

LE Magazine February 2001

ABSTRACTS

Page 1 of 3

CoQ10

Suppression of the hydrazine-induced formation of megamitochondria in the rat liver by coenzyme Q10

The effects of coenzyme Q10 (CoQ10) on the hydrazine-induced changes in the structure of mitochondria and those in antioxidant systems of the liver were investigated using rats as experimental animals. Animals were placed on a powdered diet containing 1.0% hydrazine for 7-8 days in the presence or absence of the combined treatment with CoQ10. Results obtained were as follows: (a) treatment of animals with CoQ10 prevented the hydrazine-induced formation of megamitochondria in the liver; (b) changes observed in the liver of the hydrazine-treated animals in comparison to the control were increases in the contents of alpha-tocopherol and CoQ analogs, increases in the levels of lipid peroxidation, decreases in the level of reduced glutathione with increases in that of oxidized glutathione, and increases in the ratio of unsaturated to saturated fatty acids in phospholipid domains of mitochondrial membranes; and (c) administration of CoQ10 to hydrazine-treated animals suppressed enhanced lipid peroxidation and improved lowered adenosine diphosphate/O ratios of mitochondria. The present data suggest that CoQ10 suppresses the hydrazine-induced formation of megamitochondria by scavenging free radicals generated from hydrazine and its metabolites.

Toxicol Pathol 1995 Nov-Dec;23(6):667-76

Coenzyme Q10 treatment improves the tolerance of the senescent myocardium to pacing stress in the rat

OBJECTIVE: In elderly patients the results of cardiac interventions are inferior to those in the young. A possible contributing factor is an age-related reduction in cellular energy transduction during the intervention which may induce aerobic or ischemic stress. To investigate whether coenzyme Q10 (CoQ10) improves the response to aerobic stress, functional recoveries of senescent and young rat hearts after rapid pacing were compared with or without CoQ10. **METHODS:** Young (4.8 +/- 0.1 months) and senescent (35.3 +/- 0.2 months) rats were given daily intraperitoneal injections of CoQ10 (4 mg/kg) or vehicle for 6 weeks. Their isolated hearts were rapidly paced at 510 beats per minute for 120 min to induce aerobic stress without ischemia. **RESULTS:** In senescent hearts pre-pacing cardiac work was 74% and oxygen consumption (MVO2) 66% of that in young hearts. CoQ10 treatment abolished these differences. After pacing, the untreated senescent hearts, compared to young, showed reduced recovery of pre-pacing work, (16.8 +/- 4.3 vs. 44.5 +/- 7.4%; $P < 0.01$). CoQ10 treatment in senescent hearts improved recovery of work, (48.1 +/- 4.1 vs. 16.8 +/- 4.3%; $P < 0.0001$) and MVO2 (82.1 +/- 2.8 vs. 61.3 +/- 4.0%; $P < 0.01$) in treated versus untreated hearts respectively. Post-pacing levels of these parameters in CoQ10 treated senescent hearts were as high as in young hearts. **CONCLUSIONS:** (1) Senescent rat hearts have reduced baseline function and reduced tolerance to aerobic stress compared to young hearts. (2) Pre-treatment with CoQ10 improves baseline function of the senescent myocardium and its tolerance to aerobic stress.

Cardiovasc Res 1998 Oct;40(1):165-73

Activities of vitamin Q10 in animal models and a serious deficiency in patients with cancer

New data on blood levels of vitamin Q10 in 116 cancer patients reveal an incidence of 23.1% of patients (N=17) with breast cancer whose blood levels were below 0.5 microg/ml. The incidence of breast cancer cases with levels below 0.6 microg/ml was 38.5%. The incidence is higher ($p < 0.05$) than that for a group of ordinary people. Patients (N=15) with myeloma showed a mean blood level of 0.67 +/- 0.17 microg/ml. The incidence of a vitamin Q10 blood level below 0.7 microg/ml for these 15 cases of myeloma was 53.3%, which is higher ($p < 0.05$) than the 24.5% found for a group of ordinary people.

Biochem Biophys Res Commun 1997 May 19;234(2):296-9

Progress on therapy of breast cancer with vitamin Q10 and the regression of metastases

Over 35 years, data and knowledge have internationally evolved from biochemical, biomedical and clinical research on vitamin Q10 (coenzyme Q10; CoQ10) and cancer, which led in 1993 to overt complete regression of the tumors in two cases of breast cancer. Continuing this research, three additional breast cancer patients also underwent a conventional protocol of therapy which included a daily oral dosage of 390 mg of vitamin Q10 (Bio-Quinone of Pharma Nord) during the complete trials over 3-5 years. The numerous metastases in the liver of a 44-year-old patient "disappeared," and no signs of metastases were found elsewhere. A 49-year-old patient, on a dosage of 390 mg of vitamin Q10, revealed no signs of tumor in the pleural cavity after six months, and her condition was excellent. A 75-year-old patient with carcinoma in one breast, after lumpectomy and 390 mg of CoQ10, showed no cancer in the tumor bed or metastases. Control blood levels of CoQ10 of 0.83-0.97 and of 0.62 micrograms/ml increased to 3.34-3.64 and to 3.77 micrograms/ml, respectively, on therapy with CoQ10 for patients A-MRH and EEL.

Biochem Biophys Res Commun 1995 Jul 6;212(1):172-7

Partial and complete regression of breast cancer in patients in relation to dosage of coenzyme Q10

Relationships of nutrition and vitamins to the genesis and prevention of cancer are increasingly evident. In a clinical protocol, 32 patients having -"high-risk"- breast cancer were treated with antioxidants, fatty acids, and 90 mg. of CoQ10. Six of the 32 patients showed partial tumor regression. In one of these 6 cases, the dosage of CoQ10 was increased to 390 mg. In one month, the tumor was no longer palpable and in another month, mammography confirmed the absence of tumor. Encouraged, another case having a verified breast tumor, after non-radical surgery and with verified residual tumor in the tumor bed was then treated with 300 mg. CoQ10. After 3 months, the patient was in excellent clinical condition and there was no residual tumor tissue. The bioenergetic activity of CoQ10, expressed as hematological or immunological activity, may be the dominant but not the sole molecular mechanism causing the regression of breast cancer.

Biochem Biophys Res Commun 1994 Mar 30;199(3):1504-8

Effect of hydrosoluble coenzyme Q10 on blood pressures and insulin resistance in hypertensive patients with coronary artery disease

In a randomised, double-blind trial among patients receiving antihypertensive medication, the effects of the oral treatment with coenzyme Q10 (60 mg twice daily) were compared for 8 weeks in 30 (coenzyme Q10: group A) and 29 (B vitamin complex: group B) patients known to have essential hypertension and presenting with coronary artery disease (CAD). After 8 weeks of follow-up, the following indices were reduced in the coenzyme Q10 group: systolic and diastolic blood pressure, fasting and 2-h plasma insulin, glucose, triglycerides, lipid peroxides, malondialdehyde and diene conjugates. The following indices were increased: HDL-cholesterol, vitamins A, C, E and beta-carotene (all changes $P < 0.05$). The only changes in the group taking the B vitamin complex were increases in vitamin C and beta-carotene ($P < 0.05$). These findings indicate that treatment with coenzyme Q10 decreases blood pressure possibly by decreasing oxidative stress and insulin response in patients with known hypertension receiving conventional antihypertensive drugs.

J Hum Hypertens 1999 Mar;13(3):203-8

Comparisons of coenzyme Q bound to mitochondrial membrane proteins among different mammalian species

The objective of this study was to elucidate the mechanisms that govern the variations in the rates of mitochondrial superoxide anion radical ($O_2^{\cdot -}$) generation in different species. The amounts of coenzyme Q (CoQ) associated with mitochondrial membrane proteins were compared in five different mammalian species, namely mouse, rat, rabbit, pig, and cow. Micelles of cardiac mitochondria were prepared using Triton X-100 or deoxycholate (DOC) as detergents, and the micelles containing mitochondrial proteins were sedimented by sucrose density ultracentrifugation. The amount of CoQ present in both types of micelles varied in different species, whereas alpha-tocopherol, another lipoidal molecule in mitochondrial membranes, could not be detected in the micelles of any of these species. The amounts of CoQ bound to mitochondrial proteins in DOC micelles were higher in those mammalian species where CoQ10 was the predominant CoQ homologue, and the amounts were found to be inversely correlated with the rate of mitochondrial $O_2^{\cdot -}$ generation among different species. Results also indicated that mitochondrial CoQ exists in at least two distinct pools, one of which is associated with the membrane proteins. The degree of association between CoQ and membrane proteins appears to be a factor determining the rate of mitochondrial $O_2^{\cdot -}$ generation.

Free Radic Biol Med 1999 Jul;27(1-2):220-6

Genetic and functional changes in mitochondria associated with aging

This review is devoted to the molecular genetics and bioenergetics of human mitochondria related to the mechanism of aging. Morphological and functional changes of mitochondria associated with age and age-related disease are overviewed with special reference to the changes in enzymes encoded by mitochondrial-inherent genome. The somatically acquired mutations and oxidative damage of the genome, which lead an individual to the fragmentation of mitochondrial DNA, cellular energy crisis,

naturally occurring cell death (apoptosis), and tissue degeneration and atrophy, are reviewed with relation to the inherited point mutational genotypes and the deletion types of mitochondrial DNA. Theories of aging are discussed with disclosed evidence relevant to them. Some trials to prevent age-related damage in mitochondria are introduced for the development of what may be called mitochondrial medicine.

Physiol Rev 1997 Apr;77(2):425-64Review

Mitochondrial respiratory chain inhibitors induce apoptosis

In this paper the specific mitochondrial respiratory chain inhibitors rotenone and antimycin A and the highly specific mitochondrial ATP-synthase inhibitor oligomycin are shown to induce an apoptotic suicide response in cultured human lymphoblastoid and other mammalian cells within 12-18 h. The mitochondrial inhibitors do not induce apoptosis in cells depleted of mitochondrial DNA and thus lacking an intact mitochondrial respiratory chain. Apoptosis induced by respiratory chain inhibitors is not inhibited by the presence of Bcl-2. We discuss the possible role of mitochondrial induced apoptosis in the ageing process and age-associated diseases.

FEBS Lett 1994 Feb 14;339(1-2):40-4

Relationship between mitochondrial superoxide and hydrogen peroxide production and longevity of mammalian species

The objective of this study was to examine the possible involvement of oxygen free radicals in the aging process. Rates of mitochondrial O₂⁻ and H₂O₂ production and oxygen consumption in the kidney and the heart were compared among seven different mammalian species namely, mouse, hamster, rat, guinea pig, rabbit, pig, and cow, whose maximum life span potential (MLSP) varies from 3.5 to 30 years. The rates of mitochondrial O₂⁻ and H₂O₂ generation were inversely correlated to MLSP, and directly related to specific metabolic rate and state 4 mitochondrial respiration. Results of this study indicate that under identical conditions, mitochondria from shorter-lived species produce relatively higher amounts of reactive oxygen species than those from the longer-lived species, and, thus, support the free radical hypothesis of aging.

Free Radic Biol Med 1993 Dec;15(6):621-7

Continued on Page 2

[Back to the Magazine Forum](#)

[Back to Page 1](#)

NSAIDs/Antioxidants

Acute-phase protein response and survival duration of patients with pancreatic cancer

BACKGROUND. Current methods to predict survival duration of patients with pancreatic cancer are limited. The aim of this study was to determine whether certain nutritional indices and the acute-phase protein response are prognostic factors independent of disease stage for patients with unresectable pancreatic cancer. **METHODS.** Variables at the time of diagnosis of 102 patients with unresectable pancreatic cancer were entered into a Cox's proportional hazards model. Included in the analysis were the serum concentration of C-reactive protein (CRP) and albumin, the extent of weight loss, age, sex, and disease stage (International Union Against Cancer criteria). **RESULTS.** A multivariate analysis in which each factor was adjusted for the influence of the other factors revealed the patient age, disease stage, serum albumin, and serum CRP to be independent predictors of survival. The presence of an acute-phase protein response was the most significant independent predictors of survival duration. The median survival of those with an acute-phase protein response (CRP > 10 mg/L, n = 45) was 66 days compared with 222 days for those with no acute-phase protein response (n = 57, P = 0.001, Mann-Whitney U test). **CONCLUSION.** The acute-phase protein response is a useful prognostic indicator for patients with unresectable pancreatic cancer. Moreover, the metabolic disturbances associated with an acute-phase protein response of patients with pancreatic cancer may be a worthwhile therapeutic target.

Cancer 1995 Apr 15;75(8):2077-82

A prospective study of tumor recurrence and the acute-phase response after apparently curative colorectal cancer surgery

BACKGROUND: Approximately 70% of patients who are going to develop tumor recurrence following curative colorectal surgery do so within 24 months of surgery. **PATIENTS AND METHODS:** The relationship was prospectively examined between an ongoing acute-phase response and subsequent clinical relapse in 36 colorectal cancer patients who had undergone a curative resection. Approximately 4 months after their operation, patients were grouped according to the presence (n = 15) or absence (n = 21) of an acute-phase response (C-reactive protein > 5 mg/L) and were followed-up for a minimum of 24 months. **RESULTS:** Age, tumor site, and serum carcinoembryonic antigen concentrations were similar in both groups. There was a significantly higher recurrence rate in patients with an acute-phase response (11 of 15) compared to those with no acute-phase response (2 of 21, P < 0.01). **CONCLUSIONS:** These results are consistent with the presence of an acute-phase response being an important predictive factor in the early stages of tumor recurrence in patients who have had apparently curative colorectal surgery.

Am J Surg 1995 Oct;170(4):319-22

Malnutrition and wasting, immunodepression, and chronic inflammation as independent predictors of survival in HIV-infected patients

To analyze the long-term survival factors associated with HIV infection, a prospective follow-up study of 165 HIV-infected patients was performed after a clinical, nutritional, and biological evaluation. Survival rate could be determined in 129 patients after a follow-up of 42 mo before the use of protease inhibitors. After univariate analysis, multivariate analysis was performed with the Cox regression proportional-hazard model. Survival curves were calculated and compared with the Kaplan, Meier, and log-rank tests. The study also analyzed the factors associated with impaired nutritional status at the beginning of the study and their effects on the long-term follow-up. Factors that could explain body weight loss before the study were the level of intakes, resting energy expenditure, chronic diarrhea, and the number of previous opportunistic infections. In the long-term follow-up, univariate analysis showed that nutritional status could be separated into four classes of body weight loss (BWL) by degree of loss (BWL < or = 5%, 5% < BWL < or = 10%, 10% < BWL < or = 20%, BWL > 20%); lean body mass (adjusted to height), body cell mass, CD4 count, albumin, prealbumin, and C-reactive protein (CRP) were all significant predictors. Age, stage of disease, number of previous opportunistic infections, and antiviral therapies were not associated with a change in survival. With the multivariate model, only CD4 counts, lean body mass/height squared, and CRP remained significant independent predictors of survival after controlling for other factors.

Nutrition 1999 Nov-Dec;15(11-12):865-9

Extent of inflammation predicts cardiovascular disease and overall mortality in seropositive rheumatoid arthritis - A retrospective cohort study from disease onset

OBJECTIVE: To identify predictors for cardiovascular disease (CVD) and for overall survival in patients with rheumatoid arthritis (RA) followed from disease onset. **METHODS:** A retrospective cohort of patients with seropositive RA and disease onset between 1974 and 1978 (n = 211) was followed up at the end of 1995. Potential predictors for CVD, as measured by "the first cardiovascular event," and for overall survival were registered. The predictors were identified by extended Cox regression models. **RESULTS:** In simple Cox regression analysis, male sex, higher age at disease onset, HLA-B27, high disease activity, corticosteroid treatment early in disease, and hypertension significantly increased risk of cardiovascular event. Higher educational level, extensive disease modifying antirheumatic drug (DMARD) treatment, and corticosteroids > or =1 yr before event decreased the risk. In multiple Cox regression analysis, male sex, high age at disease onset, hypertension, higher haptoglobin level at disease onset, and corticosteroid treatment early in disease increased risk of CVD. In a multiple model comprising only patients with CVD, corticosteroids delayed the event. A high last registered erythrocyte sedimentation rate (ESR) value before event increased CVD risk, in particular when early in disease progression. Decreased life span was predicted by higher age at disease onset, male sex, low education level, high disease activity, hypertension, and CVD. HLA-B27 was associated with decreased life span, as was early, but not extensive corticosteroid treatment. DMARD treatment was associated with decreased mortality risk, as was the presence of joint prosthesis. In multiple regression, male sex, higher age at disease onset, atlantoaxial subluxation early in disease, hypertension, and cardiovascular event increased mortality. A high last registered ESR value before event or death added to that risk. **CONCLUSION:** The study emphasizes the importance of inflammation as an important risk indicator for CVD and mortality in RA. The positive impact of disease activity reducing treatment on CVD risk and survival is suggested.

J Rheumatol 1999 Dec;26(12):2562-71

Changes in micronutrient concentrations following anti-inflammatory treatment in patients with gastrointestinal cancer

Circulating concentrations of vitamin antioxidants (retinol, alpha-tocopherol, lutein, lycopene, alpha- and beta-carotene) and trace elements (zinc, copper, iron and selenium) plus carrier proteins (albumin, transferrin, caeruloplasmin) in gastrointestinal cancer patients (n = 12) with an inflammatory response (as demonstrated by an elevated C-reactive protein concentration) were compared with a control group (n = 12). Further, the effect of moderating the inflammatory response, using the anti-inflammatory agent ibuprofen, on these measurements was examined in the cancer group. The control and cancer groups were similar in terms of age, sex, and body mass index. However, the cancer group had significantly higher C-reactive protein concentrations (P < 0.001). Concentrations of vitamin antioxidants and trace elements (and carrier proteins) were significantly lower (P < 0.001), except copper (ceruloplasmin) which was significantly higher (P < 0.05). After anti-inflammatory treatment, there were small but significant increases in lutein, lycopene, and beta-carotene (P < 0.05) and in iron and selenium (P < 0.05), whereas ceruloplasmin decreased (P < 0.05). The micronutrient concentrations in the cancer patients remained different from those in the control subjects. These results support the concept that the magnitude of inflammation plays an important role in the regulation of circulating concentrations of vitamin antioxidants and trace elements in patients with gastrointestinal cancer.

Nutrition 2000 Jun;16(6):425-8

Demonstration of CRP immunoreactivity in brains of Alzheimer's disease: immunohistochemical study using formic acid pretreatment of tissue sections

C-reactive protein (CRP) is a well-known serum protein which increases during inflammation and deposits in damaged tissues. To establish whether CRP appears in brain of Alzheimer's disease (AD), we immunohistochemically investigated tissue sections which were pretreated with formic acid. Positive immunostaining by anti-CRP antibodies was clearly recognized in senile plaques (SP) in the pretreated tissue sections, with very weak immunostaining in non-treated sections. These findings may suggest that the formation process of SP includes an acute-phase inflammatory state.

Neurosci Lett 1994 Aug 15;177(1-2):23-6

Prediction of male cancer mortality by plasma levels of interacting vitamins: 17-year follow-up of the prospective Basel study

Plasma vitamins C, E, retinol and carotene were measured in 1971-1973 in 2,974 men working in Basel Switzerland. In 1990, the vital status of all participants was assessed. A total of 290 men had died from cancer during the 17 years of follow-up, including 87 with lung cancer, 30 with prostate cancer, 28 with stomach cancer and 22 with colon cancer. Overall mortality from cancer was associated with low mean plasma levels of carotene (adjusted for cholesterol) and of vitamin C. Lung and stomach cancers were associated with low mean plasma carotene level. After calculation of the relative risk, using the Cox model, with exclusion of mortality during the first 2 years of follow-up, simultaneously low levels of plasma carotene (below quartile I) and lipid-adjusted retinol were related to a significantly increased mortality risk for all cancers and for lung cancer. Simultaneously, low levels of plasma vitamin C and lipid-adjusted vitamin E also were associated with a significantly increased risk for lung cancer. Additionally,

low vitamin E levels in smokers were related to an increased risk for prostate cancer. It is concluded that low plasma levels of the vitamins C, E, retinol and carotene are related to increased risk of subsequent overall and lung-cancer mortality and that low levels of vitamin E in smokers are related to an increased risk of prostate-cancer mortality.

Int J Cancer 1996 Apr 10;66(2):145-50

Continued on Page 3

[Back to the Magazine Forum](#)

[Back to Page 2](#)

ACAM

Nested case-control study of the effects of non-steroidal anti-inflammatory drugs on breast cancer risk and stage

Nested case-control study of the effects of non-steroidal anti-inflammatory drugs on breast cancer risk and stage We carried out a nested case-control study to measure the rate ratio (RR) for invasive female breast cancer in relation to non-steroidal anti-inflammatory drug (NSAID) use. The source population consisted of the female beneficiaries of the Saskatchewan Prescription Drug Plan from 1981 to 1995 with no history of cancer since 1970. Four controls/case, matched on age and sampling time, were randomly selected. Dispensing rates during successive time periods characterized NSAID exposure. RRs associated with exposure during each period were adjusted for exposure during the others. Confounding by other determinants was studied in analyses adjusted with data obtained by interviewing samples of subjects accrued from mid-1991 to mid-1995. We accrued 5882 cases and 23,517 controls. Increasing NSAID exposure 2-5 years preceding diagnosis was associated with a trend towards a decreasing RR (P-trend = 0.003); for the highest exposure level RR = 0.76, 95% confidence interval 0.63-0.92. This protective effect could not be attributed to confounding by other determinants. In analyses involving only the cases, NSAID exposure 2-5 and 6-10 years preceding diagnosis was associated with significantly reduced risks of presenting with a large tumour (> 5 cm diameter) or distant metastasis, but not regional lymph node metastasis. The use of NSAIDs may retard the growth of breast cancers and prevent distant metastasis.

Br J Cancer 2000 Jul;83(1):112-20

Inverse association of prostate cancer and non-steroidal anti-inflammatory drugs (NSAIDs): results of a case-control study

We examined the association of prostate cancer and non-steroidal anti-inflammatory drugs (NSAIDs) in a case control study of 417 prostate cancer patients and 420 group-matched control subjects. Regular daily use of over the counter NSAIDs, ibuprofen or aspirin, was associated with a 66% reduction in prostate cancer risk (odds ratio = 0.34, 95% confidence interval = 0.23-0.58, $p < 0.01$). The risk of prostate cancer was also significantly reduced in men who reported taking prescription NSAIDs (odds ratio = 0.35, 95% confidence interval = 0.15-0.84, $p < 0.05$). These results suggest that NSAIDs may have value in the chemoprevention of prostate cancer.

Oncol Rep 2000 Jan-Feb;7(1):169-70

Cancer

Therapeutic potentials of angiostatin in the treatment of cancer

The discovery of specific endothelial inhibitors such as angiostatin and endostatin not only increases our understanding of the functions of these molecules in the regulation of physiological and pathological angiogenesis, but also provides an important therapeutic strategy for cancer treatment. Recent studies have demonstrated that the angiostatin protein significantly suppresses the growth of a variety of tumors in mice. However, the dosages of angiostatin protein used in these animal studies seem to be too high for clinical trials. In addition, repeated injections and long-term treatment with angiostatin are required to reach its maximal antitumor effect. In this article, I will discuss several alternative approaches that may become feasible to move angiostatin therapy from animal experiments into the clinic. In particular, I will emphasize the therapeutic potentials of angiostatin gene therapy and more potent angiogenesis inhibitors that are related to angiostatin.

Haematologica 1999 Jul;84(7):643-50

Angiostatin induces and sustains dormancy of human primary tumors in mice

There is now considerable direct evidence that tumor growth is angiogenesis-dependent. The most compelling evidence is based on the discovery of angiostatin, an angiogenesis inhibitor that selectively instructs endothelium to become refractory to angiogenic

stimuli. Angiostatin, which specifically inhibits endothelial proliferation, induced dormancy of metastases defined by a balance of apoptosis and proliferation. We now show that systemic administration of human angiostatin potently inhibits the growth of three human and three murine primary carcinomas in mice. An almost complete inhibition of tumor growth was observed without detectable toxicity or resistance. The human carcinomas regressed to microscopic dormant foci in which tumor cell proliferation was balanced by apoptosis in the presence of blocked angiogenesis. This regression of primary tumors without toxicity has not been previously described. This is also the first demonstration of dormancy therapy, a novel anticancer strategy in which malignant tumors are regressed by prolonged blockade of angiogenesis.

Nat Med 1996 Jun;2(6):689-92

Liposomes complexed to plasmids encoding angiostatin and endostatin inhibit breast cancer in nude mice

Gene therapy transfer of angiostatin and endostatin represents an alternative method of delivering angiogenic polypeptide inhibitors. We examined whether liposomes complexed to plasmids encoding angiostatin or endostatin inhibited angiogenesis and the growth of MDA-MB-435 tumors implanted in the mammary fat pads of nude mice. We determined that plasmids expressing angiostatin (PCI-Angio) or endostatin (PCI-Endo) effectively reduced angiogenesis using an *in vivo* Matrigel assay. We then investigated the efficacy of these plasmids in reducing the size of tumors implanted in the mammary fat pad of nude mice. Both PCI-Angio and PCI-Endo significantly reduced tumor size when injected intratumorally ($P < 0.05$). Compared to the untreated control group, the mice treated with PCI-Angio and PCI-Endo exhibited a reduction in tumor size of 36% and 49%, respectively. In addition, we found that *i.v.* injections of liposomes complexed to PCI-Endo reduced tumor growth in the nude mice by nearly 40% when compared to either empty vector (PCI) or untreated controls ($P < 0.05$). These findings provide a basis for the further development of nonviral delivery of antiangiogenic genes.

Cancer Res 1999 Jul 15;59(14):3308-12

Kringle domains of human angiostatin. Characterization of the anti-proliferative activity on endothelial cells

Recently we have identified angiostatin, an endogenous angiogenesis inhibitor of 38 kDa which specifically blocks the growth of endothelial cells (O'Reilly, M. S., Holmgren, L., Shing, Y., Chen, C., Rosenthal, R. A., Moses, M., Lane, W. S., Cao, Y., Sage, E. H., and Folkman, J. (1994) *Cell* 79, 315-328; Folkman, J. (1995) *Nat. Med.* 1, 27-31). Angiostatin was shown to represent an internal fragment of plasminogen containing the first four kringle structures. We now report on the inhibitory effects of individual or combined kringle structures of angiostatin on capillary endothelial cell proliferation. Recombinant kringle 1 and kringle 3 exhibit potent inhibitory activity with half-maximal concentrations (ED₅₀) of 320 nM and 460 nM, respectively. Also, recombinant kringle 2 displays a significant inhibition, although decreased compared with both kringle 1 and kringle 3. In contrast, kringle 4 is an ineffective inhibitor of basic fibroblast growth factor-stimulated endothelial cell proliferation. Among the tandem kringle arrays, the recombinant kringle 2-3 fragment exerts inhibitory activity similar to kringle 2 alone. However, relative to kringle 2-3, a marked enhancement in inhibition is observed when individual kringle 2 and kringle 3 are added together to endothelial cells. This implies that it is necessary to open the cystine bridge between kringle 2 and kringle 3 to obtain the maximal inhibitory effect of kringle 2-3. An increased (<2-fold) inhibitory activity is observed for the kringle 1-3 fragment (ED₅₀ = 70 nM) compared with kringle 1-4 (ED₅₀ = 135 nM). These data indicate that the anti-proliferative activity of angiostatin on endothelial cells is shared by kringle 1, kringle 2, and kringle 3, but probably not by kringle 4 and that more potent inhibition results when kringle 4 is removed from angiostatin. Thus, in view of the variable lysine affinity of the homologous domains, it would appear that lysine binding capability does not correlate with the relative inhibitory effects of the kringle-containing constructs. However, as we also demonstrate, appropriate folding of kringle structures is essential for angiostatin to maintain its full anti-endothelial activity.

J Biol Chem 1996 Nov 15;271(46):29461-7

Systemic inhibition of tumor growth and tumor metastases by intramuscular administration of the endostatin gene

Tumors require ongoing angiogenesis to support their growth. Inhibition of angiogenesis by production of angiostatic factors should be a viable approach for cancer gene therapy. Endostatin, a potent angiostatic factor, was expressed in mouse muscle and secreted into the bloodstream for up to 2 weeks after a single intramuscular administration of the endostatin gene. The biological activity of the expressed endostatin was demonstrated by its ability to inhibit systemic angiogenesis. Moreover, the sustained production of endostatin by intramuscular gene therapy inhibited both the growth of primary tumors and the development of metastatic lesions. These results demonstrate the potential utility of intramuscular delivery of an antiangiogenic gene for treatment of disseminated cancers.

Nat Biotechnol 1999 Apr;17(4):343-8

Anti-angiogenesis therapy and strategies for integrating it with adjuvant therapy

Tumor angiogenesis is critical for the growth of primary cancers above 1-2 mm in diameter. A major vascular growth factor is

VEGF, and approaches to inhibit VEGF have shown encouraging results in pre-clinical studies. The mechanisms involved in switching on angiogenesis involve activation of oncogenes and upregulation of the hypoxia-sensing pathway. These provide novel targets for therapy. Many anti-angiogenic drugs are in clinical trial currently and there are problems in assessing these types of drugs if they only cause disease stabilisation. It will be important to develop methods to assess inhibition of vascular growth in vivo. New generations of anti-angiogenesis drugs such as endostatin or angiostatin, which are more potent, may cause tumor regression, but this has not yet been studied in patients. These approaches for advanced disease should be more successful when applied early in an adjuvant situation. This will also require careful monitoring of long-term toxicity.

Cancer Res 1998;152:341-52

Angiogenesis and tumor metastasis

Angiogenesis, the recruitment of new blood vessels, is an essential component of the metastatic pathway. These vessels provide the principal route by which tumor cells exit the primary tumor site and enter the circulation. For many tumors, the vascular density can provide a prognostic indicator of metastatic potential, with the highly vascular primary tumors having a higher incidence of metastasis than poorly vascular tumors. Tumor angiogenesis is regulated by the production of angiogenic stimulators including members of the fibroblast growth factor and vascular endothelial growth factor families. In addition, tumors may activate angiogenic inhibitors such as angiostatin and endostatin that can modulate angiogenesis both at the primary site and at downstream sites of metastasis. The potential use of these and other natural and synthetic angiogenic inhibitors as anticancer drugs is currently under intense investigation. Such agents may have reduced toxicity and be less likely to generate drug resistance than conventional cytotoxic drugs. Clinical trials are now underway to develop optimum treatment strategies for antiangiogenic agents.

Annu Rev Med 1998;49:407-24

Limited proteolysis of angiogenin by elastase is regulated by plasminogen

Human neutrophil elastase cleaves angiogenin at the Ile-29/Met-30 peptide bond to produce two major disulfide-linked fragments with apparent molecular weights of 10,000 and 4000, respectively. Elastase-cleaved angiogenin has slightly increased ribonucleolytic activity, but has lost its ability to undergo nuclear translocation in endothelial cells, a process essential for angiogenic activity. Cleavage appears to alter the cell-binding properties of angiogenin, despite the fact that it occurs some distance from the putative receptor-binding site, since the elastase-cleaved protein fails to compete with its native counterpart for nuclear translocation in endothelial cells. Plasminogen specifically accelerates elastase proteolysis of angiogenin. It does not enhance elastase activity toward ribonuclease A or the synthetic peptide substrate MeOSuc-Ala-Ala-Pro-Val-pNA. Plasminogen-accelerated inactivation of angiogenin by elastase might be a significant event in the process of angiogenin-induced angiogenesis since (i) angiogenin and plasminogen circulate in plasma at high concentrations, (ii) angiogenin, especially when bound to actin, activates tissue plasminogen activator to generate plasmin from plasminogen, and (iii) elastase cleaves plasminogen to produce angiostatin, a potent inhibitor of angiogenesis and metastasis. Interrelationships among angiogenin, plasminogen, plasminogen activators, elastase, and angiostatin may provide a sensitive regulatory system to balance angiogenesis and antiangiogenesis.

J Protein Chem 1997 Oct;16(7):669-79

A recombinant human angiostatin protein inhibits experimental primary and metastatic cancer

Endogenous murine angiostatin, identified as an internal fragment of plasminogen, blocks neovascularization and growth of experimental primary and metastatic tumors in vivo. A recombinant protein comprising kringles 1-4 of human plasminogen (amino acids 93-470) expressed in *Pichia pastoris* had physical properties (molecular size, binding to lysine, reactivity with antibody to kringles 1-3) that mimicked native angiostatin. This recombinant Angiostatin protein inhibited the proliferation of bovine capillary endothelial cells in vitro. Systemic administration of recombinant Angiostatin protein at doses of 1.5 mg/kg suppressed the growth of Lewis lung carcinoma-low metastatic phenotype metastases in C57BL/6 mice by greater than 90%; administration of the recombinant protein at doses of 100 mg/kg also suppressed the growth of primary Lewis lung carcinoma-low metastatic phenotype tumors. These findings demonstrate unambiguously that the antiangiogenic and antitumor activity of endogenous angiostatin resides within kringles 1-4 of plasminogen.

Cancer Res 1997 Apr 1;57(7):1329-34

Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma

To determine the mechanism responsible for the in vivo production of angiostatin that inhibits growth and metastasis in Lewis lung carcinoma (3LL), we implanted 3LL variant cells into the subcutis of syngeneic C57BL/6 mice. The tumors were infiltrated by macrophages and expressed high levels of steady-state mRNA for metalloelastase (MME). Successive passages (more than three) of cultures established from the tumors resulted in complete depletion of macrophages; steady-state MME mRNA, elastinolytic activity, and production of angiostatin (in the presence of plasminogen) were correspondingly reduced. Coculture of

macrophages with either 3LL cells or their conditioned media containing granulocyte-macrophage colony-stimulating factor resulted in secretion of MME and production of angiostatin by the macrophages, suggesting that angiostatin is produced by tumor-infiltrating macrophages whose MME expression is stimulated by tumor cell-derived granulocyte-macrophage colony-stimulating factor.

Cell 1997 Mar 21;88(6):801-10

[Back to Page 1](#)

[Back to the Magazine Forum](#)

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