N-ACETYL CYSTEINE N-ACETYL CYSTEINE INHIBITS EXERCISE-INDUCED LYMPHOCYTE APOPTOTIC PROTEIN ALTERATIONS.

PURPOSE: To investigate the effect of strenuous exercise and antioxidant administration on pro- and antiapoptotic protein expression in intestinal lymphocytes. METHODS: Female C57BL/6 mice (N = 52) were randomly assigned to receive N-acetyl cysteine (NAC; 1 g.kg(-1)) or saline (SAL) 30 min before treadmill exercise (EX) for 90 min and 2 degrees slope (30 min at 22 m.min(-1), 30 min at 25 m.min(-1), and 30 min at 28 m.min(-1)) and sacrificed immediately (Imm) or 24 h (24 h) after exercise. Control mice were exposed to treadmill noise and vibration without running (nonexercised). Intestinal lymphocytes (IL) were isolated and pro- and antiapoptotic protein expression was evaluated by Western blot analysis. RESULTS: IL protein levels of proapoptotic (caspase 3 and cytosolic cytochrome c) and antiapoptotic (Bcl-2) were significantly different among groups. Relative to nonexercised mice, protein levels of caspase 3 (P < 0.001) and cytosolic cytochrome c (P < 0.005) were significantly elevated, whereas Bcl-2 (P < 0.05) was significantly lower immediately after exercise in mice receiving saline (EX + SAL + Imm) but not in animals receiving NAC (EX + NAC + Imm) or both 24 h postgroups (EX + SAL + 24 h and EX + NAC + 24 h). CONCLUSION: These results suggest that oxidative stress acting through a mitochondrial pathway may play a role in intestinal lymphocyte apoptosis after strenuous exercise.

EXERCISE AFFECTS TISSUE LYMPHOCYTE APOPTOSIS VIA REDOX-SENSITIVE AND FAS-DEPENDENT SIGNALING PATHWAYS.

Intensive and exhaustive exercise induces an activation of blood T-lymphocytes, which seems to be terminated by apoptotic processes in the postexercise period. Here, we report that exercise-induced T-lymphocyte apoptosis is a systemic phenomenon occurring in various lymphoid and nonlymphoid tissues. The apoptosis rate could be related to exercise intensity and type. Although in some tissues, such as the spleen and Peyer’s patches, an early start of apoptosis (1-3 h postexercise) could be detected, a delayed apoptosis (24 h postexercise) was observed in lung, bone marrow, and lymph nodes. Further analysis showed a similar apoptosis distribution among lymphocyte subpopulations. We tested whether components of the extrinsic or the intrinsic apoptotic pathways or both were involved in these processes. Elevated levels of lipid peroxidation-product malondialdehyde (MDA), indicating an increased production of reactive oxygen species (ROS), were found after exercise in Peyer’s patches, lung, and spleen, but not in lymph nodes. Application of N-acetyl cysteine (NAC) prevented exercise-induced T-cell apoptosis completely in spleen and bone marrow, partially in lung and Peyer’s patches, while it was ineffective in lymph nodes. Additionally, exercise addressed the Fas-mediated apoptosis. The percentage of Fas-receptor (Fas+) and Fas-ligand positive (FasL+) lymphocytes was enhanced in Peyer’s patches after exercise. Moreover, FasL+ T cells were increased in the lung, while in lymph nodes Fas+ cells were increased. The critical role of Fas signaling in exercise-induced apoptosis was supported by using Fas-deficient MRL/lpr-mice. In Fas-deficient mice, exercise-induced T-lymphocyte apoptosis was prevented in spleen, lung, bone marrow, and lymph nodes, but not in Peyer’s patches. These data demonstrate that exercise-induced lymphocyte apoptosis is a transient systemic process with tissue-type specific apoptosis-inducing mechanisms, whose relevance for the adaptive immune competence remains to be shown.

Am J Physiol Regul Integr Comp Physiol. 2009 May;296(5):R1518-27

THE MOLECULAR BASIS FOR OXIDATIVE STRESS-INDUCED INSULIN RESISTANCE.

Reactive oxygen and nitrogen molecules have been typically viewed as the toxic by-products of metabolism. However, accumulating evidence has revealed that reactive species, including hydrogen peroxide, serve as signaling molecules that are involved in the regulation of cellular function. The chronic and/or increased production of these reactive molecules or a reduced capacity for their elimination, termed oxidative stress, can lead to abnormal changes in intracellular signaling and result in chronic inflammation and insulin resistance. Inflammation and oxidative stress have been linked to insulin resistance in vivo. Recent studies have found that this association is not restricted to insulin resistance in type 2 diabetes, but is also evident in obese, nondiabetic individuals, and in those patients with the metabolic syndrome. An increased concentration of reactive molecules triggers the activation of serine/threonine kinase cascades such as c-Jun N-terminal kinase, nuclear factor-kappaB, and others that in turn phosphorylate multiple targets, including the insulin receptor and the insulin receptor substrate (IRS) proteins. Increased serine phosphorylation of IRS reduces its ability to undergo tyrosine phosphorylation and may accelerate the
AMINOACID SUPPORT IN THE PREVENTION OF DIABETES AND DIABETIC COMPLICATIONS.

Emerging evidence suggests that amino acids may be potentially important in the prevention of diabetes and diabetes-associated complications. The pathways involved in the pathogenesis of diabetic complications include increased polyol pathway flux, increased advanced glycation end products formation, activation of protein kinase C and oxidative and carbonyl stress. This review will discuss the modulatory effects of amino acids on insulin secretion and their action in concert with insulin as signaling molecules. Evidences for the role of some amino acids in controlling glycemia and glucose-triggered pathological pathways are also included. Individual amino acids, especially the ones bestowed with antioxidant property like N-acetyl cysteine and taurine seem to have beneficial effects by their ability to reduce intracellular oxidative stress generation and glycooxidation. Other amino acids like glycine and lysine may be good candidates for the prevention of glycation. Nutritional intervention with taurine, phenyl alanine or branched chain amino acids can improve insulin sensitivity and post-prandial glucose disposal. Deficiency of one or more amino acids has been observed in diabetes and the beneficial effects of amino acids in some studies are positively correlated with the increase in plasma levels of these amino acids. Inclusion of individual amino acids/mixture, perhaps as a combinational therapy with conventional treatment protocols could be of therapeutic interest.

Curr Protein Pept Sci. 2009 Feb;10(1):8-17

METHYLGLYOXAL CONTRIBUTES TO THE DEVELOPMENT OF INSULIN RESISTANCE AND SALT SENSITIVITY IN SPRAGUE-DAWLEY RATS.

OBJECTIVES: Methylglyoxal, a metabolite of the glycolysis pathway, may play an important role in the development of diabetes and hypertension, but the exact mechanism has not been fully elucidated. The present study was designed to investigate whether methylglyoxal could directly induce insulin resistance and salt sensitivity in Sprague-Dawley rats. METHODS: Rats were allocated to four groups: control (normal drinking water), 1% methylglyoxal in drinking water, 1% methylglyoxal plus N-acetyl cysteine (NAC) (800 mg/kg per day), a methylglyoxal scavenger, or TM2002 (100 mg/kg per day), an advanced glycation endproducts (AGEs) inhibitor. After 4-week treatment insulin resistance was evaluated by an euglycemic hyperinsulinemic glucose clamp technique. In another set of rats, either a high-salt diet (4%) alone, standard rat chow with 1% methylglyoxal in drinking water or high-salt diet plus methylglyoxal was given for 4 weeks. Immunohistochemistry was performed to measure nitrotyrosine and methylglyoxal-induced AGES, N-carboxyethyl-lysine (CEL) in the kidney. RESULTS: Four-week treatment with NAC or TM2002 completely improved methylglyoxal-induced insulin resistance. Co-administration of methylglyoxal and high-salt diet significantly increased systolic blood pressure, urinary albumin excretion, urinary thiobarbituric acid-reactive substances excretion and the renal nitrotyrosine expression in the kidney (markers of oxidative stress) compared with methylglyoxal or high-salt diet alone. Renal CEL was significantly increased in methylglyoxal-treated rats compared with nonmethylglyoxal-treated rats. CONCLUSION: These results indicate that methylglyoxal-induced insulin resistance and salt sensitivity at least in part by increasing oxidative stress and/or AGEs formation in Sprague-Dawley rats. The present study provides further evidence for methylglyoxal as one of the causative factors in the pathogenesis of insulin resistance and salt-sensitive hypertension.

J Hypertens. 2009 Aug;27(8):1664-71

CHRONIC N-ACETYL CYSTEINE PREVENTS FRUCTOSE-INDUCED INSULIN RESISTANCE AND HYPERTENSION IN RATS.

We examined if administration of an antioxidant compound protects against the development of insulin resistance and hypertension. Male rats were assigned randomly into four groups, and treated for 12 weeks with normal chow, normal chow plus N-acetyl cysteine (1.5 g/day/kg), fructose (60% of diet), and fructose plus N-acetyl cysteine. After 10 weeks, plasma triglyceride and 15-F2t-isoprostane, and insulin sensitivity were measured, and after 12 weeks, pressor response to methoxamine (15-60 microg/kg min) was assessed. Relative to normal chow-fed controls, the fructose-fed rats had increased blood pressure, plasma insulin, triglyceride and 15-F2t-isoprostane, and decreased insulin sensitivity; these changes were inhibited by N-acetyl cysteine. Maximal pressor response to methoxamine was attenuated in the fructose-fed rats given N-acetyl cysteine relative to the other three groups. Therefore, chronic treatment with N-acetyl cysteine increases insulin sensitivity and prevents the blood pressure increase associated with fructose feeding in rats, the mechanism may involve the decrease of oxidative stress and alpha-adrenoceptor-mediated vasoconstriction.

Eur J Pharmacol. 2005 Jan 31;508(1-3):205-10

PROLONGED TREATMENT WITH N-ACETYLCYSTEINE AND L-ARGININE RESTORES GONADAL FUNCTION IN
Nitric oxide (NO) plays a wide spectrum of biological actions including a positive role in oocyte maturation and ovulation. Free radicals levels have been shown elevated in polycystic ovary syndrome (PCOS) and therefore would be responsible for quenching NO that, in turn, would play a role in determining oligo- or amenorrhea connoting PCOS. Eight patients with PCOS displaying oligo-amenorrhea from at least 1 yr underwent a combined treatment with N-acetyl cysteine (NAC) (1200 mg/die) plus L-arginine (ARG) (1600 mg/die) for 6 months. Menstrual function, glucose and insulin levels, and, in turn, homeostasis model assessment (HOMA) index were monitored. Menstrual function was at some extent restored as indicated by the number of uterine bleedings under treatment (3.00, 0.18-5.83 vs 0.00, 0.00-0.83; p<0.02). Also, a well-defined biphasic pattern in the basal body temperature suggested ovulatory cycles. The HOMA index decreased under treatment (2.12, 1.46-4.42 vs 3.48, 1.62-5.95; p<0.05). In conclusion, this preliminary, open study suggests that prolonged treatment with NAC+ARG might restore gonadal function in PCOS. This effect seems associated to an improvement in insulin sensitivity.


N-ACETYL CYSTEINE TREATMENT IMPROVES INSULIN SENSITIVITY IN WOMEN WITH POLYCYSTIC OVARY SYNDROME.

OBJECTIVE: To evaluate the effect of N-acetyl cysteine (NAC) on insulin secretion and peripheral insulin resistance in subjects with polycystic ovary syndrome (PCOS). DESIGN: Prospective data analysis. SETTING: Volunteer women in an academic research environment. PATIENT(S): Six lean and 31 obese subjects, aged 19-33 years. INTERVENTION(S): Patients were treated for 5-6 weeks with NAC at a dose of 1.8 g/day orally. A dose of 3 g/day was arbitrarily chosen for massively obese subjects. Six of 31 obese patients with PCOS were treated with placebo and served as controls. MAIN OUTCOME MEASURE(S): Before and after the treatment period, the hormonal and lipid blood profile and insulin sensitivity, assessed by an hyperinsulinemic euglycemic clamp, were evaluated and an oral glucose tolerance test (OGTT) was performed. RESULT(S): Fasting glucose, fasting insulin, and glucose area under curve (AUC) were unchanged after treatment. Insulin AUC after OGTT was significantly reduced, and the peripheral insulin sensitivity increased after NAC administration, whereas the hepatic insulin extraction was unaffected. The NAC treatment induced a significant fall in T levels and in free androgen index values (P<0.05). In analyzing patients according to their insulineemic response to OGTT, normoinsulinemic subjects and placebo-treated patients did not show any modification of the above parameters, whereas a significant improvement was observed in hyperinsulinemic subjects. CONCLUSION(S): NAC may be a new treatment for the improvement of insulin circulating levels and insulin sensitivity in hyperinsulinemic patients with polycystic ovary syndrome.


N-ACETYL CYSTEINE AND PENICILLAMINE INDUCE APOPTOSIS VIA THE ER STRESS RESPONSE-SIGNALING PATHWAY.

N-acetyl cysteine (NAC) and penicillamine (PEN) have been shown to induce apoptosis in multiple types of human cancer cells; however, the molecular mechanism underlying this activity is unclear. This study was designed to identify the genes responsible for apoptosis induction by NAC and PEN. We found that glucose-regulated protein 78 (GRP78) was upregulated in HeLa cells following treatment with NAC or PEN. GRP78 is a central regulator of endoplasmic reticulum (ER) stress and has been used as a marker of ER stress. Additionally, both the activating transcription factor 6 (ATF6) protein and X box-binding protein 1 (XBP1) mRNA were processed, which facilitates the expression of C/EBP homologous protein (CHOP), a key-signaling component of ER stress-induced apoptosis. Furthermore, the PERK-ATF4 pathway, which also induces the expression of CHOP, was activated in NAC-treated cells. The role of the ER stress pathway was further confirmed through the small interfering RNA (siRNA)-mediated knockdown of CHOP, which attenuated NAC and PEN-induced apoptosis. These results demonstrate that NAC- and PEN-induced apoptosis in HeLa cells is mediated by the ER stress pathway.

Mol Carcinog. 2010 Jan;49(1):68-74

N-ACETYL CYSTEINE INHIBITS HUMAN SIGNET RING CELL GASTRIC CANCER CELL LINE (SJ-89) CELL GROWTH BY INDUCING APOPTOSIS AND DNA SYNTHESIS ARREST.

BACKGROUND AND AIMS: In this study, we investigated the inhibitory effects of N-acetyl cysteine (NAC) on the growth of the human signet ring cell from the gastric-cancer cell line SJ-89, via the induction of apoptosis and the arrest of DNA synthesis. MATERIALS AND METHODS: SJ-89 cells were regularly incubated in the presence of NAC at 5, 10 and 20 mmol/l, and with IMDM as untreated control. Trypan blue-dye exclusion analysis and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay were applied to detect cell proliferation. Apoptotic morphology was observed by electron microscopy. Flow cytometry and terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labeling (TUNEL) assay were performed to detect NAC-triggered apoptosis. RESULTS: NAC could inhibit proliferation of human gastric cancer SJ-89 cells in a dose-dependent and time-dependent manner. The growth curve showed suppression by 15.8, 37.6, and 66.3% following 72 h of NAC treatment at 5, 10 and
ANTIOXIDANTS TIRON AND N-ACETYL CYSTEINE DIFFERENTIALLY MEDIATE APOPTOSIS IN MELANOMA CELLS VIA A REACTIVE OXYGEN SPECIES-INDEPENDENT NF-KAPPAB PATHWAY.

Tiron and N-acetyl cysteine (NAC) have been recognized as potential antioxidants capable of inhibiting apoptosis induced by reactive oxygen species (ROS). Although the ROS-scavenging function of tiron and NAC is clear, the mechanism for their regulation of apoptosis is still elusive. Here we demonstrate that tiron increases nuclear factor-kappaB (NF-kappaB)/DNA binding and as a result enhances NF-kappaB transcripational activity. In contrast, NAC inhibits NF-kappaB activation by reducing inhibitor of kappaB kinase (IKK) activity. Moreover, the expression of an NF-kappaB target gene, the chemokine CXCL1, is promoted by tiron and suppressed by NAC. Finally, tiron confers an antiapoptotic function, while NAC imparts a proapoptotic function in melanoma cells. These functions correlate with the alteration of mitochondrial membrane potential but not ROS production or induction of activating protein-1 (AP-1). This study underscores the potential benefits of regulating NF-kappaB activity in melanoma cells as a therapeutic approach.

N-ACETYL CYSTEINE FOSTERS INACTIVATION AND TRANSFER TO ENDOlysOSOMES OF C-SRC.

The non-receptor-protein tyrosine kinase c-Src is overexpressed and activated in a large number of human cancers, in which it is associated with tumor development and progression. Canonical regulation takes place by means of an alternative phosphorylation of tyrosine residues—Tyr419 for activation and Tyr530 for inactivation. An independent redox regulation mechanism, involving cysteine residues, has also been proposed, in which oxidation activates the enzyme. Here we present a kinetic analysis of the effect of N-acetyl cysteine (NAC) on c-Src, demonstrating that reduction reverts the oxidation-driven activation. In cancer cells, we show that NAC treatment produces an increase in specifically labeled reduced thiols of c-Src cysteines, thus confirming a redox transition. In addition to a decrease in Tyr419 phosphorylation, this leads to a massive shift of c-Src from plasma membranes—where its active form is located—to endolysosomal compartments. With the objective of deciphering the complex issue of c-Src regulation and of devising new strategies to revert its activation in cancers, redox regulation thus emerges as a promising area for study.

N-ACETYL CYSTEINE PROTECTS AGAINST IONIZING RADIATION-INDUCED DNA DAMAGE BUT NOT AGAINST CELL KILLING IN YEAST AND MAMMALS.

Ionizing radiation (IR) induces DNA strand breaks leading to cell death or deleterious genome rearrangements. In the present study, we examined the role of N-acetyl cysteine (NAC), a clinically proven safe agent, for its ability to protect against gamma-ray-induced DNA strand breaks and/or DNA deletions in yeast and mammals. In the yeast Saccharomyces cerevisiae, DNA deletions were scored by reversion to histidine prototrophy. Human lymphoblastoid cells were examined for the frequency of gamma-H2AX foci formation, indicative of DNA double strand break formation. DNA strand breaks were also measured in mouse peripheral blood by the alkaline comet assay. In yeast, NAC did not protect against cell death. NAC also reduced the frequency of IR-induced DNA deletions. However, NAC did not protect against cell death. NAC also reduced gamma-H2AX foci formation in human lymphoblastoid cells but had no protective effect in the colony survival assay. NAC administration via drinking water fully protected against DNA strand breaks in mice whole-body irradiated with 1 Gy but not with 4 Gy. NAC treatment in the absence of irradiation was not genotoxic. These data suggest that, given the safety and efficacy of NAC in humans, NAC may be useful in radiation therapy to prevent radiation-mediated genotoxicity, but does not interfere with efficient cancer cell killing.

PREVENTION OF CIGARETTE SMOKE-INDUCED LUNG TUMORS IN MICE BY BUDESONIDE, PHENETHYL ISOThIOCYANATE, AND N-ACETYL CYSTEINE.
Lung cancer is the most important cause of death among neoplastic diseases worldwide, and cigarette smoke (CS) is the major risk factor for cancer. Complementarily to avoidance of exposure to CS, chemoprevention will lower the risk of cancer in passive smokers, ex-smokers, and addicted current smokers who fail to quit smoking. Unfortunately, chemoprevention clinical trials have produced disappointing results to date and, until recently, a suitable animal model evaluating CS carcinogenicity was not available. We previously demonstrated that mainstream CS induces a potent carcinogenic response when exposure of mice starts at birth. In the present study, neonatal mice (strain H) were exposed to CS for 120 consecutive days, starting at birth. The chemopreventive agents budesonide (2.4 mg/kg diet), phenethyl isothiocyanate (PEITC, 1,000 mg/kg diet), and N-acetyl cysteine (NAC, 1,000 mg/kg body weight) were administered orally according to various protocols. The experiment was stopped after 210 days. Exposure to CS resulted in a high incidence and multiplicity of benign lung tumors and in significant increases of malignant lung tumors and other histopathological alterations. All three chemopreventive agents, administered to current smokers after weaning, were quite effective in protecting both male and female mice from CS pulmonary carcinogenicity. When given to ex-smokers after withdrawal of exposure to CS, the protective capacity of budesonide was unchanged, while PEITC lost part of its cancer chemopreventive activity. In conclusion, the proposed experimental model provides convincing evidence that it is possible to prevent CS-induced lung cancer by means of dietary and pharmacological agents.

Int J Cancer. 2010 Mar 1;126(5):1047-54

N-ACETYL CYSTEINE AND S-METHYLCYSTEINE INHIBIT MEIQX RAT HEPATOCARCINOGENESIS IN THE POST-INITIATION STAGE.

N-acetyl cysteine (NAC) and S-methylcysteine (SMC), water soluble organosulfur compounds contained in garlic, were evaluated for chemoprevention of hepatocarcinogenesis after 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) initiation in rats. Intergastric treatment with NAC or SMC five times a week resulted in decreased numbers and areas of preneoplastic, glutathione S-transferase placental form (GST-P) positive foci of the liver in a dose-dependent manner. Moreover, cell proliferation was reduced in GST-P positive foci by NAC and SMC. Insulin-like growth factor I (IGF-I) and inducible nitric oxide synthase (iNOS) mRNA expressions were found downregulated in the liver by NAC. The studies indicate that NAC can serve as a chemopreventive agent for rat hepatocarcinogenesis induced by MeIQx by reducing cell proliferation, which may involve IGF-I and iNOS downregulation.

Carcinogenesis. 2006 May;27(5):982-8