL ULCERS: AN UPDATE.

Over the last 25 years, a remarkable revolution in the pathophysiology and treatment of gastric and duodenal ulcers has occurred. Effective therapies were developed not only to heal ulcers, but also to cure most patients. The two principal causes for gastric and duodenal ulcers are either infection with *Helicobacter pylori* or the use of non-steroidal anti-inflammatory drugs (NSAIDs). With *H. pylori* eradication, gastric and duodenal ulcers are rapidly becoming historical diseases. This communication reviews the salient pharmacology of the novel anti-ulcer drugs currently in development, with particular emphasis on the treatment of gastric and duodenal ulcers. Intense research is currently focused on the development of proton pump inhibitors primarily for the treatment and prevention of gastroesophageal reflux disease. The older proton pump inhibitors, omeprazole and lansoprazole, are effective in healing gastric and duodenal ulcers. Furthermore, both drugs are effective in eradicating *H. pylori* when given with various antibiotics. Pantoprazole, rabeprazole and esomeprazole are new proton pump inhibitors, which appear to have comparable therapeutic profiles with omeprazole and lansoprazole. Rebamipide is a new mucosal protective drug, which is effective in healing gastric ulcers. Polaprezinc and nocloprost are also mucosal protective drugs, which are in clinical development. However, none of these three cytoprotective drugs have been evaluated for their efficacy in eradicating *H. pylori* when given in combination with antibiotics. Likewise, no published literature exists on the use of these drugs for preventing NSAID-induced ulcers. With the rapid eradication of *H. pylori* currently happening in the developed world, the therapeutic challenge is now directed toward preventing NSAID-associated ulcer. Significant reduction of NSAID-induced ulcers is achieved by using continuous prophylactic anti-ulcer therapy (misoprostol or omeprazole) or by using NSAIDs possessing selective COX-2 inhibitory activity. However, outcome clinical studies are needed to compare the adjuvant anti-ulcer therapies given with COX-1 inhibitors versus the selective COX-2 inhibitors given alone.

Expert Opin Investig Drugs. 2000 Jul;9(7):1537-44

PATHOGENESIS OF HELICOBACTER PYLORI INFECTION.

Helicobacter pylori is the first formally recognized bacterial carcinogen and is one of the most successful human pathogens, as over half of the world's population is colonized with this gram-negative bacterium. Unless treated, colonization usually persists lifelong. *H. pylori* infection represents a key factor in the etiology of various gastrointestinal diseases, ranging from chronic active gastritis without clinical symptoms to peptic ulceration, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma. Disease outcome is the result of the complex interplay between the host and the bacterium. Host immune gene polymorphisms and gastric acid secretion largely determine the bacterium's ability to colonize a specific gastric niche. Bacterial virulence factors such as the cytotoxin-associated gene pathogenicity island-encoded protein CagA and the vacuolating cytotoxin VacA aid in this colonization of the gastric mucosa and subsequently seem to modulate the host's immune system. This review focuses on the microbiological, clinical, immunological, and biochemical aspects of the pathogenesis of *H. pylori*.

Clin Microbiol Rev. 2006 Jul;19(3):449-90

ZINC CARNOSINE, A HEALTH FOOD SUPPLEMENT THAT STABILISES SMALL BOWEL INTEGRITY AND STIMULATES GUT REPAIR PROCESSES.

BACKGROUND: Zinc carnosine (ZnC), is a health food product claimed to possess health-promoting and gastrointestinal supportive activity. Scientific evidence underlying these claims is, however, limited. We, therefore, examined the effect of ZnC on various models of gut injury and repair and in a clinical trial. **METHODS:** In vitro studies utilised pro-migratory (wounded monolayer) and proliferation ($[^3\text{H}]$ thymidine incorporation) assays of human colonic (HT29), rat intestinal (RIE) and canine kidney (MDCK) epithelial cells. In vivo studies utilised rat gastric (indomethacin/restraint) and mouse small intestinal (indomethacin) damage models. Healthy volunteers (n=10) undertook a randomised cross-over trial comparing changes in gut permeability (lactulose/rhamnose ratios, L/R) before and after 5 days of indomethacin (50 mg tds, po.) with ZnC (37.5 mg bd) or placebo co-administration. **RESULTS:** ZnC stimulated migration and proliferation in a dose-dependent manner (maximum effects in both assays at 100microM using HT29 cells), causing an approximate three fold increase in migration or proliferation (both p < 0.01).

Oral ZnC decreased gastric (75% reduction at 5mg/ml) and small intestinal injury (50% reduction in villus shortening at 40 mg/ml), both $p < 0.01$. In volunteers, indomethacin caused a three- fold increase in gut permeability in the control arm; L/R ratio 0.35 +/- 0.035 prior to indomethacin and 0.88 +/- 0.11 (mean +/- SEM) after 5 days indomethacin ($p < 0.01$), whereas no significant increase in permeability was seen when ZnC was co-administered. CONCLUSION: These initial studies suggest that ZnC, at concentrations likely to be found in the gut lumen, stabilises gut mucosa. Further studies appear warranted.

Gut. 2006 Jun 15

ANTI-INFLAMMATORY EFFECT OF ROASTED LICORICE EXTRACTS ON LIPOPOLYSACCHARIDE-INDUCED INFLAMMATORY RESPONSES IN MURINE MACROPHAGES.

Licorice, the roots of *Glycyrrhiza inflata*, is used by practitioners of alternative medicine to treat individuals with gastric or duodenal ulcers, bronchitis, cough, arthritis, adrenal insufficiency, and allergies. We investigated the anti-inflammatory properties of 4 licorice extracts: extracts of roasted licorice obtained by ethanol (rLE) or water extraction (rLW) and extracts of raw licorice obtained by ethanol (LE) or water extraction (LW). rLE demonstrated strong anti-inflammatory activity through its ability to reduce nitric oxide and prostaglandin E(2) production in the LPS-stimulated mouse macrophage cell, RAW264.7. It also inhibited the production of pro-inflammatory cytokines and CD14 expression on the LPS-stimulated RAW264.7 cells. Further study indicated that LPS-induced degradation and phosphorylation of I κ B- α , along with DNA-binding of NF- κ B, was significantly inhibited by rLE exposure in RAW264.7 cells. In the murine model, we found that in vivo exposure to rLE-induced an increase in the survival rate, reduced plasma levels of TNF- α and IL-6, and increased IL-10 production in LPS-treated mice. Collectively, these data suggest that the use of rLE may be a useful therapeutic approach to various inflammatory diseases.

Biochem Biophys Res Commun. 2006 Jul 7;345(3):1215-23

RISK AND SAFETY ASSESSMENT ON THE CONSUMPTION OF LICORICE ROOT (GLYCYRRHIZA SP.), ITS EXTRACT AND POWDER AS A FOOD INGREDIENT, WITH EMPHASIS ON THE PHARMACOLOGY AND TOXICOLOGY OF GLYCYRRHIZIN.

Licorice (or 'liquorice') is a plant of ancient origin and steeped in history. Licorice extracts and its principle component, glycyrrhizin, have extensive use in foods, tobacco and in both traditional and herbal medicine. As a result, there is a high level of use of licorice and glycyrrhizin in the US with an estimated consumption of 0.027-3.6mg glycyrrhizin/kg/day. Both products have been approved for use in foods by most national and supranational regulatory agencies. Biochemical studies indicate that glycyrrhizates inhibit 11 β -hydroxysteroid dehydrogenase, the enzyme responsible for inactivating cortisol. As a result, the continuous, high level exposure to glycyrrhizin compounds can produce hypermineralocorticoid-like effects in both animals and humans. These effects are reversible upon withdrawal of licorice or glycyrrhizin. Other in vivo and clinical studies have reported beneficial effects of both licorice and glycyrrhizin consumption including anti-ulcer, anti-viral, and hepatoprotective responses. Various genotoxic studies have indicated that glycyrrhizin is neither teratogenic nor mutagenic, and may possess anti-genotoxic properties under certain conditions. The pharmacokinetics of glycyrrhizin have been described and show that its bioavailability is reduced when consumed as licorice; this has hampered attempts to establish clear dose-effect levels in animals and humans. Based on the in vivo and clinical evidence, we propose an acceptable daily intake of 0.015-0.229mg glycyrrhizin/kg body weight/day.

Regul Toxicol Pharmacol. 2006 Jul 31

PREVENTION OF NONSPECIFIC BACTERIAL CELL ADHESION IN IMMUNOASSAYS BY USE OF CRANBERRY JUICE.

The ability of *Vaccinium macrocarpon*, the North American cranberry, to prevent bacterial adhesion has been used to advantage in the prevention of urinary tract infections and has recently been described for the prevention of adhesion of bacteria responsible for oral infections and stomach ulcers. This report documents the ability of cranberry juice to reduce nonspecific adhesion of bacteria to the borosilicate glass microscope slides used in an immunoarray biosensor format. Nonspecific binding of analytes in the array sensor leads to high background signals that cause increased detection limits and false positives. Reduction in background-to-signal ratios can be seen as the juice concentration is increased from 0 to 50% of the sample. This impact cannot be duplicated with grape, orange, apple, or white cranberry juice. Sugar content and pH have been eliminated as the agents in the juice responsible for the anti-adhesive activity.

Anal Chem. 2006 Feb 1;78(3):853-7

INHIBITION OF HELICOBACTER PYLORI IN VITRO BY VARIOUS BERRY EXTRACTS, WITH ENHANCED SUSCEPTIBILITY TO CLARITHROMYCIN.

The objective of this study was to evaluate the effects of various berry extracts, with and without clarithromycin on *Helicobacter*

pylori. Resistance to clarithromycin by *H. pylori* has been reported, leading to interest in alternatives/adjuncts to therapy with clarithromycin. *H. pylori* American type culture collection (ATCC) strain 49503 was grown, cell suspensions were made in PBS and diluted 10-fold. One hundred microL of the suspension was then incubated for 18 h with extracts of raspberry, strawberry, cranberry, elderberry, blueberry, bilberry, and OptiBerry, a blend of the six berries, at 0.25-1% concentrations. Serially diluted cell suspensions were exposed for 1 h to clarithromycin at 15 microg/ml. Ten microl of bacterial samples from the 10(-7) dilution tube were plated and incubated for 18 h and the number of colonies were counted. Growth of *H. pylori* was confirmed by the CLO test. All berry extracts significantly ($p < 0.05$) inhibited *H. pylori*, compared with controls, and also increased susceptibility of *H. pylori* to clarithromycin, with OptiBerry demonstrating maximal effects.

Mol Cell Biochem. 2004 Oct;265(1-2):19-26

EFFICACY OF CRANBERRY JUICE ON HELICOBACTER PYLORI INFECTION: A DOUBLE-BLIND, RANDOMIZED PLACEBO-CONTROLLED TRIAL.

BACKGROUND: *Helicobacter pylori* infection is a major cause of peptic ulcer disease and gastric cancer. This study postulated that cranberry juice would be effective in the suppression of *H. pylori* in an endemically infected population at high risk for gastric cancer. **MATERIALS AND METHODS:** A prospective, randomized, double-blind, placebo-controlled trial was conducted in Linqu County of Shandong Province, China, where 189 adults aged 48.9 +/- 11.2 years (mean +/- SD) with *H. pylori* infection were randomly divided into two groups: cranberry juice (n = 97) and placebo (n = 92). Participants were assigned to orally receive two 250-ml juice boxes of cranberry juice or matching placebo beverage daily for 90 days. The degree of *H. pylori* infection was determined using the 13C-urea breath test before randomization at 35 and 90 days of intervention to assess the efficacy of cranberry juice in alleviating infection. **RESULTS:** A total of 189 subjects with positive 13C-urea breath test results prior to randomization completed the study. At day 35 of intervention, 14 of the 97 (14.43%) from the the cranberry juice treatment group and 5 of the 92 (5.44%) of the placebo recipients had negative 13C-urea breath test results. After 90 days, the study concluded that 14 of the 97 subjects in the cranberry juice treatment group versus 5 of the 92 in the placebo group yielded negative test results. Eleven individuals from the cranberry juice treatment group and only two from the placebo group were negative at 35 and 90 days of experiment. These results are significant ($p < .05$). **CONCLUSIONS:** Regular consumption of cranberry juice can suppress *H. pylori* infection in endemically afflicted populations.

Helicobacter. 2005 Apr;10(2):139-45

ENHANCING HEALTH BENEFITS OF BERRIES THROUGH PHENOLIC ANTIOXIDANT ENRICHMENT: FOCUS ON CRANBERRY.

Emerging epidemiological evidence is increasingly pointing to the beneficial effects of fruits and vegetables in managing chronic and infectious diseases. These beneficial effects are now suggested to be due to the constituent phenolic phytochemicals having antioxidant activity. Cranberry like other fruits is also rich in phenolic phytochemicals such as phenolic acids, flavonoids and ellagic acid. Consumption of cranberry has been historically been linked to lower incidences of urinary tract infections and has now been shown to have a capacity to inhibit peptic ulcer-associated bacterium, *Helicobacter pylori*. Isolated compounds from cranberry have also been shown to reduce the risk of cardiovascular diseases. Recent evidence suggests the ability of phytochemical components in whole foods in being more effective in protectively supporting human health than compared to isolated individual phenolic phytochemicals. This implies that the profile of phenolic phytochemicals determines the functionality of the whole food as a result of synergistic interaction of constituent phenolic phytochemicals. Solid state bioprocessing using food grade fungi common in Asian food cultures as well as cranberry phenolic synergies through the addition of functional biphenyls such as ellagic acid and rosmarinic acid along with processed fruit extracts have helped to advance these concepts. These strategies could be further explored to enrich cranberry and cranberry products with functional phytochemicals and further improve their functionality for enhancing health benefits.

Asia Pac J Clin Nutr. 2005;14(2):120-30

INHIBITION OF HELICOBACTER PYLORI ADHESION TO HUMAN GASTRIC MUCUS BY A HIGH-MOLECULAR-WEIGHT CONSTITUENT OF CRANBERRY JUICE.

A high-molecular-weight constituent of cranberry juice has been found to inhibit the sialyllactose specific adhesion of *Helicobacter pylori* strains to immobilized human mucus, erythrocytes, and cultured gastric epithelial cells. Different isolates of *H. pylori* differ in their affinity to the cranberry juice constituent. Cranberry juice may also inhibit adhesion of bacteria to the stomach in vivo, and may prove useful for the prevention of stomach ulcer that is caused by *H. pylori*.

Crit Rev Food Sci Nutr. 2002;42(3 Suppl):279-84

INHIBITORY EFFECT OF POLAPREZINC ON THE INFLAMMATORY RESPONSE TO HELICOBACTER PYLORI.

Helicobacter pylori-infected gastrointestinal mucosa is frequently infiltrated by polymorphonuclear leukocytes (PMN) and monocytes, and these invading cells have been implicated in gastrointestinal mucosal inflammation. To clarify the efficacy of polaprezinc, a chelate compound consisting of zinc and L-carnosine, against *H pylori*-induced inflammation including PMN infiltration, the *in vitro* effects of this drug on interleukin (IL)-8 production by an established gastric cancer cell line (MKN 45 cells) and on PMN-endothelial cell adhesive interactions was investigated. Polaprezinc and zinc sulphate inhibited IL-8 production by MKN 45 cells in response to stimulation with *H pylori* water extract (HPE) in a dose-dependent manner from 10^{-7} M to 10^{-5} M. In addition, the expression of CD11b and CD18 on PMN and PMN-dependent adhesion to endothelial cells elicited by HPE was inhibited by polaprezinc and zinc sulphate in a concentration-dependent manner. L-carnosine did not have any effects on IL-8 production or PMN-endothelial cell interactions. These results suggest that polaprezinc, mainly the zinc component, may inhibit *H pylori*-induced PMN-mediated gastric inflammation by attenuating CD11b/CD18 expression on PMN and IL-8 production from gastric epithelial cells.

Can J Gastroenterol. 2002 Nov;16(11):785-9

POLAPREZINC DOWN-REGULATES PROINFLAMMATORY CYTOKINE-INDUCED NUCLEAR FACTOR-KAPPA B ACTIVATION AND INTERLEUKIN-8 EXPRESSION IN GASTRIC EPITHELIAL CELLS.

Gastric epithelial chemokine response is a primary factor in the induction of gastric inflammation associated with *Helicobacter pylori* infection. Because sustained inflammation is a risk for gastric mucosal damage, agents that down-regulate inflammatory responses may be of therapeutic significance. We examined the effect of polaprezinc, a potent antiulcer agent, on proinflammatory cytokine-induced interleukin (IL)-8 expression in gastric epithelial cells. Because IL-8 expression is regulated by the transcription factor nuclear factor-kappaB (NF-kappaB), we also examined the effect of polaprezinc on NF-kappaB activity. MKN28 cells were used as a model of gastric epithelial cells. Secreted IL-8 was quantified by IL-8 specific enzyme-linked immunosorbent assay, and IL-8 mRNA expression was examined by Northern blot analysis. NF-kappaB activity was analyzed by electrophoretic mobility shift assay. Western blot analysis with anti-phospho-IkappaB-alpha antibody was performed to assess IkappaB-alpha phosphorylation. Polaprezinc-suppressed IL-8 secretion induced by tumor necrosis factor alpha (TNF-alpha) or IL-1beta in a dose-dependent manner. IL-8 mRNA expression also was inhibited by polaprezinc. NF-kappaB activation in response to TNF-alpha, IL-1beta, phorbol ester, and H₂O₂ was down-regulated by polaprezinc. Western blot analysis showed inhibition of TNF-alpha-induced IkappaB-alpha phosphorylation in the presence of polaprezinc. Collectively, these results suggest that polaprezinc is a novel type of anti-inflammatory agent that down-regulates inflammatory responses of gastric mucosal cells.

J Pharmacol Exp Ther. 1999 Oct;291(1):345-52

ADVANCED GLYCATION END PRODUCTS AND RAGE: A COMMON THREAD IN AGING, DIABETES, NEURODEGENERATION, AND INFLAMMATION.

The products of nonenzymatic glycation and oxidation of proteins and lipids, the advanced glycation end products (AGEs), accumulate in a wide variety of environments. AGEs may be generated rapidly or over long times stimulated by a range of distinct triggering mechanisms, thereby accounting for their roles in multiple settings and disease states. A critical property of AGEs is their ability to activate receptor for advanced glycation end products (RAGE), a signal transduction receptor of the immunoglobulin superfamily. It is our hypothesis that due to such interaction, AGEs impart a potent impact in tissues, stimulating processes linked to inflammation and its consequences. We hypothesize that AGEs cause perturbation in a diverse group of diseases, such as diabetes, inflammation, neurodegeneration, and aging. Thus, we propose that targeting this pathway may represent a logical step in the prevention/treatment of the sequelae of these disorders.

Glycobiology. 2005 Jul;15(7):16R-28R. Epub 2005 Mar 10

NEUROPATHOLOGIC CHANGES IN ALZHEIMER'S DISEASE: POTENTIAL TARGETS FOR TREATMENT.

The cognitive symptoms of Alzheimer's disease (AD) are believed to be caused not only by the loss of neurons in the cholinergic and glutamatergic neural systems but also by the irregular functioning of surviving neurons in these 2 systems. Aberrant cholinergic functioning in AD has been linked to deficits in the neurotransmitter acetylcholine, while AD-related abnormalities in glutamatergic signaling have been attributed to excitotoxicity caused by the persistent, low-level stimulation of glutamatergic neurons via the chronic influx of Ca(2+) ions through the N-methyl-D-aspartate (NMDA) receptor calcium channel. Glutamatergic abnormalities in AD can be corrected to some extent by the NMDA receptor antagonist memantine, an agent whose therapeutic efficacy is believed to be related to its low to moderate level of affinity for the NMDA receptor calcium channel, a characteristic that allows memantine to prevent excessive glutamatergic stimulation while still permitting normal glutamate-mediated neurotransmission to take place. Although the mechanism underlying the chronic stimulation of glutamatergic neurons in AD has yet to be elucidated, one hypothesis is that the characteristic neuropathologic features of AD — beta-amyloid deposits and neurofibrillary tangles — induce brain inflammation, which in turn impairs glutamatergic receptor function in such a way that the ability of these receptors to prevent the influx of Ca(2+) in the absence of an appropriate presynaptic signal is compromised. If this hypothesis is correct, and if it is correct that beta-amyloid deposits and neurofibrillary tangles arise long before the symptomatic onset of AD, then memantine, with its ability to alleviate glutamatergic receptor overstimulation, would be expected to provide therapeutic benefits beginning from the earliest stages of the disease.

J Clin Psychiatry. 2006;67 Suppl 3:3-7; quiz 23

MOLECULAR MECHANISMS FOR ALZHEIMER'S DISEASE: IMPLICATIONS FOR NEUROIMAGING AND THERAPEUTICS.

Alzheimer's disease is a progressive neurodegenerative disorder characterised by the gradual onset of dementia. The pathological hallmarks of the disease are beta-amyloid (Abeta) plaques, neurofibrillary tangles, synaptic loss and reactive gliosis. The current therapeutic effort is directed towards developing drugs that reduce Abeta burden or toxicity by inhibiting secretase cleavage, Abeta aggregation, Abeta toxicity, Abeta metal interactions or by promoting Abeta clearance. A number of clinical trials are currently in progress based on these different therapeutic strategies and they should indicate which, if any, of these approaches will be efficacious. Current diagnosis of Alzheimer's disease is made by clinical, neuropsychologic and neuroimaging assessments. Routine structural neuroimaging evaluation with computed tomography and magnetic resonance imaging is based on non-specific features such as atrophy, a late feature in the progression of the disease, hence the crucial importance of developing new approaches for early and specific recognition at the prodromal stages of Alzheimer's disease. Functional neuroimaging techniques such as functional magnetic resonance imaging, magnetic resonance spectroscopy, positron emission tomography and single photon emission computed tomography, possibly in conjunction with other related Abeta biomarkers in plasma and CSF, could prove to be valuable in the differential diagnosis of Alzheimer's disease, as well as in assessing prognosis. With the advent of new therapeutic strategies there is increasing interest in the development of magnetic resonance imaging contrast agents and positron emission tomography and single photon emission computed tomography radioligands that will permit the assessment of Abeta burden in vivo.

OXIDATIVE STRESS AND NEURODEGENERATION.

Oxidative stress is a well-studied early response in chronic neurodegenerative diseases, including Alzheimer's disease, where neuronal loss can exceed 90% in the vulnerable neuronal population. Oxidative stress affects all classes of macromolecules (sugar, lipids, proteins, and DNA), leading inevitably to neuronal dysfunction. We observed that Nepsilon-(carboxymethyl)lysine (CML), the predominant advanced glycation end product that accumulates in vivo, along with its glycation-specific precursor hexitol-lysine, are increased in neurons from cases of Alzheimer's disease, especially those containing intracellular neurofibrillary pathology. The increase in hexitol-lysine and CML can result from either lipid peroxidation or advanced glycation, whereas hexitol-lysine is solely a product of glycation, suggesting that two distinct oxidative processes act in concert in the neuropathology of the disease. Furthermore, using olfactory neurons as an experimental model, we observed an increase in glycation products in neurons derived from Alzheimer's disease patients. Our findings support the idea that aldehyde-mediated modifications, in concert with oxyradical-mediated modifications, are critical early pathogenic factors in Alzheimer's disease.

Ann N Y Acad Sci. 2005 Jun;1043:545-52

HYPERINSULINEMIA PROVOKES SYNCHRONOUS INCREASES IN CENTRAL INFLAMMATION AND BETA-AMYLOID IN NORMAL ADULTS.

BACKGROUND: Inflammation has been implicated as a pathogenetic factor in Alzheimer disease, possibly via effects on beta-amyloid (Abeta). Hyperinsulinemia induces inflammation and is a risk factor for Alzheimer disease. Thus, insulin abnormalities may contribute to Alzheimer disease pathophysiology through effects on the inflammatory network. **OBJECTIVES:** To determine the effects of induced hyperinsulinemia with euglycemia on Abeta, transthyretin, and inflammatory markers and modulators in plasma and cerebrospinal fluid (CSF). **DESIGN:** Randomized crossover trial. **SETTING:** Veterans Affairs hospital clinical research unit. **PARTICIPANTS:** Sixteen healthy adults ranging from 55 to 81 years of age (mean age, 68.2 years). **INTERVENTIONS:** On separate mornings, fasting participants received randomized infusions of saline or insulin (1.0 mU.kg(-1).min(-1)) with variable dextrose levels to maintain euglycemia, achieving plasma insulin levels typical of insulin resistance. Plasma and CSF were collected after an approximately 105-minute infusion. **MAIN OUTCOME MEASURES:** Plasma and CSF levels of interleukin 1alpha, interleukin 1beta, interleukin 6, tumor necrosis factor alpha, F2-isoprostane (CSF only), Abeta, norepinephrine, transthyretin, and apolipoprotein E. **RESULTS:** Insulin increased CSF levels of F2-isoprostane and cytokines (both $P < .01$), as well as plasma and CSF levels of Abeta42 (both $P < .05$). The changes in CSF levels of Abeta42 were predicted by increased F2-isoprostane and cytokine levels (both $P < .01$) and reduced transthyretin levels ($P = .02$). Increased inflammation was modulated by insulin-induced changes in CSF levels of norepinephrine and apolipoprotein E (both $P < .05$). **CONCLUSION:** Moderate hyperinsulinemia can elevate inflammatory markers and Abeta42 in the periphery and the brain, thereby potentially increasing the risk of Alzheimer disease.

Arch Neurol. 2005 Oct;62(10):1539-44

ALPHA-LIPOIC ACID INCREASES NA+K+ATPASE ACTIVITY AND REDUCES LIPOFUSCIN ACCUMULATION IN DISCRETE BRAIN REGIONS OF AGED RATS.

A convincing link between oxidative stress and neurodegenerative diseases has been found with the knowledge that it actually damages neuronal cells in culture. We analyzed the effect of DL-alpha-lipoic acid on lipofuscin and Na(+)K(+) ATPase in discrete brain regions of young and aged rats. In aged rats, the level of lipofuscin was increased, and the activity of Na(+)K(+)ATPase was decreased. Intraperitoneal administration of lipoic acid to aged rats led to a duration-dependent reduction and elevation in lipofuscin and enzyme activity, respectively, in the cortex, cerebellum, striatum, hippocampus, and hypothalamus of the brain. These results suggest that lipoic acid, a natural metabolic antioxidant, should be useful as a therapeutic tool in preventing neuronal dysfunction in aged individuals.

Ann N Y Acad Sci. 2004 Jun;1019:350-4.

USE OF CARNOSINE AS A NATURAL ANTI-SENESCENCE DRUG FOR HUMAN BEINGS.

Carnosine is an endogenous free-radical scavenger. The latest research has indicated that apart from the function of protecting cells from oxidation-induced stress damage, carnosine appears to be able to extend the lifespan of cultured cells, rejuvenate senescent cells, inhibit the toxic effects of amyloid peptide (A beta), malondialdehyde, and hypochlorite to cells, inhibit glycosylation of proteins and protein-DNA and protein-protein cross-linking, and maintain cellular homeostasis. Also, carnosine seems to delay the impairment of eyesight with aging, effectively preventing and treating senile cataract and other age-related diseases. Therefore, carnosine may be applied to human being as a drug against aging.

Biochemistry (Mosc). 2000 Jul;65(7):869-71

CINNAMON IMPROVES GLUCOSE AND LIPIDS OF PEOPLE WITH TYPE 2 DIABETES.

OBJECTIVE: The objective of this study was to determine whether cinnamon improves blood glucose, triglyceride, total cholesterol, HDL cholesterol, and LDL cholesterol levels in people with type 2 diabetes. **RESEARCH DESIGN AND METHODS:** A total of 60 people with type 2 diabetes, 30 men and 30 women aged 52.2 +/- 6.32 years, were divided randomly into six groups. Groups 1, 2, and 3 consumed 1, 3, or 6 g of cinnamon daily, respectively, and groups 4, 5, and 6 were given placebo capsules corresponding to the number of capsules consumed for the three levels of cinnamon. The cinnamon was consumed for 40 days followed by a 20-day washout period. **RESULTS:** After 40 days, all three levels of cinnamon reduced the mean fasting serum glucose (18-29%), triglyceride (23-30%), LDL cholesterol (7-27%), and total cholesterol (12-26%) levels; no significant changes were noted in the placebo groups. Changes in HDL cholesterol were not significant. **CONCLUSIONS:** The results of this study demonstrate that intake of 1, 3, or 6 g of cinnamon per day reduces serum glucose, triglyceride, LDL cholesterol, and total cholesterol in people with type 2 diabetes and suggest that the inclusion of cinnamon in the diet of people with type 2 diabetes will reduce risk factors associated with diabetes and cardiovascular diseases.

Diabetes Care. 2003 Dec;26(12):3215-8

ELEVATED INTAKES OF SUPPLEMENTAL CHROMIUM IMPROVE GLUCOSE AND INSULIN VARIABLES IN INDIVIDUALS WITH TYPE 2 DIABETES.

Chromium is an essential nutrient involved in normal carbohydrate and lipid metabolism. The chromium requirement is postulated to increase with increased glucose intolerance and diabetes. The objective of this study was to test the hypothesis that the elevated intake of supplemental chromium is involved in the control of type 2 diabetes. Individuals being treated for type 2 diabetes (180 men and women) were divided randomly into three groups and supplemented with: 1) placebo, 2) 1.92 micromol (100 microg) Cr as chromium picolinate two times per day, or 3) 9.6 micromol (500 microg) Cr two times per day. Subjects continued to take their normal medications and were instructed not to change their normal eating and living habits. HbA1c values improved significantly after 2 months in the group receiving 19.2 pmol (1,000 microg) Cr per day and was lower in both chromium groups after 4 months (placebo, 8.5 +/- 0.2%; 3.85 micromol Cr, 7.5 +/- 0.2%; 19.2 micromol Cr, 6.6 +/- 0.1%). Fasting glucose was lower in the 19.2-micromol group after 2 and 4 months (4-month values: placebo, 8.8 +/- 0.3 mmol/l; 19.2 micromol Cr, 7.1 +/- 0.2 mmol/l). Two-hour glucose values were also significantly lower for the subjects consuming 19.2 micromol supplemental Cr after both 2 and 4 months (4-month values: placebo, 12.3 +/- 0.4 mmol/l; 19.2 micromol Cr, 10.5 +/- 0.2 mmol/l). Fasting and 2-h insulin values decreased significantly in both groups receiving supplemental chromium after 2 and 4 months. Plasma total cholesterol also decreased after 4 months in the subjects receiving 19.2 micromol/day Cr. These data demonstrate that supplemental chromium had significant beneficial effects on HbA1c, glucose, insulin, and cholesterol variables in subjects with type 2 diabetes. The beneficial effects of chromium in individuals with diabetes were observed at levels higher than the upper limit of the Estimated Safe and Adequate Daily Dietary Intake.

Diabetes. 1997 Nov;46(11):1786-91

ORAL ADMINISTRATION OF RAC-ALPHA-LIPOIC ACID MODULATES INSULIN SENSITIVITY IN PATIENTS WITH TYPE-2 DIABETES MELLITUS: A PLACEBO-CONTROLLED PILOT TRIAL.

Alpha-lipoic acid (ALA), a naturally occurring compound and a radical scavenger was shown to enhance glucose transport and utilization in different experimental and animal models. Clinical studies described an increase of insulin sensitivity after acute and short-term (10 d) parenteral administration of ALA. The effects of a 4-week oral treatment with alpha-lipoic acid were evaluated in a placebo-controlled, multicenter pilot study to determine whether oral treatment also improves insulin sensitivity. Seventy-four patients with type-2 diabetes were randomized to either placebo (n = 19); or active treatment in various doses of 600 mg once daily (n = 19), twice daily (1200 mg; n = 18), or thrice daily (1800 mg; n = 18) alpha-lipoic acid. An isoglycemic glucose-clamp was done on days 0 (pre) and 29 (post). In this explorative study, analysis was done according to the number of subjects showing an improvement of insulin sensitivity after treatment. Furthermore, the effects of active vs. placebo treatment on insulin sensitivity was compared. All four groups were comparable and had a similar degree of hyperglycemia and insulin sensitivity at baseline. When compared to placebo, significantly more subjects had an increase in insulin-stimulated glucose disposal (MCR) after ALA treatment in each group. As there was no dose effect seen in the three different alpha-lipoic acid groups, all subjects receiving ALA were combined in the "active" group and then compared to placebo. This revealed significantly different changes in MCR after treatment (+27% vs. placebo; p < .01). This placebo-controlled explorative study confirms previous observations of an increase of insulin sensitivity in type-2 diabetes after acute and chronic intravenous administration of ALA. The results suggest that oral administration of alpha-lipoic acid can improve insulin sensitivity in patients with type-2 diabetes. The encouraging findings of this pilot trial need to be substantiated by further investigations.

Free Radic Biol Med. 1999 Aug;27(3-4):309-14

FREE RADICAL THEORY OF AGING: AN UPDATE: INCREASING THE FUNCTIONAL LIFE SPAN.

Aging is the progressive accumulation of diverse, deleterious changes with time that increase the chance of disease and death. The basic chemical process underlying aging was first advanced by the free radical theory of aging (FRTA) in 1954: the reaction of active free radicals, normally produced in the organisms, with cellular constituents initiates the changes associated with aging. The involvement of free radicals in aging is related to their key role in the origin and evolution of life. Aging changes are commonly attributed to development, genetic defects, the environment, disease, and an inborn aging process (IAP). The latter produces aging changes at an exponentially increasing rate with age, becoming the major risk factor for disease and death for humans after the age of 28 years in the developed countries. In them the IAP limits human average life expectancy at birth (ALE-B)—a rough measure of the healthy life span—to about 85 years; few reach 100 years and only one is known to have lived to 122 years. In these countries, improvements in living conditions (ILC) have gradually raised ALE-Bs to 76-79 years, 6-9 years less than the limit imposed by aging, with no change in the maximum life span (MLS). The extensive studies based on the FRTA hold promise that ALE-B and the MLS can be extended, the ALE-B possibly by a few years, and the MLS somewhat less.

Ann N Y Acad Sci. 2006 May;1067:10-21

VITAMIN C DEFICIENCY INCREASES THE LUNG PATHOLOGY OF INFLUENZA VIRUS-INFECTED GULO^{-/-} MICE.

This study was designed to determine the effects of vitamin C deficiency on the immune response to infection with influenza virus. l-Gulono-gamma-lactone oxidase gene-inactivated mice (gulo^{-/-} mice) require vitamin C supplementation for survival. Five-wk-old male and female gulo^{-/-} mice were provided water or water containing 1.67 mmol/L vitamin C for 3 wk before inoculation with influenza A/Bangkok/1/79. There were no differences in lung influenza virus titers between vitamin C-adequate and -deficient mice; however, lung pathology in the vitamin C-deficient mice was greater at 1 and 3 d after infection but less at d 7 compared with vitamin C-adequate mice. Male vitamin C-deficient mice had higher expression of mRNA for regulated upon activation normal T expressed and secreted (RANTES), IL-1beta, and TNF-alpha in the lungs at d 1 after infection compared with male controls. However, at d 3 after infection, male vitamin C-deficient mice had less expression of mRNA for RANTES, monocyte chemoattractant protein-1 (MCP-1), and IL-12 compared with male controls. None of these differences were observed in female mice. Vitamin C-deficient male mice also had greater nuclear factor-kappaB activation as early as 1 d after infection compared with male controls. These data suggest that vitamin C is required for an adequate immune response in limiting lung pathology after influenza virus infection.

J Nutr. 2006 Oct;136(10):2611-6

A PILOT CLINICAL STUDY OF CONTINUOUS INTRAVENOUS ASCORBATE IN TERMINAL CANCER PATIENTS.

Case studies suggest that vitamin C, given intravenously at doses of 10-100 grams/day can improve patient well being and in some cases, reduce tumor size. While ascorbate is generally considered safe, clinical data on high intravenous doses is limited. Twenty-four late stage terminal cancer patients were given continuous infusions of 150 to 710 mg/kg/day for up to eight weeks. Blood chemistry and blood count profiles were obtained at roughly one-week intervals while patient health, adverse events and tumor progression were monitored. The majority of patients were vitamin C deficient prior to treatment. Intravenous infusions increased plasma ascorbate concentrations to a mean of 1.1 mM. The most common adverse events reported were nausea, edema, and dry mouth or skin; and these were generally minor. Two Grade 3 adverse events 'possibly related' to the agent were reported: one patient with a history of renal calculi developed a kidney stone after thirteen days of treatment and another patient experienced hypokalemia after six weeks of treatment. White blood cell counts were stable while hemoglobin and hematocrit levels dropped slightly during treatment, consistent with trends observed prior to therapy. Blood creatinine, BUN, glucose, and uric acid concentrations decreased or remained stable during therapy, suggesting that ascorbate infusions did not adversely affect renal function. One patient had stable disease and continued the treatment for forty-eight weeks. These data suggest that intravenous vitamin C therapy for cancer is relatively safe, provided the patient does not have a history of kidney stone formation.

P R Health Sci J. 2005 Dec;24(4):269-76

SUPPRESSION OF HUMAN CERVICAL CANCER CELL LINES HELA AND DOTC2 4510 BY A MIXTURE OF LYSINE, PROLINE, ASCORBIC ACID, AND GREEN TEA EXTRACT.

Cervical cancer, the second most common cancer in women, once metastasized, leads to poor prognosis. We investigated the antitumor effect of a nutrient mixture (NM) containing lysine, proline, arginine, ascorbic acid, and green tea extract on human cervical cancer cells Hela (CCL-2) and DoTc2 4510 by measuring cell proliferation (MTT assay), modulation of matrix metalloproteinases (MMP)-2 and MMP-9) expression (gelatinase zymography), and cancer cell invasive potential (Matrigel). NM showed significant antiproliferative effect on CCL-2 and DoTc2 4510 cancer cells. The NM inhibited CCL-2 expression of MMP-2 and MMP-9 in a dose-dependent fashion, with virtual total inhibition of MMP-2 at 1000 microg/mL and MMP-9 at 500 microg/mL NM. Untreated DoTc2 4510 cells showed MMP-9 expression, which was enhanced with phorbol 12-myristate 13-acetate treatment. NM inhibited MMP-9 expression in a dose-dependent fashion, with virtual inhibition at 500 microg/mL. Invasion of human cervical cancer cells CCL-2 and DoTc2 4510 through Matrigel decreased in a dose-dependent fashion, with 100% inhibition at 500 microg/mL NM ($P < 0.0001$) and 1000 microg/mL NM ($P < 0.0001$), respectively. Our results suggest that the mixture of lysine, proline, arginine, ascorbic acid, and green tea extract has potential in the treatment of cervical cancer by inhibiting critical steps in cancer development and spread.

Int J Gynecol Cancer. 2006 May-Jun;16(3):1241-7

INHIBITION OF MALIGNANT MESOTHELIOMA CELL MATRIX METALLOPROTEINASE PRODUCTION AND INVASION BY A NOVEL NUTRIENT MIXTURE.

Malignant mesothelioma (MM), an asbestos-associated cancer with no known cure, is a highly aggressive tumor causing profound morbidity and nearly universal mortality. Extracellular matrix (ECM) matrix metalloproteinases (MMPs) produced by tumor and stromal cells play a key role in tumor invasion and metastasis. Prevention of ECM degradation by MMP inhibition has been shown to be a promising therapeutic approach to inhibition of cancer development. Based on reported anticancer properties, the authors investigated the effect of a mixture (NM) containing lysine, proline, ascorbic acid, and green tea extract on MM cell line MSTO-211 H proliferation (by [MTT] [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay), MMP secretion (by gelatinase zymography), invasion (through Matrigel), and morphology (by hematoxylin and eosin [H&E] staining). MMP-2 and phorbol 12-myristate 13-acetate (PMA)-induced MMP-9 secretion were inhibited by NM in a dose-dependent fashion, with virtual total inhibition at 500 microg/ml NM. Invasion through Matrigel was inhibited at 50, 100, and 500 microg/ml by 27%, 36%, and 100%, respectively. NM was not toxic to the MM cell line, and H&E staining did not indicate any changes at and below 100 microg/ml concentration. In conclusion, NM significantly inhibited MM cell MMP secretion and invasion-both important parameters for cancer prevention, suggesting NM is an effective treatment strategy for MM.

Exp Lung Res. 2006 Mar-Apr;32(3-4):69-79

ANTITUMOR EFFECT OF ASCORBIC ACID, LYSINE, PROLINE, ARGININE, AND GREEN TEA EXTRACT ON BLADDER CANCER CELL LINE T-24.

AIMS: Bladder cancer, the fourth highest incident cancer in men and tenth in women, is associated with a high rate of recurrence, even when treated in situ, and prognosis is poor once the cancer metastasizes to distant sites. Based on anticancer properties, we investigated the effect of a mixture of lysine, proline, arginine, ascorbic acid, and green tea extract on human bladder cancer cells T-24 by measuring: proliferation, matrix metalloproteinase (MMP) expression, and cancer cell invasive potential. METHODS: Human bladder cancer cells T-24 (ATCC) were grown in McCoy medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL) and streptomycin (100 mg/mL) in 24-well tissue culture plates. At near confluence, the cells were treated with the nutrient mixture dissolved in media and tested at 0, 10, 50, 100, 500, and 1000 microg/mL in triplicate at each dose. Cells were also treated with PMA 200 ng/mL to study enhanced MMP-9 activity. Cell proliferation was evaluated by MTT assay, MMP activity by gelatinase zymography, and invasion through Matrigel. RESULTS: Nutrient mixture inhibited the T-24 cell secretion of MMP-2 and -9, with virtual total inhibition of MMP-2 at 500 microg/mL and MMP-9 at 100 microg/mL. The nutrient mixture significantly reduced the invasion of human bladder cancer cells T-24 through Matrigel in a dose-dependent fashion, with 95% inhibition at 500 microg/mL and 100% at 1000 microg/mL nutrient mixture ($P < 0.001$). CONCLUSION: Our results suggest that our nutrient mixture is an excellent candidate for therapeutic use in the treatment of bladder cancer, by inhibiting critical steps in cancer development and spread, such as MMP secretion and invasion.

Int J Urol. 2006 Apr;13(4):415-9

IN VIVO AND IN VITRO ANTITUMOR EFFECT OF ASCORBIC ACID, LYSINE, PROLINE, ARGININE, AND GREEN TEA EXTRACT ON HUMAN FIBROSARCOMA CELLS HT-1080.

Current treatment of fibrosarcoma, an aggressive cancer of the connective tissue, is generally associated with poor prognosis. Matrix metalloproteinases (MMPs), vascular endothelial growth factor (VEGF), and constituents of the extracellular matrix (ECM), such as fibronectin, play a critical role in angiogenesis and underlie neoplastic invasion and metastasis. This and anticancer properties of lysine, proline, arginine, ascorbic acid, and green tea extract (NM) prompted us to investigate the effect of these nutrients in vitro on human fibrosarcoma cells HT-1080 by measuring cell proliferation, modulation of MMP-2 and MMP-

9, and invasive potential. In vivo, we studied the growth of human fibrosarcoma HT-1080 cells in athymic nude mice and the expression of MMPs and VEGF. Cell proliferation was evaluated by MTT assay, MMP expression by gelatinase zymography, and invasion through Matrigel and migration by scratch assay. Tumors were excised, weighed, and processed for histology in both the control and nutrient-supplemented groups. Results showed NM inhibited the growth and reduced the size of tumors in nude mice; decreased MMP-9 and VEGF secretion was found in the supplemented group tissues. NM inhibited invasion through Matrigel and migration with total inhibition at 1,000 microg/mL. These results offer promise in the therapeutic use of the nutrient mixture of lysine, proline, arginine, ascorbic acid, and green tea extract tested in the treatment of fibrosarcoma.

Med Oncol. 2006;23(1):105-11

INHIBITION OF MATRIX METALLOPROTEINASE-2 SECRETION AND INVASION BY HUMAN OVARIAN CANCER CELL LINE SK-OV-3 WITH LYSINE, PROLINE, ARGININE, ASCORBIC ACID AND GREEN TEA EXTRACT.

AIMS: Based on the poor prognosis associated with ovarian cancer and reported anticancer properties of specific nutrients, we investigated the effect of a nutrient mixture (NM) containing lysine, proline, arginine, ascorbic acid and epigallocatechin gallate on human ovarian cancer cells SK-OV-3 by measuring: cell proliferation, modulation of matrix metalloproteinase (MMP)-2 and -9 expression, and cancer cell invasive potential. **METHODS:** Cell proliferation was evaluated by MTT assay, MMP activity by gelatinase zymography, and invasion through Matrigel. **RESULTS:** Human ovarian cancer cell growth was not significantly affected by the NM. Zymography demonstrated inhibition of MMP-2 secretion in a dose-dependent fashion with virtual total inhibition at 50 microg/mL NM concentration. Invasion of human ovarian cancer cells through Matrigel decreased in a dose-dependent fashion, with 90% inhibition at 500 microg/mL NM and 100% inhibition at 1000 microg/mL NM ($P < 0.0001$). **CONCLUSION:** The combination of lysine, proline, arginine, ascorbic acid and green tea extract tested inhibited critical steps in cancer development and spread, such as MMP expression and invasion, indicating its potential as a treatment modality against ovarian cancer.

J Obstet Gynaecol Res. 2006 Apr;32(2):148-54

IN VIVO AND IN VITRO ANTITUMOR EFFECT OF ASCORBIC ACID, LYSINE, PROLINE AND GREEN TEA EXTRACT ON HUMAN MELANOMA CELL LINE A2058.

BACKGROUND: Melanoma, a very serious form of skin cancer, causes the most skin cancer-related deaths, due to metastasis. Structural changes in the extracellular matrix (ECM) are necessary for cell migration during tissue remodeling. MMPs, VEGF, Ki-67 (proliferative protein) and constituents of ECM play a critical role in angiogenesis, and are crucial in neoplastic invasion and metastasis. **MATERIALS AND METHODS:** The effect of a diet (NM) containing lysine, proline, arginine, ascorbic acid and green tea extract on the growth of tumors induced by implanting human melanoma A2058 cells in athymic nude mice was examined and, also, on the expression of MMPs, VEGF and Ki-67 in these tumors. The effect of NM in vitro on the melanoma A2058 cell line was tested by measuring: cell proliferation by the MTT assay, expression of MMPs by gelatinase zymography and invasion through Matrigel. **RESULTS:** Nutrient supplementation strongly suppressed the growth of tumors (by 57%) without adverse effects in nude mice. Histological studies supported these findings by showing inhibition of MMP-9 and VEGF secretion and mitotic index. In vitro, NM inhibited melanoma cell growth by 64% at 500 microg/ml and Matrigel invasion by 95% at 100 microg/ml NM. **CONCLUSION:** These results suggest that NM may have a therapeutic potential in melanoma.

In Vivo. 2006 Jan-Feb;20(1):25-32

INHIBITORY EFFECT OF A MIXTURE CONTAINING ASCORBIC ACID, LYSINE, PROLINE AND GREEN TEA EXTRACT ON CRITICAL PARAMETERS IN ANGIOGENESIS.

Degradation of extracellular matrix (ECM) is a hallmark of tumor invasion, metastasis and angiogenesis. Based on the Rath multitargeted approach to cancer using natural substances to control ECM stability and enhancing its strength, we developed a novel formulation (NM) of lysine, proline, ascorbic acid and green tea extract that has shown significant anti-cancer activity against a number of cancer cell lines. The aim of the present study was to determine whether NM exhibits anti-angiogenic and anti-metastatic effects using in vitro and in vivo experimental models. Angiogenesis was measured using a chorioallantoic membrane (CAM) assay in chick embryos and bFGF-induced vessel growth in C57BL/6J female mice. To determine the in vivo effect of NM on the tumor xenograft growth, male nude mice were inoculated with 3×10^6 MNNG-HOS cells. Control mice were fed a mouse chow diet, while the test group was fed a mouse chow diet supplemented with 0.5% NM for 4 weeks. In vitro studies on cell proliferation (MTT assay), MMP expression (zymography) and Matrigel invasion were conducted on human osteosarcoma U2OS, maintained in McCoy medium, supplemented with 10% FBS, penicillin and streptomycin in 24-well tissue culture plates and tested with NM at 0, 10, 50, 100, 500, and 1000 microg/ml in triplicate at each dose. NM at 250 microg/ml caused a significant ($p < 0.05$) reduction in bFGF-induced angiogenesis in CAM. NM inhibited tumor growth of osteosarcoma MNNG-HOS cell xenografts in nude mice by 53%; furthermore, tumors in NM-treated mice were less vascular and expressed lower levels of VEGF and MMP-9 immunohistochemically than tumors in the control group. In addition, NM exhibited a dose-dependent inhibition of osteosarcoma U2OS cell proliferation (up to 60% at 1000 microg/ml), MMP-2 and -9 expression (with virtual total

inhibition at 500 microg/ml NM), and invasion through Matrigel (with total inhibition at 100 microg/ml NM). Moreover, NM decreased U2OS cell expression of VEGF, angiopoietin-2, bFGF, PDGF and TGFbeta-1. These results together with our earlier findings suggest that NM is a relatively non-toxic formulation, which inhibits growth, invasion, metastasis, and angiogenesis of tumor cells.

Oncol Rep. 2005 Oct;14(4):807-15

MODULATION OF N-METHYL-N-NITROSOUREA INDUCED MAMMARY TUMORS IN SPRAGUE-DAWLEY RATS BY COMBINATION OF LYSINE, PROLINE, ARGININE, ASCORBIC ACID AND GREEN TEA EXTRACT.

INTRODUCTION: The limited ability of current treatments to control metastasis and the proposed antitumor properties of specific nutrients prompted us to examine the effect of a specific formulation (nutrient supplement [NS]) of lysine, proline, arginine, ascorbic acid, and green tea extract in vivo on the development of N-methyl-N-nitrosourea (MNU)-induced mammary tumors in rats. **METHODS:** A single intraperitoneal dose of MNU was injected into each of 20 female Sprague-Dawley rats (aged 50 days) to induce tumors. Two weeks after MNU treatment, a time by which the animals had recovered from MNU-induced toxicity, the rats were divided into two groups. Rats in group 1 (n = 10) were fed Purina chow diet, whereas those in group 2 (n = 10) were fed the same diet supplemented with 0.5% NS. After a further 24 weeks, the rats were killed and tumors were excised and processed. **RESULTS:** NS reduced the incidence of MNU-induced mammary tumors and the number of tumors by 68.4%, and the tumor burden by 60.5%. The inhibitory effect of NS was also reflected by decreased tumor weight; the tumor weights per rat and per group were decreased by 41% and 78%, respectively. In addition, 30% of the control rats developed ulcerated tumors, in contrast to 10% in the nutrient supplemented rats. **CONCLUSION:** These findings suggest that the specific formulation of lysine, proline, arginine, ascorbic acid, and green tea extract tested significantly reduces the incidence and growth of MNU-induced mammary tumors, and therefore has strong potential as a useful therapeutic regimen for inhibiting breast cancer development.

Breast Cancer Res. 2005;7(3):R291-5. Epub 2005 Jan 31

IN VITRO AND IN VIVO ANTITUMORIGENIC ACTIVITY OF A MIXTURE OF LYSINE, PROLINE, ASCORBIC ACID, AND GREEN TEA EXTRACT ON HUMAN BREAST CANCER LINES MDA-MB-231 AND MCF-7.

Current treatments are generally ineffective once breast cancer has metastasized; median survival is reduced to 2-3 yr. Previous research studies demonstrating potent synergistic antitumor activity of lysine, proline, ascorbic acid, and epigallocatechin gallate prompted us to investigate the in vivo inhibitory effect of a nutrient mixture containing lysine, proline, arginine, ascorbic acid, and epigallocatechin gallate (NM) on the growth of human cancer xenografts in female athymic nude mice. Five to six week old female mice were inoculated with 3x10⁶ breast cancer cells MDA-MB-231. After injection, the mice were randomly divided into two groups A and B; group A was fed a regular diet and group B with the regular diet supplemented with 0.5% of the nutrient mixture (NM). Four weeks later, the mice were sacrificed, and their tumors were excised, weighed, and processed for histology. We also tested the effect of NM in vitro on estrogen-receptor positive (ER+) MCF-7 and estrogen-receptor negative (ER-) MDA-MB-231 breast cancer cell lines by measuring: cell proliferation by MTT assay, expression of MMPs by gelatinase zymography, invasion through Matrigel, and VEGF by ELISA. MCF-7 cells were also treated with estradiol to study enhanced invasion and expression of MMPs and VEGF. Results showed that NM inhibited the growth and reduced the size of tumors in female nude mice by 27%. Furthermore, histological evaluation revealed increased mitotic index, MMP-9 and VEGF secretion, and PAS material (mucin) in the control group tissues. In vitro studies showed NM inhibited MDA-MB-231 cell growth by 34% at 500 microg/mL and MCF-7 cell growth by 18% at 1000 microg/mL. Invasion of MDA-MB-231 through Matrigel was inhibited by 50%, 60%, and 95% by 10, 50, and 100 microg/mL of NM, respectively. The results of this study demonstrated that the nutrient mixture tested significantly suppressed tumor growth of breast cancer cells in female athymic nude mice and significantly inhibited MMP expression, angiogenesis, and invasion in breast cancer cells, in vitro, offering promise for therapeutic use in the treatment of breast cancer.

Med Oncol. 2005;22(2):129-38.

ANTITUMOR EFFECT OF A COMBINATION OF LYSINE, PROLINE, ARGININE, ASCORBIC ACID, AND GREEN TEA EXTRACT ON PANCREATIC CANCER CELL LINE MIA PACA-2.

BACKGROUND: Current treatment of pancreatic cancer is generally associated with poor prognosis, even if diagnosed early, owing to its aggressive rate of metastasis and non-responsiveness to chemotherapy and radiotherapy. Matrix metalloproteinases (MMPs) have received much attention in recent years for their role in various malignancies, and have been implicated in tumor invasion, metastasis, and angiogenesis. **AIM OF STUDY:** Reported antitumor properties of ascorbic acid, lysine, proline, and green tea extract prompted us to investigate the effect of a combination of lysine, proline, arginine, ascorbic acid, and green tea extract on pancreatic cancer cell line MIA PaCa-2 for viability, MMP expression, invasion, and morphology. **METHODS:** Viability was evaluated based on cell proliferation by MTT assay and MMP expression in condition media by gelatinase zymography. Invasion through Matrigel was assayed and morphology was observed by hematoxylin and eosin (H+E) staining. Data was analyzed by independent sample "t" test. **RESULTS:** The nutrient mixture (NM) did not inhibit cell proliferation at 10 microg/mL

and exhibited a dose-dependent antiproliferative effect with maximum inhibition of 38% over the control at 1000 microg/mL. Zymography demonstrated production of only MMP-9, which showed a dose-dependent decreased expression that was abolished at 100 microg/mL of NM. Invasion through Matrigel was inhibited at 10, 50, 100, and 500 microg/mL by 66%, 66%, 87% and 100%, respectively. H&E staining did not indicate changes even at the highest concentration of NM. **CONCLUSION:** Our results suggest that the formulation of green tea extract, lysine, proline, and ascorbic acid, tested as a promising adjunct to standard treatment of pancreatic cancer, by inhibiting MMP expression and invasion without toxic effects important parameters in cancer metastasis.

Int J Gastrointest Cancer. 2005;35(2):97-102

IN VIVO ANTITUMOR EFFECT OF ASCORBIC ACID, LYSINE, PROLINE AND GREEN TEA EXTRACT ON HUMAN PROSTATE CANCER PC-3 XENOGRAPTS IN NUDE MICE: EVALUATION OF TUMOR GROWTH AND IMMUNOHISTOCHEMISTRY.

BACKGROUND: Matrix metalloproteinases (MMPs), vascular endothelial growth factor (VEGF), Ki 67 (proliferative protein) and constituents of ECM play a critical role in angiogenesis, and are crucial in neoplastic invasion and metastasis. Based on the antitumor properties of certain nutrients, we investigated the effect of a diet containing lysine, proline, arginine, ascorbic acid and green tea extract on the growth of tumors induced by implanting human prostate cancer PC-3 cells in athymic nude mice and on the expression of MMPs, VEGF, Ki 67 and fibronectin in these tumors, as well as the production of mucin (by PAS staining). **MATERIALS AND METHODS:** Male nude mice (n =12) were inoculated with 3x10(6) prostate cancer PC-3 cells and randomly divided into two groups; Group A was fed a regular diet and Group B was fed a regular diet supplemented with 0.5% of the nutrient mixture (NM). Four weeks later, tumors were excised, weighed and processed for histology. **RESULTS:** The results showed inhibition of tumor growth in Group B. Histological studies revealed inhibition of MMP-9 and VEGF secretion and mitosis in Group B tissues. **CONCLUSION:** Nutrient supplementation strongly suppressed the growth of tumors without any adverse effects in nude mice, suggesting strong potential as an anticancer agent.

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