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Journal ABSTRACTS

Homocysteine

HOMOCYSTEINE, APOLIPOPROTEIN E AND METHYLENETETRAHYDROFOLATE REDUCTASE IN ALZHEIMER'S DISEASE AND MILD COGNITIVE IMPAIRMENT.

BACKGROUND: Alzheimer's disease (AD) is the most common dementia disorder in elderly people. Currently, the only known genetic factor associated with the development of sporadic AD is the apolipoprotein E (ApoE) 4 allele. There is a need to identify other environmental and genetic risk factors that could modulate the risk of developing sporadic AD. **OBJECTIVE:** To analyse the correlation between the ApoE and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and plasma homocysteine levels and vitamins (B(12) and folic acid) concentrations in serum from patients with AD and mild cognitive impairment (MCI) as compared with control group. **METHODS:** The study was carried out in 99 AD patients, 98 subjects with MCI and 100 healthy subjects. Diagnosis of probable AD was made according to the NINCDS-ADRDA and DSM-IV criteria. The following factors were analysed: age, gender, duration of disease, concentration of plasma total homocysteine, folic acid and vitamin B(12) in the serum and the polymorphism of MTHFR and ApoE genes. The results obtained were analysed by multivariate analysis of regression. **RESULTS:** We found that plasma total homocysteine is increased in AD patients ($p < 0.0001$) and depended on the MTHFR T/T genotype in the presence of low folate levels ($p < 0.05$). The increased frequency of ApoE4 allele in the AD population was independent of homocysteine, folic acid and vitamin B(12) levels and MTHFR status. **CONCLUSIONS:** We conclude that the concentration of plasma total homocysteine is increased in AD patients. This may be associated with the T/T genotype in the MTHFR gene; however, the distribution of the MTHFR C677T polymorphism in the Polish population does not differ in AD and controls.

Dement Geriatr Cogn Disord. 2003;16(2):64-70

COMPARATIVE EFFECTS OF HYDROXOCOBALAMIN AND CYANOCOBALAMIN ON PLASMA HOMOCYSTEINE CONCENTRATIONS IN END-STAGE RENAL DISEASE.

End-stage renal disease (ESRD) is associated with marked hyperhomocysteinemia which is only partially corrected by folic acid and pyridoxine supplementation. We and others have reported that various forms of parenteral cobalamin reduce plasma total homocysteine (tHcy) concentrations of patients with ESRD substantially below the lowest levels attainable with folic acid. We here report a 16-week randomized controlled crossover trial which directly compared the Hcy-lowering effect of intravenous hydroxocobalamin (HC) with that of cyanocobalamin (CC). Folic acid- and vitamin B12-replete maintenance hemodialysis patients were randomly assigned to receive either 1 mg intravenous HC weekly for 8 weeks followed by CC for a further 8 weeks, or CC for 8 weeks followed by HC for 8 weeks. Hydroxocobalamin increased serum cobalamin concentrations 40-fold, whereas CC increased them only 10-fold, but both treatments reduced plasma tHcy concentrations similarly by 33% ($P < .001$). Crossover to the alternate form of the vitamin greatly affected the serum cobalamin concentration but was without further effect on the plasma tHcy concentration. These results confirm that weekly cobalamin injections lower plasma tHcy concentrations of hemodialysis patients well below the level attainable with folic acid. Hydroxocobalamin and CC are equipotent despite producing very different serum cobalamin concentrations.

Metabolism. 2005 Oct;54(10):1362-7

ORALLY ADMINISTERED BETAINE HAS AN ACUTE AND DOSE-DEPENDENT EFFECT ON SERUM BETAINE AND PLASMA HOMOCYSTEINE CONCENTRATIONS IN HEALTHY HUMANS.

Betaine, i.e., trimethylglycine, is linked to homocysteine metabolism. A 3-mo daily betaine supplementation decreased even normal plasma total homocysteine (tHcy) concentrations in humans. The pharmacokinetic characteristics and metabolism of betaine in humans have not been investigated in detail. The aim of this study was to assess the pharmacokinetics of orally administered betaine and its acute effect on plasma tHcy concentrations. Healthy volunteers ($n = 10$; 3 men, 7 women) with normal body weight (mean \pm SD, 69.5 \pm 17.0 kg), 40.8 \pm 12.4 y old, participated in the study. The betaine doses were 1, 3, and 6 g. The doses were mixed with 150 mL of orange juice and ingested after a 12-h overnight fast by each volunteer according to a randomized double-blind crossover design. Blood samples were drawn for 24 h and a 24-h urine collection was performed.

Orally administered betaine had an immediate and dose-dependent effect on serum betaine concentration. Single doses of 3 and 6 g lowered plasma tHcy concentrations ($P = 0.019$ and $P < 0.001$, respectively), unlike the 1-g dose. After the highest dose, the concentrations remained low during the 24 h of monitoring. The change in plasma tHcy concentration was linearly associated with betaine dose ($P = 0.006$) and serum betaine concentration ($R^2 = 0.17$, $P = 0.025$). The absorption and elimination of betaine were dose dependent. The urinary excretion of betaine seemed to increase with an increasing betaine dose, although a very small proportion of ingested betaine was excreted via urine. In conclusion, a single dose of orally administered betaine had an acute and dose-dependent effect on serum betaine concentration and resulted in lowered plasma tHcy concentrations within 2 h in healthy subjects.

J Nutr. 2006 Jan;136(1):34-8

INCREASED PLASMA PROTEIN HOMOCYSTEINYLACTION IN HEMODIALYSIS PATIENTS.

Hyperhomocysteinemia, an independent cardiovascular risk factor, is present in the majority of hemodialysis patients. Among the postulated mechanisms of toxicity, protein homocysteinylation is potentially able to cause significant alterations in protein function. Protein homocysteinylation occurs through various mechanisms, among which is the post-translational acylation of free amino groups (protein-N-homocysteinylation, mediated by homocysteine (Hcy) thiolactone). Another type of protein homocysteinylation occurs through the formation of a covalent -S-S- bond, found primarily with cysteine residues (protein-S-homocysteinylation). Scant data are available in the literature regarding the extent to which alterations in protein homocysteinylation are present in uremic patients on hemodialysis, and the effects of folate treatment are not known. Protein homocysteinylation was measured in a group of hemodialysis patients ($n=28$) compared to controls ($n=14$), with a new method combining protein reduction, gel filtration and Hcy derivatization. Chemical hydrolysis was performed, followed by high-pressure liquid chromatography separation. The effects of folate treatment on protein homocysteinylation, as well as in vitro binding characteristics were evaluated. Plasma Hcy, protein-N-homocysteinylation and protein-S-homocysteinylation were significantly higher in patients vs controls. Plasma Hcy and protein-S-homocysteinylation were significantly correlated. After 2 months of oral folate treatment, protein-N-homocysteinylation was normalized, and protein-S-homocysteinylation was significantly reduced. Studies on albumin-binding capacity after in vitro homocysteinylation show that homocysteinylated albumin is significantly altered at the diazepam-binding site. In conclusion, increased protein homocysteinylation is present in hemodialysis patients, with possible consequences in terms of protein function. This alteration can be partially reversed after folate treatment.

Kidney Int. 2006 Mar;69(5):869-76

PLASMA REDUCED HOMOCYSTEINE AND OTHER AMINOTHIOL CONCENTRATIONS IN PATIENTS WITH CKD.

BACKGROUND: Hyperhomo-cysteinemia, a risk factor for cardiovascular disease, is present in the majority of patients with chronic kidney disease (CKD). Several studies indicated that the moiety of homocysteine (Hcy) with an unbound -SH group (reduced Hcy [rHcy]) is the atherogenic molecule. This study is designed to examine the relation between different forms of Hcy and other aminothiols in hemodialysis (HD) patients, peritoneal dialysis (PD) patients, and nondialyzed patients with CKD. **METHODS:** rHcy, free Hcy (fHcy), and total Hcy (tHcy), as well as different forms of cysteine, cysteinyl-glycine, and glutathione, were studied by using a high-performance liquid chromatography technique in 19 HD patients, 12 PD patients, 47 patients with CKD, and 15 control subjects. **RESULTS:** In PD patients, tHcy levels were 2.8 times greater compared with controls, and in HD patients and those with CKD, 2.1 and 1.9 times greater, respectively. Mean rHcy/tHcy ratios were significantly greater in both HD ($P < 0.05$) and PD patients ($P < 0.01$), but did not differ in patients with CKD compared with controls. The decrease in rHcy levels during 1 HD treatment was smaller than that in tHcy and fHcy levels, and rHcy/tHcy ratio increased (before HD, 1.25% +/- 0.44%; after HD, 1.44% +/- 0.66%; $P < 0.05$). **CONCLUSION:** Levels of rHcy and other aminothiols are markedly increased in patients with impaired renal function. In dialysis patients, rHcy/tHcy ratio is markedly elevated and shows greater variability than in patients with CKD and controls. We conclude that because rHcy is believed to induce endothelial dysfunction and may be part of the accelerated atherogenic process in patients with CKD, plasma rHcy level could be a more relevant marker of cardiovascular disease risk than tHcy level.

Am J Kidney Dis. 2006 Jan;47(1):60-71

HYPERHOMOCYSTEINEMIA AND RESPONSE OF METHIONINE CYCLE INTERMEDIATES TO VITAMIN TREATMENT IN RENAL PATIENTS.

The role of hyperhomocysteinemia (HHcy) as a risk marker for cardiovascular diseases in renal patients is a matter of controversy. The remethylation of homocysteine (Hcy) to methionine in the kidneys is of great importance for Hcy clearance. Hcy remethylation is markedly decreased in patients on hemodialysis, but transsulfuration remains mostly unaffected. Markedly increased concentrations of methylmalonic acid (MMA), as a metabolic marker of vitamin B12 deficiency, have been found in approximately 70% of renal patients. This is in contrast to normal concentrations of vitamin B12 usually reported in such patients. We demonstrated in cell culture experiments that the uptake of vitamin B12 by mononuclear cells from renal patients was lower than that taken up by cells from controls. The lowering of MMA and Hcy concentrations in renal patients after B12

administration may indicate the presence of intracellular pre-treatment deficiency. We administered folic acid (5 mg) plus vitamin B6 (50 mg) and B12 (0.7 mg) three times per week intravenously to hyperhomocysteinemic dialysis patients. Hcy decreased after 4 weeks by 51%. Hcy was normalized in almost all patients, while serum concentrations of MMA and cystathionine were reduced by 28% and 26%, respectively. Cystathionine, an indicator for the transsulfuration pathway, showed a drastic increase in renal disease and was only slightly lowered by B-vitamin treatment. The increased cystathionine/cysteine ratio in renal patients indicates possible impairment of the catabolism of cystathionine by cystathionase. Moreover, renal failure is associated with severe abnormalities in plasma concentrations of S-adenosyl Hcy (SAH) and S-adenosyl methionine (SAM), as well as the SAM/SAH ratio. This ratio is an indicator of the availability of methyl groups from SAM. Therapeutic doses of B-vitamins in dialysis patients led to a limited improvement in the biomarkers of methylation and probably did not have a significant effect on transmethylation potential in the cells. Furthermore, elevated serum levels of asymmetric dimethylarginine (ADMA) in renal patients, which are associated with a poor outcome for such patients, could be lowered, but this effect was confined to patients who had no anemia. Future studies may consider extending the duration of vitamin treatment, as well as agents that may enhance the hydrolysis of SAH and cystathionine.

Clin Chem Lab Med. 2005;43(10):1039-47

CAUSES OF HYPERHOMOCYSTEINEMIA IN PATIENTS WITH CHRONIC KIDNEY DISEASES.

Plasma homocysteine (Hcy) levels are increased significantly in patients with moderate renal failure and increase markedly in patients with end-stage renal disease. An increase in plasma Hcy level theoretically could be caused by an increased production rate (ie, transmethylation), a decreased rate of removal by transsulfuration or remethylation, or a decrease in the excretion of Hcy. Current evidence indicates that the major mechanism for hyperhomocysteinemia in renal failure is a decrease in Hcy removal from the body. However, it is debated whether this effect is the result of a decrease in the renal metabolic clearance or a result of extrarenal metabolic changes. The human kidney plays a major role in the removal of several aminothiols or Hcy-related compounds from the circulation (eg, cysteine-glycine, glutathione, AdoMet, and AdoHcy). However, the glomerular filtration of Hcy seems to be restricted because of protein binding. Besides glomerular filtration, the normal kidney can remove Hcy by plasma flow and peritubular uptake. Although in the low normal range in absolute terms, the flow through the transsulfuration pathway is reduced if related to Hcy levels in uremia; in addition, the remethylation pathway also is impaired. Besides the potential effect of the reduced renal mass on Hcy removal, available evidence suggests the occurrence of a generalized down-regulation of the methionine cycle and catabolism in uremia. AdoHcy, sulfate, and dimethylglycine currently are being investigated as retained solutes that can inhibit 1 or more pathways of Hcy metabolism. In addition, the high Hcy levels decrease in malnourished end-stage renal disease patients and change according to nutrient intake and several other nutritional parameters, indicating that circulating Hcy levels become an expression of nutritional status.

Semin Nephrol. 2006 Jan;26(1):3-7

HOMOCYSTEINE AND ITS DETERMINANTS IN NONDIALYZED CHRONIC KIDNEY DISEASE PATIENTS.

This cross-sectional study aimed to investigate the prevalence of hyperhomocysteinemia, the determinants of plasma total homocysteine concentrations, and the relationship of total homocysteine with nutritional parameters in a sample of patients with chronic kidney disease (CKD) and not yet on dialysis. The study was done with outpatients from the Nephrology Division of the Federal University of Sao Paulo and Oswaldo Ramos Foundation. Sixty-six patients with CKD (70% male; age 58.6 \pm 15.6 years [mean \pm standard deviation]) with moderate to severe renal impairment (creatinine clearance=29.8 \pm 14.3 mL/min [0.5 \pm 0.24 mL/sec]), clinically stable, and older than 18 years were included. A group of 20 healthy subjects from the clinic staff was also studied for reference values for plasma homocysteine, folate, and vitamin B-12 concentration. Fasting blood samples were collected to determine plasma total homocysteine, folate, vitamin B-12, and creatinine. To calculate creatinine clearance, a 24-hour urine collection sample was obtained. The assessment of nutritional status included anthropometric parameters. Pearson correlation, Mann-Whitney test, and multiple linear regression analysis were used for statistical analyses. The main results showed that the concentration of total homocysteine in the patients was significantly increased compared with the healthy subjects (3.4 \pm 1.7 vs 1.41 \pm 0.42 mg/L [25.4 \pm 12.2 vs 10.4 \pm 3.1 micromol/L]; P <0.001). Plasma folate and plasma vitamin B-12 were in the normal range and did not differ between patients and healthy individuals. A high prevalence of hyperhomocysteinemia (total homocysteine >1.89 mg/L [14 micromol/L]) was found in the patients (89%). Plasma total homocysteine did not correlate with any of the nutritional parameters studied and did not differ between patients in terms of whether they were using or not using folic acid supplementation (3.07 \pm 1.09 vs 3.55 \pm 1.78 mg/L [22.7 \pm 8.1 vs 26.3 \pm 13.2 micromol/L]; P =0.47), although plasma folate was significantly higher in the supplemented group (12.6 \pm 3.0 vs 8.0 \pm 3.6 ng/mL [28.5 \pm 6.8 nmol/L vs 18.1 \pm 8.2 nmol/L]; P <0.001). According to the multiple regression analysis, the determinants of total homocysteine were only plasma folate, plasma vitamin B-12, and creatinine clearance (r^2 =0.20). In conclusion, a high prevalence of hyperhomocysteinemia was found in our sample of nondialyzed patients with CKD. The determinants of total homocysteine levels were plasma folate, plasma vitamin B-12, and creatinine clearance. No association between nutritional parameters and total homocysteine was observed.

J Am Diet Assoc. 2006 Feb;106(2):267-70

HOMOCYSTEINE, METHYLENETETRAHYDROFOLATE REDUCTASE AND RISK OF SCHIZOPHRENIA: A META-ANALYSIS.

Elevated plasma homocysteine concentration has been suggested as a risk factor for schizophrenia, but the results of epidemiological studies have been inconsistent. The most extensively studied genetic variant in the homocysteine metabolism is the 677C>T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene, resulting in reduced enzyme activity and, subsequently, in elevated homocysteine. A meta-analysis of eight retrospective studies (812 cases and 2,113 control subjects) was carried out to examine the association between homocysteine and schizophrenia. In addition, a meta-analysis of 10 studies (2,265 cases and 2,721 control subjects) on the homozygous (TT) genotype of the MTHFR 677C>T polymorphism was carried out to assess if this association is causal. A 5 micromol/l higher homocysteine level was associated with a 70% (95% confidence interval, CI: 27-129) higher risk of schizophrenia. The TT genotype was associated with a 36% (95% CI: 7-72) higher risk of schizophrenia compared to the CC genotype. The performed meta-analyses showed no evidence of publication bias or excessive influence attributable to any given study. In conclusion, our study provides evidence for an association of homocysteine with schizophrenia. The elevated risk of schizophrenia associated with the homozygous genotype of the MTHFR 677C>T polymorphism provides support for causality between a disturbed homocysteine metabolism and risk of schizophrenia.

Mol Psychiatry. 2006 Feb;11(2):143-9

HOMOCYSTEINE-REDUCING STRATEGIES IMPROVE SYMPTOMS IN CHRONIC SCHIZOPHRENIC PATIENTS WITH HYPERHOMOCYSTEINEMIA.

BACKGROUND: An elevated homocysteine level is reported to be a risk factor for several diseases, including Alzheimer's and cerebrovascular disease. Recently, several studies have reported that homocysteine levels are elevated in many schizophrenic patients. Homocysteine levels can be lowered by oral folic acid, B-12, and pyridoxine. **METHODS:** Forty-two schizophrenic patients with plasma homocysteine levels >15 micromol/L were treated with these vitamins for 3 months and placebo for 3 months in a study with a randomized, double-blind, placebo-controlled, crossover design. **RESULTS:** Homocysteine levels declined with vitamin therapy compared with placebo in all patients except for one noncompliant subject. Clinical symptoms of schizophrenia as measured by the Positive and Negative Syndrome Scale declined significantly with active treatment compared with placebo. Neuropsychological test results overall, and Wisconsin Card Sort (Categories Completed) test results in particular, were significantly better after vitamin treatment than after placebo. **CONCLUSIONS:** A subgroup of schizophrenic patients with hyperhomocysteinemia might benefit from the simple addition of B vitamins.

Biol Psychiatry. 2006 Aug 1;60(3):265-9.

PLASMA TOTAL HOMOCYSTEINE LEVEL AND BONE MINERAL DENSITY: THE HORDALAND HOMOCYSTEINE STUDY.

BACKGROUND: Plasma total homocysteine (tHcy) has been associated with hip fracture but not directly with bone mineral density (BMD). We examined the association of hip BMD with levels of plasma tHcy, folate, and vitamin B12 and the methylenetetrahydrofolate reductase (MTHFR) 677C->T and 1298A->C polymorphisms. **METHODS:** Bone mineral density was measured between 1997 and 2000 in 2,268 men and 3,070 women, aged 47 to 50 and 71 to 75 years, from the Hordaland Homocysteine Study cohort. Low BMD was defined as BMD in the lowest quintile for each sex and age group. Linear, logistic, and generalized additive regression models were used. **RESULTS:** Plasma levels of tHcy were inversely related to BMD among middle-aged and elderly women ($P < .001$) but not among men. The multiple adjusted odds ratio for low BMD among subjects with high (≥ 15 micromol/L [≥ 2.02 mg/L]) compared with low (< 9 micromol/L [< 1.22 mg/L]) tHcy level was 1.96 (95% confidence interval, 1.40-2.75) for women and was not significant for men. Additional adjustments for plasma folate level or intake of calcium and vitamin D did not substantially alter the results. Plasma folate level was associated with BMD in women only. We observed no association between BMD and vitamin B12 level or the MTHFR polymorphisms. **CONCLUSIONS:** Elevated tHcy and low folate levels were associated with reduced BMD in women but not in men. These findings suggest that tHcy may be a potential modifiable risk factor for osteoporosis in women.

Arch Intern Med. 2006 Jan 9;166(1):88-94

EVALUATION OF PLASMA HOMOCYSTEINE AND RISK OF AGE-RELATED MACULAR DEGENERATION.

PURPOSE: To assess the relationship between plasma levels of homocysteine and age-related macular degeneration (AMD). **DESIGN:** Cross-sectional, case-control study. **METHODS:** Fasting plasma homocysteine levels were measured at two centers in 934 individuals who were participating in an ancillary study of the Age-Related Eye Disease Study. There were 547 cases and 387 control subjects, who were determined by fundus photography. Conditional logistic regression analyses were conducted to assess the association of homocysteine with AMD. **RESULTS:** Median values of homocysteine were higher among advanced AMD cases (9.51 mmol/l) compared with persons with no AMD (8.81 mmol/l; $P = .01$). Values of > 12 mmol/l vs ≤ 12 mmol/l were also associated with an increased risk of AMD ($P = .023$), when controlled for other covariates. **CONCLUSION:** Results are

consistent with a possible small, independent association between higher homocysteine levels and AMD. Homocysteine may be a modifiable risk factor for AMD.

Am J Ophthalmol. 2006 Jan;141(1):201-3

ASSOCIATION OF PLASMA HOMOCYSTEINE WITH CORONARY ARTERY CALCIFICATION IN DIFFERENT CATEGORIES OF CORONARY HEART DISEASE RISK.

OBJECTIVE: To Investigate the association of plasma homocysteine with coronary artery calcification (CAC) in strata based on 10-year risk of coronary heart disease (CHD) in a cohort enriched in persons with hypertension. **PARTICIPANTS AND METHODS:** Fasting plasma homocysteine was measured by liquid chromatography electrospray tandem mass spectrometry. Coronary artery calcification was measured noninvasively by electron beam computed tomography and CAC score calculated using the method of Agatston et al. The 10-year CHD risk was calculated based on the Framingham risk score. The association of homocysteine with log-transformed CAC score was assessed in the pooled sample and within each risk stratum by linear regression after adjustment for conventional risk factors. **RESULTS:** In the 1,071 participants studied, homocysteine was associated with CAC quantity ($P = .01$) after adjustment for CHD risk factors (age, male sex, total and high-density lipoprotein cholesterol, diabetes, history of smoking, body mass Index, and systolic blood pressure), serum creatinine, and statin and hypertension medication use. When the association was assessed in strata based on 10-year CHD risk, homocysteine was significantly ($P = .003$) associated with CAC quantity in participants at Intermediate 10-year risk of CHD (6%-20%) independent of other risk factors but not in those at lower risk or higher risk. **CONCLUSION:** Plasma homocysteine is associated with quantity of CAC Independent of CHD risk factors. When studied in categories of 10-year CHD risk, the association was significant in participants at intermediate risk but not in those at low or high risk. Plasma homocysteine levels may have clinical utility as a marker of CHD risk in such individuals.

Mayo Clin Proc. 2006 Feb;81(2):177-82

EFFECTS OF VITAMIN C ON INTRACORONARY L-ARGININE DEPENDENT CORONARY VASODILATATION IN PATIENTS WITH STABLE ANGINA.

OBJECTIVE: To assess the effects of intravenous vitamin C administration on the vasomotor responses to intracoronary L-arginine infusion in epicardial coronary arteries. **METHODS:** 28 patients with coronary artery disease and stable angina were enrolled in the study. Eight patients received intracoronary infusions of 150 micromol/min L-arginine before and after intravenous infusion of vitamin C, 10 patients received intracoronary infusions of 150 micromol/min L-arginine before and after intravenous infusion of normal saline, and 10 patients received intracoronary normal saline before and after intravenous infusion of vitamin C. The diameter of proximal and distal coronary artery segments was measured by quantitative angiography. **RESULTS:** Infusion of L-arginine caused significant dilatation of both proximal (4.87 (0.96)%, $p < 0.01$ v normal saline) and distal (6.33 (1.38)%, $p < 0.01$ v normal saline) coronary segments. Co-infusion of vitamin C and L-arginine dilated proximal coronary segments by 8.68 (1.40)% ($p < 0.01$ v normal saline, $p < 0.01$ v L-arginine) and distal segments by 13.07 (2.15)% ($p < 0.01$ v normal saline, $p < 0.01$ v L-arginine). Intravenous infusion of vitamin C caused a borderline increase in proximal and distal coronary segment diameters (1.93 (0.76)% and 2.09 (1.28)%, respectively, not significant). **CONCLUSIONS:** L-arginine dependent coronary segment vasodilatation was augmented by the antioxidant vitamin C in patients with coronary artery disease. Thus, vitamin C may have beneficial effects on nitric oxide bioavailability induced by L-arginine.

Heart. 2005 Oct;91(10):1319-23

VITAMIN C-INDUCED ACTIVATION OF PHOSPHOLIPASE D IN LUNG MICROVASCULAR ENDOTHELIAL CELLS: REGULATION BY MAP KINASES.

Our earlier studies have shown that vitamin C at pharmacological doses (mM) induces loss of redox-dependent viability in bovine lung microvascular endothelial cells (BLMVECs) that is mediated by oxidative stress. Therefore, here, we investigated the vitamin C-induced activation of the lipid signaling enzyme, phospholipase D (PLD) in BLMVECs. Monolayer cultures of BLMVECs were treated with vitamin C (0-10 mM) for different time periods (0-2 h) and the activity of PLD was determined. Vitamin C induced activation of PLD in BLMVECs in a time- and dose-dependent fashion that was significantly attenuated by antioxidants, p38 mitogen-activated protein kinase (p38 MAPK)-specific inhibitor (SB203580), extracellular signal-regulated protein kinase (ERK)-specific inhibitor (PD98059), and transient transfection of cells with dominant-negative (DN)-p38 MAPK and DN-ERK1/ERK2. Vitamin C also induced phosphorylation and enhanced the activities of p38 MAPK and ERK in BLMVECs in a time-dependent fashion. It was also evident that vitamin C induced translocation of PLD(1) and PLD(2), association of p38 MAPK and ERK with PLD(1) and PLD(2), threonine phosphorylation of PLD(1) and PLD(2) and SB203580- and PD98059-inhibitable threonine phosphorylation of PLD(1) in BLMVECs. Transient transfection of BLMVECs with DN-p38 MAPK and DN-ERK1/ERK2 resulted in marked attenuation of vitamin C-induced phosphorylation of threonine in PLD(1) and PLD(2). We, for the first time, showed that vitamin C at pharmacological doses, activated PLD in the lung microvascular ECs through oxidative stress and MAPK activation.

Cell Signal. 2006 Sep;18(9):1396-407

ANTIOXIDANT EFFECTS OF COMBINED VITAMINS C AND E IN ACUTE MYOCARDIAL INFARCTION. THE RANDOMIZED, DOUBLE-BLIND, PLACEBO CONTROLLED, MULTICENTER PILOT MYOCARDIAL INFARCTION AND VITAMINS (MIVIT) TRIAL.

AIMS: There is a large body of evidence that reactive oxygen species (ROS) produced during myocardial ischemia and reperfusion play a crucial role in myocardial damage and endothelial dysfunction. The MIVIT pilot trial was designed to test the effects of antioxidant vitamins C and E on the clinical outcome of patients with AMI. **METHODS and RESULTS:** In this randomized, double-blind, multicenter trial, 800 patients (mean age 62) with AMI were randomly allocated to receive, on top of routine medication, one of two treatments: vitamin C (1000 mg/12 h infusion) followed by 1200 mg/24 h orally and vitamin E (600 mg/24 h) or matching placebo for 30 days. Primary end point (composite of in-hospital cardiac mortality, non-fatal new myocardial infarction, VT/VF/asystole, shock/pulmonary edema) occurred less frequently in patients treated with antioxidants (55 [14%] vs 75 [19%], OR 0.82 [95% CI, 0.68-1.00], $p=0.048$). **CONCLUSIONS:** This randomized pilot trial shows that supplementation with antioxidant vitamins is safe and seems to positively influence the clinical outcome of patients with AMI. A larger study is warranted to provide further evidence of this promising and inexpensive regimen.

ASCORBIC ACID (VITAMIN C) AND ILOPROST ATTENUATE THE LUNG INJURY CAUSED BY ISCHEMIA/REPERFUSION OF THE LOWER EXTREMITIES OF RATS.

The objectives of this study were to compare the protective effects of ascorbic acid and iloprost on lung injury caused by ischemia reperfusion (I/R) of the lower extremities of rats. Wistar albino rats ($n = 34$) were divided into five groups. In the I/R group ($n = 6$), the aorta was cross-clamped for 3 hr, followed by 1 hr of reperfusion. In the vitamin C group ($n = 8$), animals were pretreated with 100 mg/kg ascorbic acid via the left jugular vein before aortic cross-clamping. In the iloprost group ($n = 8$), animals were pretreated with 20 ng/(kg x min) iloprost by constant intravenous infusion via the left jugular venous cannula. In the sham group ($n = 6$), the abdomen was left open at the same period and a jugular venous line was established. In the control group ($n = 6$), lungs were removed and blood samples taken immediately after sternotomy. No treatment was given in this group. After both lungs were removed, biochemical parameters were measured and histopathological evaluation was made. Although the arterial blood pO₂ and HCO₃ levels were statistically significantly high in both the vitamin C and iloprost groups compared to the I/R group, plasma malondialdehyde (MDA) levels were significantly low. Meanwhile, the MDA levels in the lung tissue were significantly low in the vitamin C group compared to the I/R group. The MDA level in the lung tissue in the iloprost group was also low compared to the I/R group, but it was not statistically significant. The lungs of the I/R group displayed intense interstitial leukocytic infiltration in histopathological examination compared to the other groups. Pretreatment of animals with iloprost and vitamin C significantly decreased the pulmonary injury characterized by decreased plasma leukocyte sequestration. The results suggest that both vitamin C and iloprost are useful agents for attenuating the lung injury caused by increased oxidative stress and neutrophil accumulation after a period of I/R of the lower extremities.

Ann Vasc Surg. 2006 Jan;20(1):49-55

ORAL VITAMIN C ADMINISTRATION REDUCES EARLY RECURRENCE RATES AFTER ELECTRICAL CARIOVERSION OF PERSISTENT ATRIAL FIBRILLATION AND ATTENUATES ASSOCIATED INFLAMMATION.

BACKGROUND: Inflammation and oxidative stress have been recently implicated in the pathophysiology of atrial fibrillation (AF). The aim of this study was to examine the potential benefit of vitamin C on the early recurrence rates and on inflammatory indices after successful cardioversion of persistent AF, as well as to investigate the time course of changes in these indices post-cardioversion. **METHODS:** We prospectively studied 44 consecutive patients after successful electrical cardioversion of persistent AF. All patients received standard treatment and were randomised in one to one fashion to either oral vitamin C administration or no additional therapy. We followed-up the patients for 7 days performing successive measurements of white blood cell (WBC) count, C-reactive protein (CRP), fibrinogen, and ferritin levels. **RESULTS:** One week after successful cardioversion, AF recurred in 4.5% of patients in the vitamin C group and in 36.3% of patients in the control group ($p=0.024$). Compared to baseline values, inflammatory indices decreased after cardioversion in patients receiving vitamin C but did not change significantly in the control group. A significant variance was found in the serial measurements of WBC counts ($F=5.86$, $p=0.001$) and of fibrinogen levels ($F=4.10$, $p=0.0084$) in the two groups. In the vitamin C group CRP levels were lower on the seventh day ($p<0.05$). CRP and fibrinogen levels were higher in patients who relapsed into AF compared to patients who maintained sinus rhythm ($F=2.77$, $p=0.044$ and $F=3.51$, $p=0.017$, respectively). **CONCLUSIONS:** These findings suggest that vitamin C reduces the early recurrence rates after cardioversion of persistent AF and attenuates the associated low-level inflammation. These effects indicate that therapeutic approaches targeting at inflammation and oxidative stress may exert favourable effects on atrial electrical remodeling.

Int J Cardiol. 2005 Jul 10;102(2):321-6

THE RELATIONSHIP BETWEEN POTENCY OF OXIDATIVE STRESS AND SEVERITY OF DILATED CARDIOMYOPATHY.

BACKGROUND: It has been suggested that oxidative stress may have a role in the etiopathogenesis of congestive heart failure. **OBJECTIVES:** To investigate and compare the oxidative-antioxidative status and oxidative stress index (OSI) of patients with idiopathic dilated cardiomyopathy (IDC) with those of healthy volunteers, and to determine the relationship between total antioxidant capacity (TAC) and ejection fraction (EF). **METHODS:** Twenty-eight patients with IDC and 24 control subjects were enrolled in the study. Antioxidative status was evaluated by measuring the TAC and the vitamin C and thiol levels in the plasma. Oxidative status was evaluated by measuring the total peroxide level. The per cent ratio of TAC to total peroxide level was accepted as the OSI. EF was measured using Simpson's method. **RESULTS:** TAC and vitamin C and thiol levels of plasma were found to be significantly lower in patients with IDC than in control subjects ($P < 0.001$). In contrast, total peroxide levels and OSIs were significantly higher in patients with IDC than in control subjects ($P = 0.002$ and $P = 0.002$, respectively). An important positive correlation was found between TAC and EF ($r = 0.772$; $P < 0.001$). On the other hand, significant negative correlations were found between EF and OSI and between EF and total peroxide levels in patients. **CONCLUSIONS:** Oxidants are increased and antioxidants are decreased in patients with IDC; as a result, the oxidative-antioxidative balance is shifted to the oxidative side. There is a significant correlation between the potency of oxidative stress and the severity of IDC. It is believed that

supplementation of antioxidants in the treatment of IDC may be helpful to these patients.

Can J Cardiol. 2005 Aug;21(10):851-5

MOLECULAR TARGETS AND ANTICANCER POTENTIAL OF INDOLE-3-CARBINOL AND ITS DERIVATIVES.

Indole-3-carbinol (I3C) is produced by members of the family Cruciferae, and particularly members of the genus Brassica (e.g., cabbage, radishes, cauliflower, broccoli, Brussels sprouts, and daikon). Under acidic conditions, I3C is converted to a series of oligomeric products (among which 3,3'-diindolylmethane is a major component) thought to be responsible for its biological effects in vivo. In vitro, I3C has been shown to suppress the proliferation of various tumor cells including breast cancer, prostate cancer, endometrial cancer, colon cancer, and leukemic cells; induce G1/S arrest of the cell cycle, and induce apoptosis. The cell cycle arrest involves downregulation of cyclin D1, cyclin E, cyclin-dependent kinase (CDK)2, CDK4, and CDK6 and upregulation of p15, p21, and p27. Apoptosis by I3C involves downregulation antiapoptotic gene products, including Bcl-2, Bcl-xL, survivin, inhibitor-of-apoptosis protein (IAP), X chromosome-linked IAP (XIAP), and Fas-associated death domain protein-like interleukin-1-beta-converting enzyme inhibitory protein (FLIP); upregulation of proapoptotic protein Bax; release of mitochondrial cytochrome C; and activation of caspase-9 and caspase-3. This agent inhibits the activation of various transcription factors including nuclear factor-kappaB, SP1, estrogen receptor, androgen receptor and nuclear factor-E2-related factor 2 (Nrf2). This indole potentiates the effects of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) through induction of death receptors and synergises with chemotherapeutic agents through downregulation of P-glycoprotein (P-gp). In vivo, I3C was found to be a potent chemopreventive agent for hormonal-dependent cancers such as breast and cervical cancer. These effects are mediated through its ability to induce apoptosis, inhibit DNA-carcinogen adduct formation, and suppress free-radical production, stimulate 2-hydroxylation of estradiol, inhibit invasion and angiogenesis. Numerous studies have indicated that I3C also has a strong hepatoprotective activity against various carcinogens. Initial clinical trials in women have shown that I3C is a promising agent against breast and cervical cancers.

Cell Cycle. 2005 Sep;4(9):1201-15

EFFECTS OF INDOLE-3-CARBINOL AND PHENETHYL ISOTHIOCYANATE ON COLON CARCINOGENESIS INDUCED BY AZOXYMETHANE IN RATS.

Indole-3-carbinol (I3C) and phenethyl isothiocyanate (PEITC) are breakdown products of the glucosinolates glucobrassicin and gluconasturtiin, respectively, and are thought to reduce carcinogen activation by P450 enzymes. To assess the effects of these compounds on colon cancer risk, rats were divided into five groups and fed the following diets: control diet (AIN-93G), or diets with PEITC or I3C added to the control diet: high-PEITC (3.37 mmols/kg diet-high level of PEITC), low-PEITC (0.67 mmols/kg-low level of PEITC), high-I3C (6.8 mmols/kg-high level of I3C) and low-I3C (1.36 mmols/kg-low level of I3C). Diets were fed for 2 weeks before and 10 weeks after administration of the colon carcinogen azoxymethane. Precancerous lesion (aberrant crypt foci, ACF) number in the distal colon was significantly lower in both high-I3C and low-I3C groups (6.9 +/- 0.8 and 5.9 +/- 0.59 per cm², respectively) when compared with the control group (10.4 +/- 0.9). No significant difference in ACF number was found between the PEITC group and the control group. ACF expressing sialomucin, thought to indicate ACF more likely to progress to tumors, were greater in the high-PEITC group (13 +/- 3) than the control (5.6 +/- 2). Mucin-depleted ACF, suggested to have the greatest tumorigenic potential, tended to be lower in the low-I3C group (P < 0.06) compared with the control group. Mucosal apoptotic and cell proliferation labeling indices did not differ among groups, suggesting that reduction in the ACF number by I3C does not involve alterations in mucosal cell kinetics. No significant differences were found among the groups in hepatic cytochrome P450 2E1 (CYP2E1) activity, the first enzyme involved in activation of azoxymethane. However, there was increased activity of NADPH- and NADH reductases with high-I3C, which are the enzymes involved in the transfer of reducing equivalents to cytochrome P450. These results suggest that I3C lowers colon cancer risk through a mechanism not involving reduction of carcinogen activation by CYP2E1.

Carcinogenesis. 2006 Feb;27(2):287-92

INDOLE-3-CARBINOL ACTIVATES THE ATM SIGNALING PATHWAY INDEPENDENT OF DNA DAMAGE TO STABILIZE P53 AND INDUCE G1 ARREST OF HUMAN MAMMARY EPITHELIAL CELLS.

The phytochemical indole-3-carbinol (I3C), from cruciferous vegetables such as broccoli, has been shown to elicit a potent anti-proliferative response in human breast cancer cell lines. Treatment of the immortalized human mammary epithelial cell line MCF10A with I3C induced a G1 cell cycle arrest, elevated p53 tumor suppressor protein levels and stimulated expression of downstream transcriptional target, p21. I3C treatment also elevated p53 levels in several breast cancer cell lines that express

mutant p53. I3C did not arrest MCF10A cells stably transfected with dominant-negative p53, establishing a functional requirement for p53. Cell fractionation and immunolocalization studies revealed a large fraction of stabilized p53 protein in the nucleus of I3C-treated MCF10A cells. With I3C treatment, phosphatidylinositol-3-kinase family member ataxia telangiectasia-mutated (ATM) was phosphorylated, as were its substrates p53, CHK2 and BRCA1. Phosphorylation of p53 at the N-terminus has previously been shown to disrupt the interaction between p53 and its ubiquitin ligase, MDM2, and therefore stabilizing p53. Coimmunoprecipitation analysis revealed that I3C reduced by 4-fold the level of MDM2 protein that associated with p53. The p53-MDM2 interaction and absence of p21 production were restored in cells treated with I3C and the ATM inhibitor wortmannin. Significantly, I3C does not increase the number of 53BP1 foci or H2AX phosphorylation, indicating that ATM is activated independent of DNA double-strand breaks. Taken together, our results demonstrate that I3C activates ATM signaling through a novel pathway to stimulate p53 phosphorylation and disruption of the p53-MDM2 interaction, which releases p53 to induce the p21 CDK inhibitor and a G1 cell cycle arrest.

Int J Cancer. 2006 Feb 15;118(4):857-68

BRASSICA VEGETABLE CONSUMPTION REDUCES URINARY F2-ISOPROSTANE LEVELS INDEPENDENT OF MICRONUTRIENT INTAKE.

Isothiocyanates and indoles (e.g., indole-3-carbinol) from Brassica vegetables (e.g., broccoli) induce Phase I and Phase II enzymes responsible for the oxidation, reduction, and metabolism of endogenous and exogenous carcinogens. Brassica vegetables also contain micronutrients that may provide additional DNA protection from reactive oxygen species. This randomized cross-over trial (n=20) compares the effects of a Brassica Vegetable Intervention (BV) against a Micronutrient and Fiber Supplementation Intervention (M+F) on urinary F2-isoprostane levels (F2-iP), a stable biomarker of systemic oxidative stress. Brassica intake was monitored by repeated 24-hour recalls, urinary ITC levels, and questionnaire. Urinary F2-iP levels were measured by mass spectrometry from first-morning urine samples collected at Baseline and after each intervention, and change in natural log-transformed urinary F2-iP levels were analyzed using repeated measures regression. Brassica consumption increased from 2 grams/day during the Baseline or M+F Intervention periods to 218 grams/day during the BV Intervention, while exposure to most antioxidant vitamins and minerals was greatest during the M+F Intervention. F2-iP levels significantly decreased 22.0% or 21.8% during the BV Intervention compared to Baseline or the M+F Intervention (p=0.05, p=0.05, respectively). Urinary F2-iP levels did not significantly differ between Baseline and the M+F Intervention (difference = 0.2%; p=0.98). Brassica intake has been associated with reduced risk of colon, lung, bladder, breast, prostate, and other cancers. Our results suggest that Brassica consumption reduces systemic oxidative stress independent of the vitamin and mineral content of these vegetables.

Carcinogenesis. 2006 May 15

THE IMMUNE SYSTEM AS A TARGET FOR ENVIRONMENTAL CHEMICALS: XENOESTROGENS AND OTHER COMPOUNDS.

The immune system in higher organisms is under integrated control and has the capacity to rapidly respond to the environment. Recently, there has been a significant increase in the prevalence of allergic diseases. Environmental factors likely play a major role in the explosion of allergy. Although the "hygiene hypothesis" may explain the increase in allergic diseases which are prone to T helper 2 (Th2) immune responses, recent findings highlight the possible involvement of environmental xenobiotic chemicals which can modulate normal immune function. Interestingly, several reports suggest that the prevalence of systemic lupus erythematosus, a Th2-type autoimmune disease, is also increasing, although the development of high-sensitivity immunological tests may be a possible cause. The increased prevalence of autoimmune disease in women, the sexual dimorphism of the immune response, and the immunomodulatory effects of sex steroids, have focused attention on the role of chemicals which influence sex steroids in the development of immune diseases. Moreover, recent reports indicate that some environmental chemicals can work on nuclear hormone receptors, other than sex hormone receptors, and modulate immune reactions. This review focuses on the impact of environmental chemicals on immune system function and pathogenesis of immune diseases, including allergy and autoimmune diseases.

Toxicol Lett. 2006 Jul 14;164(3):191-206

ESTROGENIC ACTIVITY OF POLYCHLORINATED BIPHENYLS PRESENT IN HUMAN TISSUE AND THE ENVIRONMENT.

This study evaluated the estrogenicity of polychlorinated biphenyls (PCBs) present in environmental media and human tissue and assessed exposure pathways for PCB-derived estrogenic potency in air, soil, and dust from New Bedford, MA, an area with a PCB-contaminated Superfund site. Thirty-four PCB congeners were assayed for estrogenic potency using E-SCREEN, an assay based on the estrogen-dependent proliferation of MCF-7 cells in vitro. Childhood exposure to estradiol-equivalents via PCBs in environmental media was estimated by weighting previously reported New Bedford congener-specific concentrations by their relative estrogenic potency and published inhalation and soil ingestion rates. Thirteen congeners were weakly estrogenic in E-

SCREEN: PCBs 17, 18, 30, 44, 49, 66, 74, 82, 99, 103, 110, 128, and 179. These PCBs were typically 6 orders of magnitude less potent than 17beta-estradiol, with proliferative potencies ranging from 0.0007% to 0.0040%. Of the environmental media assessed, air (inhalation) had the highest PCB-derived estradiol-equivalent exposure. PCB estrogenic potency information from this study provides an important resource both for preliminary estimation of routes of human exposure to xenoestrogens and for application to human health studies focused on estrogen-responsive health outcomes, such as reproductive development and related malignancies.

Environ Sci Technol. 2006 Apr 15;40(8):2819-25

BIOMAGNIFICATION OF PBDES AND PCBs IN FOOD WEBS FROM THE BALTIC SEA AND THE NORTHERN ATLANTIC OCEAN.

Biomagnification of polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs) in food webs from the Baltic Sea and the northern Atlantic Sea was investigated. For this, we used PCB and PBDE concentration data, together with data on fish body weight and delta(15)N of fish and zooplankton as a measure of trophic position. In the Baltic Sea material, consisting of zooplankton, sprat, herring and salmon, we report biomagnification of all PCB congeners but PCB #209 and of PBDEs with 3-6 or 7 bromine atoms. Higher brominated PBDEs and PCB 209 did not biomagnify likely due to their high molecular weights or sizes and subsequent inefficient dietary uptake in fish. If salmon was excluded from the statistical analysis, strong biomagnification of PCB #209 was evident, indicating species differences in biomagnification. In the Baltic Sea material delta(15)N and body weight covaried. In the Atlantic Sea material, consisting of fish samples (herring and salmon) of larger body sizes, we show positive correlation between concentrations of most PCBs and PBDEs and body weight without increasing delta(15)N. This shows that biomagnification in some cases depends on body size and not trophic position. We conclude that there probably is trophic position dependence in biomagnification, which was manifested in a food chain from zooplankton to piscivores, but no further trophic position influence on biomagnification in fish at the highest trophic levels. In these fish, there was a body size effect leading to biomagnification, probably due to slower clearance in larger fish. PCB concentrations were generally between 2 and 6 times higher in Baltic Sea salmon than in Atlantic Sea salmon. Higher PBDE concentrations in the Baltic compared to the Atlantic Sea salmon were also found, but with a larger variation between congeners. Nona- to deca-BDEs were found in most investigated samples, which illustrates the bioavailability of these compounds. Unidentified penta-, hexa-, hepta-, and octa- BDEs were found in several samples.

Sci Total Environ. 2006 Aug 1;366(2-3):659-72

INDOLE-3-CARBINOL, BUT NOT ITS MAJOR DIGESTIVE PRODUCT 3,3'-DIINDOLYLMETHANE, INDUCES REVERSIBLE HEPATOCYTE HYPERTROPHY AND CYTOCHROMES P450.

Indole-3-carbinol (I-3-C) and 3,3'-diindolylmethane (DIM) have been shown to reduce the incidence and multiplicity of cancers in laboratory animal models. Based on the observation that I-3-C induced hepatocyte hypertrophy when administered orally for 13 weeks to rats, a treatment and recovery study was undertaken to test the hypothesis that the induction of hepatocyte hypertrophy and cytochrome P450 (CYP) activity by I-3-C are adaptive, reversible responses. Additionally, we directly compared the effects of I-3-C to those of its principle metabolite DIM. Rats were treated orally for 28 days with 2 doses of I-3-C (5 and 50 mg I-3-C/kg body weight/day) and DIM (7.5 and 75 mg DIM/kg body weight/day) and then one-half of the animals were not treated for an additional 28 days. Organ weights, histopathology, and the CYP enzyme activities of 1A1/2, 2B1/2, 2C9, 2D6, 2E1, 3A4, and 19 A were measured both after treatment and after recovery. Oral administration of 50 mg I-3-C/kg body weight/day to rats for 28 days significantly increased liver weights and CYP enzyme activities. The effects in males were more pronounced and persistent after recovery than the effects in females. The increased organ weights returned to control values after treatment. Conversely, DIM did not alter liver weights and had no effect on CYP activities after the 28-day treatment. Some changes in CYP activities were measured after the DIM recovery period but the magnitudes of the changes were considered biologically insignificant. The results show that I-3-C, but not DIM, induces reversible adaptive responses in the liver.

Toxicol Appl Pharmacol. 2006 Mar 1;211(2):115-23

1ALPHA,25-DIHYDROXYVITAMIN D3 INHIBITS PROSTATE CANCER CELL INVASION VIA MODULATION OF SELECTIVE PROTEASES.

Inhibition of invasion and metastasis has become a new approach for treatment of advanced prostate cancer in which secondary hormone therapy has failed. Accumulating evidence indicates that 1alpha,25-dihydroxyvitamin D3 (1,25-V_D) suppresses prostate cancer progression by inhibition of tumor growth and metastasis. However, the detailed mechanisms underlying these effects remain to be determined. Here, we used the in vitro cell invasion assay to demonstrate that 1,25-V_D inhibits the invasive ability of human prostate cancer cell lines, LNCaP, PC-3 and DU 145. Three major groups of proteases, the matrix metalloproteinases (MMPs), the plasminogen activators (PAs) and the cathepsins (CPs), that are involved in tumor invasion were then examined for changes in activity and expression after 1,25-V_D treatment. We found that 1,25-V_D decreased MMP-9 and CPs, but not PAs activities, while it increased the activity of their counterparts, tissue inhibitors of metalloproteinase-1 (TIMP-1) and cathepsin

inhibitors. Mechanistic studies showed that 1,25-VD did not suppress MMP-9 expression at the transcriptional level, but reduced its mRNA stability. In addition, 1,25-VD increased AP-1 complexes binding to TIMP-1 promoter, which contributed to the enhancement of TIMP-1 activity, and thus resulted in inhibition of MMP activity and tumor invasion. These findings support the idea that vitamin D-based therapies might be beneficial in the management of advanced prostate cancer, especially among patients who have higher MMP-9 and CPs activities.

Carcinogenesis. 2006 Jan;27(1):32-42

3,3'-DIINDOLYLMETHANE DOWNREGULATES PRO-SURVIVAL PATHWAY IN HORMONE INDEPENDENT PROSTATE CANCER.

Epidemiological evidences suggest that the progression and promotion of prostate cancer (CaP) can be modulated by diet. Since all men die with prostate cancer rather than of the disease, it is of particular interest to prevent or delay the progression of the disease by chemopreventive strategies. We have been studying the anticancer properties of compounds present in cruciferous vegetables such as indole-3-carbinol (I3C). Diindolylmethane (DIM) is a dimer of I3C that is formed under acidic conditions and unlike I3C is more stable with higher anti-cancer effects. In the present report, we demonstrate that DIM is a potent anti-proliferative agent compared to I3C in the hormone independent DU 145 CaP cells. The anti-prostate cancer effect is mediated by the inhibition of the Akt signal transduction pathway as DIM, in sharp contrast to I3C, induces the downregulation of Akt, p-Akt, and PI3 kinase. DIM also induced a G1 arrest in DU 145 cells by flow cytometry and downstream concurrent inhibition of cell cycle parameters such as cyclin D1, cdk4, and cdk6. Our data suggest a need for further development of DIM, as a chemopreventive agent for CaP, which justifies epidemiological evidences and molecular targets that are determinants for CaP dissemination/progression. The ingestion of DIM may benefit CaP patients and reduce disease recurrence by eliminating micro-metastases that may be present in patients who undergo radical prostatectomy.

Biochem Biophys Res Commun. 2006 Feb 10;340(2):718-25

FATE OF 3,3'-DIINDOLYLMETHANE IN CULTURED MCF-7 HUMAN BREAST CANCER CELLS.

3,3'-Diindolylmethane (DIM) is a major *in vivo* product of the cancer preventative agent indole-3-carbinol that is found in vegetables of the genus Brassica. Here, we report on the metabolic fate of radiolabeled DIM in MCF-7 cells. DIM was slowly metabolized to several sulfate conjugates of oxidized DIM products that were primarily detected in the medium. The radioactivity detected in cells was predominantly unmodified DIM (81-93%) at all time intervals up to 72 h treatment. Co-treatment of MCF-7 cells with quercetin slowed the rate that oxidized DIM products accumulated in the medium, while indole[3,2-b]carbazole (ICZ) co-treatment accelerated their production. ICZ is an inducer of P450 1A2, while quercetin is a specific inhibitor of this isoform, suggesting that P450 1A2 is primarily responsible for the oxidation of DIM, probably through 2,3-epoxidation similar to 3-methylindole. Sulfate conjugates of oxidized DIM metabolites were cleaved by sulfatase digestion and identified by LC/MS as 3-(1H-indole-3-ylmethyl)-2-oxindole (2-ox-DIM), bis(1H-indol-3-yl)methanol (3-methylenedioxy-DIM), 3-[hydroxy-(1H-indol-3-yl)-methyl]-1,3-dihydro-2-oxindole (3-methylenedioxy-2-ox-DIM), and 3-hydroxy-3-(1H-indole-3-ylmethyl)-2-oxindole (3-hydroxy-2-ox-DIM). Derivatives of 2-ox-DIM represented greater than 30% of the radioactivity in the sulfatase-digested medium. Although oxindole formation was the primary metabolic pathway in MCF-7 cells, synthetic 2-ox-DIM was inactive in a 4-ERE-luciferase reporter assay and, therefore, probably not responsible for the estrogenic activity previously observed for DIM. Unmodified DIM rapidly accumulated in the nuclear membranes representing approximately 35-40% of the radioactivity after 0.5-2 h treatment. Uptake of radiolabeled DIM appeared to be a passive partitioning into the nuclear membranes and was not dependent upon the cell cytosol. The nuclear uptake of DIM was not saturable and could not be blocked by pretreatment with unlabeled DIM (100 microM). Further, treatments in serum-free medium increased the uptake of radiolabeled DIM by the MCF-7 cells. These findings show that the uptake of DIM by membranes significantly increases its localized concentration, which may contribute to its biological activities.

Chem Res Toxicol. 2006 Mar;19(3):436-42

A RANDOMIZED PHASE II TRIAL OF INDOLE-3-CARBINOL IN THE TREATMENT OF VULVAR INTRAEPITHELIAL NEOPLASIA.

The aim of this study was to determine the potential therapeutic benefits of indole-3-carbinol (I3C) in the management of vulvar intraepithelial neoplasia (VIN). Women with histologically confirmed high-grade VIN were randomized to receive 200 and 400 mg/day of I3C. Symptomatology by visual analog scale and vulvoscopic appearance were assessed at recruitment, 6 weeks, 3 months, and 6 months. Tissue biopsy to determine histologic response was obtained at completion of the study period. Urine samples were obtained at each visit to determine 2-hydroxyestrone to 16-alpha-hydroxyestrone ratios. Data from 12 women were suitable for analysis. There was a significant improvement in symptomatology with the introduction of I3C (itch, $P=0.018$; pain, $P=0.028$). Lesion size and severity were also significantly reduced (size, $P=0.005$; appearance, $P=0.046$). In addition, there was a significant increase in 2-hydroxyestrone to 16-alpha-hydroxyestrone ratio following commencement of I3C, $P=0.05$. However, tissue biopsy from the worst-affected vulvar areas revealed no improvement in grade of VIN during the 6-month period, $P=0.317$. There were no significant differences in results between those women taking 200 mg/day of I3C and those on 400

mg/day. This study has shown significant clinical improvement in symptomatology and vulvoscopic appearance of VIN with I3C therapy. Further clinical and scientific investigations are required to support these preliminary findings.

Int J Gynecol Cancer. 2006 Mar-Apr;16(2):786-90

ACTIVATION AND POTENTIATION OF INTERFERON-GAMMA SIGNALING BY 3,3'-DIINDOLYLMETHANE IN MCF-7 BREAST CANCER CELLS.

3,3'-Diindolylmethane (DIM), a natural autolytic product in plants of the Brassica genus, including broccoli, cauliflower, and Brussels sprouts, exhibits promising cancer protective activities, especially against mammary neoplasia in animal models. We observed previously that DIM induced a G(1) cell-cycle arrest and strong induction of cell-cycle inhibitor p21 expression and promoter activity in both estrogen-responsive and -independent breast cancer cell lines. We showed recently that DIM up-regulates the expression of interferon gamma (IFN γ) in human MCF-7 breast cancer cells. This novel effect may contribute to the anticancer effects of DIM because IFN γ plays an important role in preventing the development of primary and transplanted tumors. In this study, we observed that DIM activated the IFN γ signaling pathway in human breast cancer cells. DIM activated the expression of the IFN γ receptor (IFNGR1) and IFN γ -responsive genes p56- and p69-oligoadenylate synthase (OAS). In cotreatments with IFN γ , DIM produced an additive activation of endogenous p69-OAS and of an OAS-Luc reporter and a synergistic activation of a GAS-Luc reporter. DIM synergistically augmented the IFN γ induced phosphorylation of signal transducer and activator of transcription factor 1, further evidence of DIM activation of the IFN γ pathway. DIM and IFN γ produced an additive inhibition of cell proliferation and a synergistic increase in levels of major histocompatibility complex class-1 (MHC-1) expression, accompanied by increased levels of mRNAs of MHC-1-associated proteins and transporters. These results reveal novel immune activating and potentiating activities of DIM in human tumor cells that may contribute to the established effectiveness of this dietary indole against various tumors types.

Mol Pharmacol. 2006 Feb;69(2):430-9

CHEMICAL AND BIOLOGICAL CHARACTERISATION OF NUTRACEUTICAL COMPOUNDS OF BROCCOLI.

People's diet offers a greater and more diverse group of plant bioactives than do drugs, and they often do not realise that many drugs are derived from the compounds originally discovered in plant foods. Numerous epidemiological studies indicate that Brassica vegetables in general, and broccoli in particular, protect humans against cancer since they are rich sources of glucosinolates as well as possessing a high content of flavonoids, vitamins and mineral nutrients. One unusual phytotherapeutic role of broccoli is for skin diseases-the juice of the leaves is used to treat warts. However, the main use of broccoli stems from its health-promoting properties. Some criteria have been proposed to evaluate the possibilities of developing new "functional foods" to reduce the risk of specific cancers; largely in broccoli, which is associated with cancer protection. Processing conditions, transport, domestic cooking, etc., affect the health-promoting properties of broccoli and these have been widely studied. This review makes an in-depth study of the chemical and biological characterization of the phytochemicals of broccoli and the effects on the bioactive composition of broccoli.

J Pharm Biomed Anal. 2006 May 17

BIOLOGY AND BIOCHEMISTRY OF GLUCOSINOLATES.

Glucosinolates are sulfur-rich, anionic natural products that upon hydrolysis by endogenous thioglucosidases called myrosinases produce several different products (e.g., isothiocyanates, thiocyanates, and nitriles). The hydrolysis products have many different biological activities, e.g., as defense compounds and attractants. For humans these compounds function as cancer-preventing agents, biopesticides, and flavor compounds. Since the completion of the Arabidopsis genome, glucosinolate research has made significant progress, resulting in near-complete elucidation of the core biosynthetic pathway, identification of the first regulators of the pathway, metabolic engineering of specific glucosinolate profiles to study function, as well as identification of evolutionary links to related pathways. Although much has been learned in recent years, much more awaits discovery before we fully understand how and why plants synthesize glucosinolates. This may enable us to more fully exploit the potential of these compounds in agriculture and medicine.

Annu Rev Plant Biol. 2006 Jun 2;57:303-333

UNCARIA TOMENTOSA (WILLD.) DC.— ETHNOMEDICINAL USE AND NEW PHARMACOLOGICAL, TOXICOLOGICAL AND BOTANICAL RESULTS.

The medicinal system of the Ashaninka Indians in Peru is portrayed. Three categories of medical disorders and healers are recognized. A human is viewed to consist of a physical and a spiritual being who communicate with each other by means of a regulating element. The significance of *Uncaria tomentosa* (Willd.) DC. (Rubiaceae), locally known as *una de gato*, in traditional medicine is emphasized by its exclusive use by priests to influence this regulation. Pharmacological and toxicological results

obtained with extracts or isolated compounds are summarized. Pentacyclic oxindole alkaloids stimulate endothelial cells in vitro to produce a lymphocyte-proliferation-regulating factor. Tetracyclic oxindole alkaloids act as antagonists. A significant normalization of lymphocyte percentage was observed in vivo although total leucocyte numbers did not change.

J Ethnopharmacol. 1999 Jan;64(1):23-34

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