

## ABSTRACTS

## Anti-inflammatory

Interleukin-1 beta induces cytosolic phospholipase A2 and prostaglandin H synthase in rheumatoid synovial fibroblasts. Evidence for their roles in the production of prostaglandin E2.

**OBJECTIVE.** In order to investigate potential regulatory mechanisms for the increased production of prostaglandin E2 (PGE2) in interleukin-1 beta (IL-1 beta)-stimulated rheumatoid synovial fibroblasts (RSF), this study examined the induction of phospholipase A2 (PLA2) and prostaglandin H synthase (PGHS) enzymes and the correlation of these events with PGE2 production in IL-1 beta-stimulated RSF. **METHODS.** Protein and messenger RNA (mRNA) levels of cytosolic PLA2 (cPLA2) and PGHS-2 enzymes in IL-1 beta-stimulated RSF were measured by Western and Northern blotting, respectively, using specific antisera and complementary DNA probes. Enzymatic activity of cPLA2 was determined in cell-free reaction mixtures utilizing mixed micelles of 14C-phosphatidylcholine and Triton X-100 as the substrate. PGE2 levels were quantitated using a commercial enzyme immunoassay kit. **RESULTS.** Incubation of RSF with IL-1 beta increased the mRNA and protein levels for the high molecular weight cPLA2 as well as for the mitogen/growth factor-responsive PGHS (PGHS-2). The IL-1 receptor antagonist completely abolished the induction of these two enzymes and the stimulation of PGE2 production by IL-1 beta in RSF. In contrast, levels of the other known forms of these enzymes, i.e., the 14-kd secretory group II PLA2 (sPLA2) and the constitutive form of PGHS (PGHS-1), were unaffected by IL-1 beta treatment. **CONCLUSION.** These are the first data to demonstrate the coordinate induction by IL-1 of cPLA2 and PGHS-2 in RSF. The time-course for the induction of these enzymes suggests that their increase contributes to the increased production of PGE2 in IL-1-treated RSF, and may help explain the capacity of RSF to produce large amounts of PGE2.

Arthritis Rheum 1994 May;37(5):653-61

Interleukin-1-mediated phospholipid breakdown and arachidonic acid release in human synovial cells.

**OBJECTIVE.** Interleukin-1 (IL-1), an important mediator contributing to joint destruction in rheumatoid arthritis, is known to stimulate the release of arachidonic acid (AA) and prostaglandin E2 (PGE2) from adherent synoviocytes. To study the intracellular pathways involved in these functions, we stimulated cultures of human synovial cells with recombinant IL-1 beta. **METHODS.** AA liberation was measured after labeling synovial cells with 3H-AA, and PGE2 levels were determined by high performance liquid chromatography or radioimmunoassay. Identification of 3H-AA-labeled phospholipids was performed by thin layer chromatography. Cell-associated phospholipase A2 (PLA2) enzymatic activity was determined by an assay with cell-free systems and exogenous substrates. **RESULTS.** Stimulation of synovial cells with recombinant IL-1 beta induced a decrease in phosphatidylcholine (PC), phosphatidylinositol (PI), and phosphatidylethanolamine (PE), and a marked increase in cell-associated PLA2 activity as compared with controls. In the presence of either quinacrine, an inhibitor of PLA2 pathway activation, or neomycin, which binds to PI mono- and biphosphate thus blocking their degradation by phospholipases, AA and PGE2 secretion were reduced in a dose-dependent manner. Kinetic studies revealed that quinacrine had little blocking activity on the IL-1-mediated AA release after one hour of stimulation but completely abolished it after five or eight hours. In contrast, neomycin exerted a partial but significant inhibitory effect from the first hour of stimulation onward. Addition of quinacrine was also demonstrated to abolish the IL-1-induced hydrolysis of PC and PE but not PI, indicating that PC and PE are the preferred substrates for PLA2 enzymatic activity in human synovial cells. **CONCLUSION.** Our findings strongly suggest that AA and PGE2 production by IL-1-triggered synoviocytes are largely dependent upon PLA2-mediated hydrolysis of PC and PE and to a lesser extent upon the earlier degradation of PI.

Arthritis Rheum 1993 Feb;36(2):158-67

The analgesic efficacy of topical capsaicin is enhanced by glyceryl trinitrate in painful osteoarthritis: a randomized, double blind, placebo controlled study.

The aim of this study was to assess if the pain of osteoarthritis is reduced by topical capsaicin and to determine whether addition of glyceryl trinitrate has an effect on analgesic efficacy and tolerability of capsaicin. A randomized, double blind, placebo controlled study was carried out on 200 adult patients attending a Pain Clinic with osteoarthritis pain. Patients applied one of four creams topically over the affected joint over a six-week period. Creams contained either placebo (vehicle), 0.025% capsaicin, 1.33% glyceryl

trinitrate or 0.025% capsaicin + 1.33% glyceryl trinitrate. Analgesic efficacy, tolerability of cream and analgesic consumption were assessed. One hundred and sixty-seven of 200 patients completed the study. Baseline visual analogue scores (0-10 scale) for pain were 6.40. There was a significant reduction in pain scores in the glyceryl trinitrate group (mean decrease 0.59,  $p < 0.05$ , 95% confidence limits 0.04-1.14), 0.025% capsaicin group (mean decrease 0.5,  $p < 0.05$ , 95% confidence limits 0.05-1.05) and the glyceryl trinitrate capsaicin group (mean decrease 1.1,  $p < 0.05$ , 95% confidence limits 0.22-1.98). Baseline discomfort of application scores were similar for all but the capsaicin groups (they were significantly higher (by 2.1 units,  $p < 0.001$ )). The odds ratio in favor of continuing treatment was 2.1 (95% confidence limits 1.0-4.4) for glyceryl trinitrate and 2.4 (95% confidence limits 1.2-5.1) for capsaicin and 5.0 (95% confidence limits 3.8-6.4) for capsaicin GTN combination. The study showed that topical capsaicin and glyceryl trinitrate have an analgesic effect in painful osteoarthritis. When used together this effect is increased with the combination being more tolerable than capsaicin alone. Analgesic consumption is decreased by capsaicin, glyceryl trinitrate and to a greater extent by both combined.

Eur J Pain 2000;4(4):355-60

Use of topical non-steroidal anti-inflammatory drugs in aggravated and decompensated arthroses.

Pain in osteoarthritis of the big weight bearing joints is either derived from periarticular ligaments, tendons, fascias, muscles, bursae—periarthropathy as sign of decompensation or from the reactive synovitis with or without effusion. NSAIDs (ibuprofen, diclofenac, indometacin, some salicylates, etofenamate and piroxicam) have demonstrated relevant advantages of the percutaneous route over the systemic one in soft tissue rheumatism. NSAIDs, mentioned above, locally administered as cream, gel or spray, quickly penetrate through the corneal layer of the skin and the site of application, reach highly effective concentrations in subcutis, fascias, tendons, ligaments and muscles, less in joint-capsule and -fluid indicating direct penetration. The blood levels of topical NSAIDs are extremely low with no systemic side effects, especially no gastric toxicity; however, local skin irritation is observed (1% to 2%). In contrast to this, systemic (oral) NSAIDs lead primarily via high blood levels to a lower concentration—only one tenth—in periarticular soft tissues with a high incidence of side effects. In conclusion the percutaneous application of certain NSAIDs has become a well established therapeutic regimen in painful osteoarthritis and in all other inflammatory degenerative and posttraumatic alterations of soft tissue structure.

Wien Med Wochenschr 1999;149(19-20):546-7

Getting control of osteoarthritis pain. An update on treatment options.

Osteoarthritis consists of a heterogeneous group of disorders that result in articular cartilage degeneration and is diagnosed on the basis of clinical findings. Pathogenesis involves an imbalance between the synthetic and degradative processes that occur in joints. Current interest in the role of cytokines and metalloproteinases may lead to improved treatment of osteoarthritis. For now, management consists of combinations of pharmacologic and nonpharmacologic therapies. A general pharmacologic approach is to begin with acetaminophen and add a low-dose NSAID, nonacetylated salicylate, selective COX-2 inhibitor, or topical capsaicin cream if needed. If pain persists, full-dose NSAID therapy, with the addition of a protective agent in patients at risk for gastrointestinal bleeding, or full-dose COX-2 inhibitor therapy may be tried. Joint injections, irrigation or arthroscopy may be beneficial in some cases. In patients who continue to have pain and limited function despite these measures, surgical intervention should be considered.

Postgrad Med 1999 Oct 1;106(4):127-34

Treatment of arthritis with topical capsaicin: a double-blind trial.

The neuropeptide substance P has been implicated in the pathogenesis of inflammation and pain in arthritis. In this double-blind randomized study, 70 patients with osteoarthritis (OA) and 31 with rheumatoid arthritis (RA) received capsaicin (a substance P depletor) or placebo for four weeks. The patients were instructed to apply 0.025% capsaicin cream or its vehicle (placebo) to painful knees four times daily. Pain relief was assessed using visual analog scales for pain and relief, a categorical pain scale, and physicians' global evaluations. Most of the patients continued to receive concomitant arthritis medications. Significantly more relief of pain was reported by the capsaicin-treated patients than the placebo patients throughout the study; after four weeks of capsaicin treatment, RA and OA patients demonstrated mean reductions in pain of 57% and 33%, respectively. These reductions in pain were statistically significant compared with those reported with placebo ( $P = 0.003$  and  $P = 0.033$ , respectively). According to the global evaluations, 80% of the capsaicin-treated patients experienced a reduction in pain after two weeks of treatment. Transient burning was felt at the sites of drug application by 23 of the 52 capsaicin-treated patients; two patients withdrew from treatment because of this side effect. It is concluded that capsaicin cream is a safe and effective treatment for arthritis.

Clin Ther 1991 May-Jun;13(3):383-95

Anti-inflammatory effect of diclofenac-sodium ointment (cream) in topical application.

This study was performed to develop a topical ointment of diclofenac-Na which has a potent anti-inflammatory activity by oral administration. At first, research was carried out on the ointment base which influences the external anti-inflammatory effect of the drug. Ointments of diclofenac-Na were prepared with three kinds of bases: lipophilic, emulsion (cream) and gel bases; and their anti-inflammatory effects were compared. The cream was found to have the most potent effect. Therefore, in the next experiment, an optimum concentration of diclofenac-Na in cream was determined comparing the anti-inflammatory effect among the cream preparations containing 0.5, 0.75, 1.0 and 1.5% of the drug. Obvious effects were observed with the cream containing 1.0% and 1.5% of the drug concentration, and there was no significant difference in the anti-inflammatory activities of these two concentrations. Based on these results, the cream preparation containing 1.0% of diclofenac-Na (DF cream) was adopted as the external ointment of the drug. The anti-inflammatory effect of this cream was compared with that of existing anti-inflammatory ointments, i.e., indomethacin gel (IM gel), bnfexamac cream (BM cream) and mobilat ointment (ML ointment). DF cream produced obvious inhibition on increased vascular permeability and on acute edema and remarkable suppression of ultraviolet erythema. These activities of DF cream were similar to those of IM gel and more potent than those of BM cream and ML ointment. The inhibitory effect of DF cream on the proliferation of granulation tissue was almost equal to that of ML ointment and more distinguishable than that of IM gel and BM cream. In adjuvant arthritis, DF cream reduced the swelling remarkably in the treated paw and slightly in the untreated paw. The anti-adjuvant activity of DF cream was equal to that of IM gel and more potent than that of BM cream and ML ointment. In pain to pressure stimulation, an analgesic effect was observed in the early stage of DF cream application, and its activity was slightly stronger than that of the other ointments. These results show that DF cream has an obvious anti-inflammatory effect as an external preparation, and the activity is comparable or superior to that of similar existing anti-inflammatory ointments. This cream may be considered as useful in the clinical field as a topical anti-inflammatory preparation.

Jpn J Pharmacol 1983 Feb;33(1):121-32

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Phospholipase A2 is a major component of the salt-extractable pool of matrix proteins in adult human articular cartilage.

Adult human articular cartilage contains a component with an apparent molecular weight of 16 kd, which is extractable with high ionic strength buffers. This protein, which, in addition to lysozyme, is one of the most prominent components in salt extracts of adult cartilage, is not detectable in cartilage from newborns. We performed N-terminal sequence analysis to identify the protein. The amino acid sequence obtained for the first 20 residues was identical to that reported for phospholipase A2 (PLA2) from human placenta and human synovial cells. The extractable PLA2 was found to be active. The lack of PLA2 in salt extracts from newborn cartilage observed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis was confirmed by the very low levels of PLA2 activity detectable in these preparations. PLA2 was clearly present in cartilage extracts from an 18-year-old subject and a 19-year-old subject, suggesting that its accumulation begins at some stage during the adolescent growth period. The enzyme does not appear to be released from cartilage matrix under normal physiologic conditions, and it is possible that the accumulation of PLA2 in maturing cartilage is a result of the decreased matrix turnover associated with the termination of skeletal growth. Whether PLA2 is active in the cartilage matrix, its precise localization, and its effects on the resident chondrocytes remain to be determined.

Arthritis Rheum 1991 Sep;34(9):1106-15

Anti-inflammatory activity of orpanoxin administered orally and topically to rodents.

Orpanoxin, a nonsteroidal anti-inflammatory drug (NSAID) lacking gastric ulcerogenic effects in the therapeutic dose range in rats, was compared with six reference NSAIDs for oral activity in the rat paw carrageenin-induced edema assay. Tested NSAIDs were ranked on the basis of oral mg/kg ED50 values: piroxicam, 0.55; orpanoxin, 35.6; diflunisal, 59.6; benoxaprofen, greater than 300; tolmetin sodium, greater than 300; and sulindac, greater than 300. Zomepirac sodium was inactive. Only the three most potent compounds produced greater than 60% inhibition of edema. Inhibition was generally greater at 4 h than at 6 h post carrageenin for all compounds. Oral activity of orpanoxin was also demonstrated in the guinea-pig u.v.-induced erythema model (ED50 = 24.2 mg/kg p.o. when given 1 h before irradiation) and in the mouse ear croton oil induced edema test (ED50 value = 131 mg/kg p.o.). Topical activity of orpanoxin was assessed in both the guinea-pig and mouse models. In the guinea-pig u.v.-induced erythema model, application (1 h after u.v.) of 1, 5, and 10% (w/v) orpanoxin creams (containing 10% urea) significantly inhibited erythema at 2, 3, and 4 h post-irradiation. Orpanoxin, mefenamic acid, and indomethacin as 1% creams inhibited total erythema scores 70, 92 and 74%, respectively. Evidence for topical activity in the mouse ear assay was also obtained for orpanoxin in diethyl ether or 10% urea cream, but not in dimethylsulfoxide. It was concluded that orpanoxin has anti-inflammatory activity comparable to reference NSAIDs in the rat paw edema test, is active orally in rat, mouse, and guinea-pig models, and shows topical activity in the guinea-pig and the mouse.

Agents Actions 1985 Jul;16(5):369-76

Percutaneous therapy of painful arthritis.

Pain in osteoarthritis of the big weight bearing joints is either derived from periarticular ligaments, tendons, fasciae, muscles, bursae—peri-arthropathy as sign of decompensation—or from the reactive synovitis with or without effusion. NSAIDs systemically administered have been so far considered as first choice medication together with physical therapy. New pharmacokinetic data on the topical, percutaneous application of NSAIDs (ibuprofen, diclofenac, indomethacin, some salicylates and to a lesser degree for etofenamate and piroxicam) have demonstrated relevant advantages of the percutaneous route over the systemic one in soft tissue rheumatism. NSAIDs, mentioned above, locally administered as cream, gel or spray, quickly penetrate through the corneal layer of the skin at the site of application, reach high effective concentrations in subcutis, fasciae, tendons, ligaments and muscles, lesser in joint-capsule and -fluid indicating direct penetration. The blood levels of topical NSAIDs are extremely low with no systemic side effects, especially no gastric toxicity; however, local skin irritation is observed (1 to 2%). In contrast to this, systemic (oral) NSAIDs lead primarily via high blood levels to a much lesser concentration—only one tenth—in particular soft tissues with a high incidence of side effects. In conclusion the percutaneous application of certain NSAIDs has become a well established therapeutic regimen in painful osteoarthritis and in all other inflammatory degenerative and posttraumatic alterations of soft tissue structures.

Ther Umsch 1991 Jan;48(1):42-5

Comparative tissue absorption of oral <sup>14</sup>C-aspirin and topical triethanolamine <sup>14</sup>C-salicylate in human and canine knee joints.

The local, articular, and systemic absorption of oral and topical salicylates was studied in dogs and humans using radioisotope techniques. Topical triethanolamine 14C-salicylate was found capable of percutaneous absorption into the knee joint and surrounding tissues. In dogs, topical salicylate application resulted in higher salicylate concentrations than oral aspirin in a number of tissues, despite lower blood levels. In patients with rheumatoid arthritis, intraarticular 14C-salicylate levels after triethanolamine 14C-salicylate cream were 60% of those obtained with oral aspirin. Four of six patients reported equal improvement in local discomfort after oral and topical salicylates. A potential role for topical salicylate cream in the treatment of localized rheumatic disorders is suggested.

J Clin Pharmacol 1982 Jan;22(1):42-8

Effect of topical capsaicin in the therapy of painful osteoarthritis of the hands.

Topical capsaicin 0.075% was evaluated for the treatment of the painful joints of rheumatoid arthritis (RA) and osteoarthritis (OA) in a four week double blind, placebo controlled randomized trial. Twenty-one patients were selected, all of whom had either RA (n = 7) or OA (n = 14) with painful involvement of the hands. Assessments of pain (visual analog scale), functional capacity, morning stiffness, grip strength, joint swelling and tenderness (dolorimeter) were performed before randomization. Treatment was applied to each painful hand joint four times daily with reassessment at one, two and four weeks after entry. One subject did not complete the study. Capsaicin reduced tenderness (p less than 0.02) and pain (p less than 0.02) associated with OA, but not RA as compared with placebo. A local burning sensation was the only adverse effect noted. These findings suggest that topical capsaicin is a safe and potentially useful drug for the treatment of painful OA of the hands.

J Rheumatol 1992 Apr;19(4):604-7

Topical application of doxepin hydrochloride, capsaicin and a combination of both produces analgesia in chronic human neuropathic pain: a randomized, double-blind, placebo-controlled study.

AIMS: To assess the analgesic efficacy of topical administration of 3.3% doxepin hydrochloride, 0.025% capsaicin and a combination of 3.3% doxepin and 0.025% capsaicin in human chronic neuropathic pain. METHODS: A randomized, double-blind, placebo-controlled study of 200 consenting adult patients. Patients applied placebo, doxepin, capsaicin or doxepin/capsaicin cream daily for four weeks. Patients recorded on a daily basis overall pain, shooting, burning, paraesthesia and numbness using a 0-10 visual analogue scale during the week prior to cream application (baseline levels) and for the four-week study period. Side-effects and desire to continue treatment were also recorded. RESULTS: Overall pain was significantly reduced by doxepin, capsaicin and doxepin/capsaicin to a similar extent. The analgesia with doxepin/capsaicin was of more rapid onset. Capsaicin significantly reduced sensitivity and shooting pain. Burning pain was increased by doxepin and by capsaicin and to a lesser extent by doxepin/capsaicin. Side-effects were minor. One patient requested to continue placebo cream, 17 doxepin cream, 13 capsaicin and nine the combination of doxepin and capsaicin. CONCLUSIONS: Topical application of 3.3% doxepin, 0.025% capsaicin and 3.3% doxepin/0.025% capsaicin produces analgesia of similar magnitude. The combination produces more rapid analgesia.

Br J Clin Pharmacol 2000 Jun;49(6):574-9

Topical capsaicin for chronic neck pain. A pilot study.

Substance P is thought to be the principle neurotransmitter of nociceptive impulses in type C sensory neurons. Prolonged repeated applications of capsaicin cream depletes the sensory C-fibers of substance P. In an open-labeled prospective pilot study, 23 patients with chronic neck pain (greater than three mo) completed the study. Patients applied topical capsaicin (0.025%) cream four times a day to painful areas in the neck and shoulder girdle for a five-wk treatment period. One patient dropped out because of intolerable burning. Statistically significant improvement was obtained in two primary outcome variables, the visual analog pain scale (P = 0.00013) and the pain relief scale (P = 0.002). Paired t tests failed to show a significant improvement in the McGill Pain Questionnaire. This study demonstrated that topically applied capsaicin cream may decrease subjective neck pain. A double-blind, placebo-controlled trial is needed to confirm this treatment effect.

Am J Phys Med Rehabil 1995 Jan-Feb;74(1):39-44

Treatment of painful diabetic neuropathy with topical capsaicin. A multicenter, double-blind, vehicle-controlled study. The Capsaicin Study Group.

A multicenter study was conducted to establish the efficacy of topical 0.075% capsaicin cream in relieving the pain associated with diabetic neuropathy. Capsaicin or vehicle cream was applied to painful areas four times per day for eight weeks in patients randomly assigned to one of two groups. Pain intensity and relief were recorded at two-week intervals using physician's global evaluation and visual analog scales. Analysis at final visit for 252 patients showed statistical significance favoring capsaicin compared with vehicle for the following: 69.5% vs 53.4% pain improvement by the physician's global evaluation scale, 38.1% vs 27.4% decrease in pain intensity, and 58.4% vs 45.3% improvement in pain relief. With the exception of transient burning, sneezing, and coughing,

capsaicin was well tolerated. Study results suggest that topical capsaicin cream is safe and effective in treating painful diabetic neuropathy.

Arch Intern Med 1991 Nov;151(11):2225-9

A double-blind evaluation of topical capsaicin in pruritic psoriasis.

**BACKGROUND:** Substance P, an undecapeptide neurotransmitter, has been implicated in the pathophysiology of psoriasis and pruritus. **OBJECTIVE:** Safety and efficacy of topical capsaicin, a potent substance P depletor, were evaluated in patients with pruritic psoriasis. **METHODS:** Patients applied capsaicin 0.025% cream (n = 98) or vehicle (n = 99) four times a day for six weeks in this double-blind study. Efficacy was based on a physician's global evaluation and a combined psoriasis severity score including scaling, thickness, erythema and pruritus. **RESULTS:** Capsaicin-treated patients demonstrated significantly greater improvement in global evaluation (p = 0.024 after four weeks and p = 0.030 after six weeks) and in pruritus relief (p = 0.002 and p = 0.060, respectively), as well as a significantly greater reduction in combined psoriasis severity scores (p = 0.030 and p = 0.036, respectively). The most frequently reported side effect in both treatment groups was a transient burning sensation at application sites. **CONCLUSION:** Topically applied capsaicin effectively treats pruritic psoriasis, a finding that supports a role for substance P in this disorder.

J Am Acad Dermatol 1993 Sep;29(3):438-42

Gamma tocopherol

Gamma tocopherol, the major form of vitamin E in the U.S. diet, deserves more attention.

Gamma tocopherol is the major form of vitamin E in many plant seeds and in the U.S. diet, but has drawn little attention compared with alpha tocopherol, the predominant form of vitamin E in tissues and the primary form in supplements. However, recent studies indicate that gamma tocopherol may be important to human health and that it possesses unique features that distinguish it from alpha tocopherol. gamma tocopherol appears to be a more effective trap for lipophilic electrophiles than is alpha tocopherol. Gamma tocopherol is well absorbed and accumulates to a significant degree in some human tissues; it is metabolized, however, largely to 2,7,8-trimethyl-2-(beta-carboxyethyl)-6-hydroxychroman (gamma-CEHC), which is mainly excreted in the urine. gamma-CEHC, but not the corresponding metabolite derived from alpha tocopherol, has natriuretic activity that may be of physiologic importance. Both gamma tocopherol and gamma-CEHC, but not alpha tocopherol, inhibit cyclooxygenase activity and, thus, possess anti-inflammatory properties. Some human and animal studies indicate that plasma concentrations of gamma tocopherol are inversely associated with the incidence of cardiovascular disease and prostate cancer. These distinguishing features of gamma tocopherol and its metabolite suggest that gamma tocopherol may contribute significantly to human health in ways not recognized previously. This possibility should be further evaluated, especially considering that high doses of alpha tocopherol deplete plasma and tissue gamma tocopherol, in contrast with supplementation with gamma tocopherol, which increases both. We review current information on the bioavailability, metabolism, chemistry and nonantioxidant activities of gamma tocopherol and epidemiologic data concerning the relation between gamma tocopherol and cardiovascular disease and cancer.

Am J Clin Nutr 2001 Dec;74(6):714-22

Association between alpha tocopherol, gamma tocopherol, selenium and subsequent prostate cancer.

**BACKGROUND:** Selenium and alpha tocopherol, the major form of vitamin E in supplements, appear to have a protective effect against prostate cancer. However, little attention has been paid to the possible role of gamma tocopherol, a major component of vitamin E in the U.S. diet and the second most common tocopherol in human serum. A nested case-control study was conducted to examine the associations of alpha tocopherol, gamma tocopherol and selenium with incident prostate cancer. **METHODS:** In 1989, a total of 10,456 male residents of Washington County, MD, donated blood for a specimen bank. A total of 117 of 145 men who developed prostate cancer and 233 matched control subjects had toenail and plasma samples available for assays of selenium, alpha tocopherol and gamma tocopherol. The association between the micronutrient concentrations and the development of prostate cancer was assessed by conditional logistic regression analysis. All statistical tests were two-sided. **RESULTS:** The risk of prostate cancer declined, but not linearly, with increasing concentrations of alpha tocopherol (odds ratio (highest versus lowest fifth) = 0.65; 95% confidence interval = 0.32--1.32; P(trend) = .28). For gamma tocopherol, men in the highest fifth of the distribution had a fivefold reduction in the risk of developing prostate cancer than men in the lowest fifth (P:(trend) = .002). The association between selenium and prostate cancer risk was in the protective direction with individuals in the top four fifths of the distribution having a reduced risk of prostate cancer compared with individuals in the bottom fifth (P(trend) = .27). Statistically significant protective associations for high levels of selenium and alpha tocopherol were observed only when gamma tocopherol concentrations were high. **CONCLUSIONS:** The use of combined alpha and gamma tocopherol supplements should be considered in upcoming prostate cancer prevention trials, given the observed interaction between alpha tocopherol, gamma tocopherol and selenium.

J Natl Cancer Inst 2000 Dec 20;92(24):2018-23

Carotenoids, retinol and vitamin E and risk of proliferative benign breast disease and breast cancer.

We investigated the relationship between serum levels of retinol, beta-carotene, alpha-carotene, lycopene, alpha tocopherol, and gamma tocopherol as well as intakes of retinol, carotene, and vitamin E and the risks of breast cancer and proliferative benign breast disease (BBD) in a case-control study of postmenopausal women in the Boston, MA (United States) area. Serum nutrient data were available for 377 women with newly diagnosed stage I or II breast cancer and 173 women with proliferative BBD. Controls were 403 women who were evaluated at the same institutions but did not require a breast biopsy or whose biopsy revealed nonproliferative BBD. We observed no significant associations between serum levels of these micronutrients and risk of proliferative BBD or breast cancer. The risk of breast cancer was decreased among women in the highest quintile of intake of vitamin E from food sources only (odds ratio [OR] for the highest quintile = 0.4, 95 percent confidence interval [CI] = 0.2-0.9; P, trend across quintiles = 0.02) but less so for total vitamin E intake including supplements (OR = 0.7, CI = 0.4-1.3; P, trend = 0.07).

Cancer Causes Control 1992 Nov;3(6):503-12

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Serum micro nutrients and upper aerodigestive tract cancer.

Numerous dietary studies have found that vegetables and fruits protect against upper aerodigestive tract cancer. To evaluate the role of beta-carotene and other specific carotenoids, a nested case-control study using prediagnostic serum was conducted among 6832 American men of Japanese ancestry examined from 1971 to 1975. During a surveillance period of 20 years, the study identified 28 esophageal, 23 laryngeal and 16 oral-pharyngeal cancer cases in this cohort. The 69 cases were matched to 138 controls. A liquid chromatography technique, designed to optimize recovery and separation of the individual carotenoids, was used to measure serum levels of lutein, zeaxanthin, beta-cryptoxanthin, lycopene, alpha-carotene, beta-carotene, retinol, retinyl palmitate, and alpha-, delta-, and gamma tocopherol. With adjustment for cigarette smoking and alcohol intake, we found that alpha-carotene, beta-carotene, beta-cryptoxanthin, total carotenoids and gamma tocopherol levels were significantly lower in the 69 upper aerodigestive tract cancer patients than in their controls. Trends in risk by tertile of serum level were significant for these five micro nutrients. These significant trends persisted in cases diagnosed 10 or more years after phlebotomy for the three individual carotenoids and total carotenoid measurements. The odds ratios for the highest percentile were 0.19 (95% confidence interval, 0.05-0.75) for alpha-carotene, 0.10 (0.02-0.46) for beta-carotene, 0.25 (0.06-1.04) for beta-cryptoxanthin, and 0.22 (0.05-0.88) for total carotenoids. When the cases were separated into esophageal, laryngeal, and oral-pharyngeal cancer, both alpha-carotene and beta-carotene were consistently and strongly associated with reduced risk at each site. The findings suggest that alpha-carotene and other carotenoids, as well as beta-carotene, may be involved in the etiology of upper aerodigestive tract cancer.

Cancer Epidemiol Biomarkers Prev 1997 Jun;6(6):407-12

Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women.

**BACKGROUND:** The role of dietary antioxidant vitamins in preventing coronary heart disease has aroused considerable interest because of the knowledge that oxidative modification of low-density lipoprotein may promote atherosclerosis. **METHODS.** We studied 34,486 postmenopausal women with no cardiovascular disease who in early 1986 completed a questionnaire that assessed, among other factors, their intake of vitamins A, E and C from food sources and supplements. During approximately seven years of follow-up (ending December 31, 1992), 242 of the women died of coronary heart disease. **RESULTS.** In analyses adjusted for age and dietary energy intake, vitamin E consumption appeared to be inversely associated with the risk of death from coronary heart disease. This association was particularly striking in the subgroup of 21,809 women who did not consume vitamin supplements (relative risks from lowest to highest quintile of vitamin E intake, 1.0, 0.68, 0.71, 0.42 and 0.42; P for trend 0.008). After adjustment for possible confounding variables, this inverse association remained (relative risks from lowest to highest quintile, 1.0, 0.70, 0.76, 0.32 and 0.38; P for trend, 0.004). There was little evidence that the intake of vitamin E from supplements was associated with a decreased risk of death from coronary heart disease, but the effects of high-dose supplementation and the duration of supplement use could not be definitely addressed. Intake of vitamins A and C did not appear to be associated with the risk of death from coronary heart disease. **CONCLUSIONS.** These results suggest that in postmenopausal women the intake of vitamin E from food is inversely associated with the risk of death from coronary heart disease and that such women can lower their risk without using vitamin supplements. By contrast, the intake of vitamins A and C was not associated with lower risks of dying from coronary disease.

N Engl J Med 1996 May 2;334(18):1156-62

Differential effects of alpha and gamma tocopherol on low-density lipoprotein oxidation, superoxide activity, platelet aggregation and arterial thrombogenesis.

**OBJECTIVES:** This study was designed to examine the differential effects of alpha and gamma tocopherol on parameters of oxidation-antioxidation and thrombogenesis. **BACKGROUND:** Experimental studies have shown that antioxidants, such as vitamin E (alpha tocopherol), improve atherosclerotic plaque stability and vasomotor function, and decrease platelet aggregation and tendency to thrombus formation. **METHODS:** Sprague Dawley rats were fed chow mixed with alpha or gamma tocopherol (100 mg/kg/day) for 10 days. A filter soaked in 29% FeCl<sub>3</sub> was applied around the abdominal aorta to study the patterns of arterial thrombosis. The aortic blood flow was observed and continuously recorded using an ultrasonic Doppler flow probe. ADP-induced platelet aggregation, low-density lipoprotein oxidation induced by phorbol 12-myristate 13-acetate (PMA)-stimulated leukocytes, superoxide anion generation and superoxide dismutase (SOD) activity were also measured. **RESULTS:** Both alpha and gamma tocopherol decreased platelet aggregation and delayed time to occlusive thrombus (all p < 0.05 vs. control). Both alpha and gamma tocopherol decreased arterial superoxide anion generation, lipid peroxidation and LDL oxidation (all p < 0.05 vs. control), and

increased endogenous SOD activity ( $p < 0.05$ ). The effects of gamma tocopherol were more potent than those of alpha tocopherol ( $p < 0.05$ ). CONCLUSIONS: This study indicates that both alpha- and gamma tocopherol decrease platelet aggregation and delay intraarterial thrombus formation, perhaps by an increase in endogenous antioxidant activity. Alpha tocopherol is significantly more potent than alpha-tocopherol in these effects.

J Am Coll Cardiol 1999 Oct;34(4):1208-15

Optimal nutrition: vitamin E.

Interest in the role of vitamin E in disease prevention has encouraged the search for reliable indices of vitamin E status. Most studies in human subjects make use of static markers, usually alpha-tocopherol concentrations in plasma or serum. Plasma or serum alpha-tocopherol concentrations of  $< 11.6$ ,  $11.6-16.2$ , and  $> 16.2$   $\mu\text{mol/l}$  are normally regarded as indicating deficient, low and acceptable vitamin E status respectively, although more recently it has been suggested that the optimal plasma alpha-tocopherol concentration for protection against cardiovascular disease and cancer is  $> 30$   $\mu\text{mol/l}$  at common plasma lipid concentrations in combination with plasma vitamin C concentrations of  $> 50$   $\mu\text{mol/l}$  and  $> 0.4$   $\mu\text{mol}$  beta-carotene/l. Assessment of vitamin E status has also been based on alpha-tocopherol concentrations in erythrocytes, lymphocytes, platelets, lipoproteins, adipose tissue, buccal mucosal cells and LDL, and on alpha-tocopherol: gamma-tocopherol in serum or plasma. Erythrocyte susceptibility to haemolysis or lipid oxidation, breath hydrocarbon exhalation, oxidative resistance of LDL, and alpha-tocopheryl quinone concentrations in cerebrospinal fluid have been used as functional markers of vitamin E status. However, many of these tests tend to be non-specific and poorly standardized. The recognition that vitamin E has important roles in platelet, vascular and immune function in addition to its antioxidant properties may lead to the identification of more specific biomarkers of vitamin E status.

Proc Nutr Soc 1999 May;58(2):459-68

Gamma-tocopherol decreases ox-LDL-mediated activation of nuclear factor-kappaB and apoptosis in human coronary artery endothelial cells.

Gamma-tocopherol, produced by many plants, is the major form of tocopherol in the United States diet. It is an efficient protector of lipids against peroxidative damage. Epidemiologic studies show that supplementation of diet with gamma-tocopherol is inversely related to the risk of death from cardiovascular disease. This study was conducted to examine the role of gamma-tocopherol in oxidized LDL (ox-LDL)-induced nuclear factor (NF)-kappaB activation and apoptosis in human coronary artery endothelial cells (HCAECs). Cultured HCAECs were treated with ox-LDL (10-40 microgram/ml). Incubation of HCAECs with ox-LDL resulted in apoptosis of HCAECs, as determined by TUNEL and DNA laddering. Ox-LDL degraded IkappaB protein and activated NF-kappaB in HCAECs (both  $P < 0.01$  vs control), as determined by Western blot. Treatment of cells with gamma-tocopherol attenuated ox-LDL-mediated degradation of IkappaB and activation of NF-kappaB (both  $P < 0.01$  vs ox-LDL alone). The presence of gamma-tocopherol also reduced ox-LDL-induced apoptosis ( $P < 0.01$  vs ox-LDL alone). A high concentration of gamma-tocopherol (50 micromol/L) was more effective than the low concentration of gamma-tocopherol (10 micromol/L) in this process. These observations show that ox-LDL induces apoptosis of HCAECs at least partially by activation of NF-kappaB signal transduction pathway. Gamma-tocopherol significantly decreases ox-LDL-induced apoptosis of HCAECs by inhibiting the activation of NF-kappaB.

Biochem Biophys Res Commun 1999 May 27;259(1):157-61

Vitamin E deficiency in variant angina.

BACKGROUND: Oxidative modification of LDL has been suggested to increase coronary vasoreactivity to agonists. A deficiency of vitamin E, a major antioxidant, may be related to the occurrence of coronary artery spasm. METHODS AND RESULTS: Vitamin E levels were determined with the use of high-performance liquid chromatography in normolipidemic subjects, including 29 patients with active variant angina (group 1), 13 patients with inactive stage of variant angina without anginal attacks during the past 6 months (group 2), 32 patients with a significant ( $>75\%$ ) organic coronary stenosis and stable effort angina (group 3), and 30 patients without coronary artery disease (group 4). Total lipid levels in blood were calculated as total cholesterol plus triglyceride levels. The plasma alpha-tocopherol levels as well as alpha-tocopherol/lipids were significantly lower in group 1 than in groups 2 through 4. Also, the plasma gamma-tocopherol levels were significantly lower in group 1 than in groups 2 through 4. The vitamin E levels were not significantly different between group 1 patients with and those without a significant organic stenosis. In group 1, both alpha- and gamma-tocopherol levels were significantly elevated after a  $>$  or  $=$  six-month angina-free period. The alpha-tocopherol levels in the LDL fraction were significantly lower in group 1 than in group 4. Plasma alpha-tocopherol levels were significantly correlated with those in the LDL fractions. In six patients of group 1 still having anginal attacks while receiving calcium channel blockers, the addition of vitamin E acetate (300 mg/d) significantly elevated plasma alpha-tocopherol levels and inhibited the occurrence of angina. CONCLUSIONS: Plasma vitamin E levels were significantly lower in patients with active variant angina than in subjects without coronary spasm, suggesting an association between vitamin E deficiency and coronary artery spasm.

Circulation 1996 Jul 1;94(1):14-8

Gamma-tocopherol: an efficient protector of lipids against nitric oxide-initiated peroxidative damage.

Nitric oxide released by macrophages during inflammation reacts with active oxygen to form peroxynitrite. Peroxynitrite nitrates protein and peroxidizes lipids. Gamma-tocopherol traps peroxynitrite and is more effective than alpha-tocopherol in protecting lipids against such peroxidation.

Nutr Rev 1997 ct;55(10):376-8

Gamma-tocopherol traps mutagenic electrophiles such as NO(X) and complements alpha-tocopherol: physiological implications.

Peroxynitrite, a powerful mutagenic oxidant and nitrating species, is formed by the near diffusion-limited reaction of .NO and O<sub>2</sub>· during activation of phagocytes. Chronic inflammation induced by phagocytes is a major contributor to cancer and other degenerative diseases. We examined how gamma-tocopherol (gammaT), the principal form of vitamin E in the United States diet, and alpha-tocopherol (alphaT), the major form in supplements, protect against peroxynitrite-induced lipid oxidation. Lipid hydroperoxide formation in liposomes (but not isolated low-density lipoprotein) exposed to peroxynitrite or the .NO and O<sub>2</sub>· generator SIN-1 (3-morpholinisydnonimine) was inhibited more effectively by gammaT than alphaT. More importantly, nitration of gammaT at the nucleophilic 5-position, which proceeded in both liposomes and human low density lipoprotein at yields of approximately 50% and approximately 75%, respectively, was not affected by the presence of alphaT. These results suggest that despite alphaT's action as an antioxidant gammaT is required to effectively remove the peroxynitrite-derived nitrating species. We postulate that gammaT acts in vivo as a trap for membrane-soluble electrophilic nitrogen oxides and other electrophilic mutagens, forming stable carbon-centered adducts through the nucleophilic 5-position, which is blocked in alphaT. Because large doses of dietary alphaT displace gammaT in plasma and other tissues, the current wisdom of vitamin E supplementation with primarily alphaT should be reconsidered.

Proc Natl Acad Sci U S A 1997 Apr 1;94(7):3217-22

Effect of alpha- and gamma-tocopherol as well as cholesterol on lipid peroxidation.

For a period of 15 weeks growing rats were fed low fat diets containing equimolar doses of alpha- and gamma-tocopherol (180 and 174 ppm) as well as mixtures of alpha- and gamma-tocopherol (3:1; 1:1; 1:3) without cholesterol or with 1% cholesterol. The influence of these supplements on lipid peroxidation and tocopherol retention in the liver were investigated. The tocopherol status was estimated by measuring the activities of creatine kinase and transaminases (GOT, GPT) in plasma as well as by in vitro hemolysis of erythrocytes. The in vitro hemolysis rate was only lowered by alpha-tocopherol and the mixtures of alpha- and gamma-tocopherol. In response to lipid peroxidation in the liver, alpha-tocopherol was the more efficient antioxidant, whereas gamma-tocopherol was more efficient in the diet. Cholesterol had a lowering effect on lipid peroxidation in vitro and in vivo; cholesterol in combination with alpha-tocopherol had a stabilizing effect on the erythrocyte membrane. Moreover, there was a positive effect of cholesterol on tocopherol retention in the liver. The biological activity of gamma-tocopherol in relation to alpha-tocopherol was calculated according to the test criterium; it ranged from 22% to 100%.

Z Ernährungswiss 1986 Mar;25(1):47-62

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## ABSTRACTS

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Cellular aging

Telomere position effect in human cells.

In yeast, telomere position effect (TPE) results in the reversible silencing of genes near telomeres. Here we demonstrate the presence of TPE in human cells. HeLa clones containing a luciferase reporter adjacent to a newly formed telomere express 10 times less luciferase than do control clones generated by random integration. Luciferase expression is restored by trichostatin A, a histone deacetylase inhibitor. Over expression of a human telomerase reverse transcriptase complementary DNA results in telomere elongation and an additional 2- to 10-fold decrease in expression in telomeric clones but not control clones. The dependence of TPE on telomere length provides a mechanism for the modification of gene expression throughout the replicative life span of human cells.

Science 2001 Jun 15;292(5524):2075-7

Extension of life span by introduction of telomerase into normal human cells.

Normal human cells undergo a finite number of cell divisions and ultimately enter a nondividing state called replicative senescence. It has been proposed that telomere shortening is the molecular clock that triggers senescence. To test this hypothesis, two telomerase-negative normal human cell types, retinal pigment epithelial cells and foreskin fibroblasts, were transfected with vectors encoding the human telomerase catalytic subunit. In contrast to telomerase-negative control clones, which exhibited telomere shortening and senescence, telomerase-expressing clones had elongated telomeres, divided vigorously, and showed reduced straining for beta-galactosidase, a biomarker for senescence. Notably, the telomerase-expressing clones have a normal karyotype and have already exceeded their normal life span by at least 20 doublings, thus establishing a causal relationship between telomere shortening and in vitro cellular senescence. The ability to maintain normal human cells in a phenotypically youthful state could have important applications in research and medicine.

Science 1998 Jan 16;279(5349):349-52

Telomerase, checkpoints and cancer.

Telomere dynamics and changes in telomerase activity are consistent elements of cellular alterations associated with changes in proliferative state. In particular, the highly specific correlations and early causal relationships between telomere loss in the absence of telomerase activity and replicative senescence or crisis, on the one hand, and telomerase reactivation and cell immortality, on the other, point to a new and important paradigm in the complementary fields of ageing and cancer. Although the signaling pathways between telomeres and transcriptional and cell cycle machinery remain undefined, recently described homologies between telomeric proteins and lipid/protein kinase activities important in chromosome stability provide evidence for the existence of pathways transducing signals originating in chromosome structure to cell cycle regulatory processes. Similarities between cell cycle arrest at senescence and the response of mortal cells to DNA/oxidative damage suggest overlap in the signal transduction mechanisms culminating in irreversible and stable cell cycle arrest. The feasibility of targeting telomeres/telomerase as a strategy for antiproliferative therapeutics has been shown in studies in yeast, in which mutations in specific telomere associated genes result in delayed cell death. Similarly, antisense oligonucleotide inhibition of telomerase activity in human tumor cells (HeLa) results in delayed cell death. The mechanism of cell death and possible escape from this fate require further study. In human cells, however, it would seem reasonable to predict that in these circumstances, apoptosis is induced in the vast majority of cells either directly in response to a DNA damage signal arising from critically shortened telomeres or as a secondary consequence of genetic instability.

Cancer Surv 1997;29:263-84

Inhibition of human telomerase in immortal human cells leads to progressive telomere shortening and cell death.

The correlation between telomerase activity and human tumors has led to the hypothesis that tumor growth requires reactivation of telomerase and that telomerase inhibitors represent a class of chemotherapeutic agents. Herein, we examine the effects of inhibition of telomerase inside human cells. Peptide nucleic acid and 2'-O-MeRNA oligomers inhibit telomerase, leading to progressive telomere shortening and causing immortal human breast epithelial cells to undergo apoptosis with increasing frequency until no cells remain. Telomere shortening is reversible: if inhibitor addition is terminated, telomeres regain their initial lengths. Our

results validate telomerase as a target for the discovery of anticancer drugs and supply general insights into the properties that successful agents will require regardless of chemical type. Chemically similar oligonucleotides are in clinical trials and have well characterized pharmacokinetics, making the inhibitors we describe practical lead compounds for testing for an antitelomerase chemotherapeutic strategy.

Proc Natl Acad Sci U S A 1999 Dec 7;96(25):14276-81

Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype.

Expression of the human telomerase catalytic component, hTERT, in normal human somatic cells can reconstitute telomerase activity and extend their replicative life span. We report here that at twice the normal number of population doublings, telomerase-expressing human skin fibroblasts (BJ-hTERT) and retinal pigment epithelial cells (RPE-hTERT) retain normal growth control in response to serum deprivation, high cell density, G1 or G2 phase blockers and spindle inhibitors. In addition, we observed no cell growth in soft agar and detected no tumor formation in vivo. Thus, we find that telomerase expression in normal cells does not appear to induce changes associated with a malignant phenotype.

Nat Genet 1999 Jan;21(1):111-4

Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription.

*S. cerevisiae* chromosomes end with the telomeric repeat (TG1-3)<sub>n</sub>. When any of four Pol II genes was placed immediately adjacent to the telomeric repeats, expression of the gene was reversibly repressed as demonstrated by phenotype and mRNA analyses. For example, cells bearing a telomere-linked copy of ADE2 produced predominantly red colonies (a phenotype characteristic of ade2- cells) containing white sectors (characteristic of ADE2+ cells). Repression was due to proximity to the telomere itself since an 81 bp tract of (TG1-3)<sub>n</sub> positioned downstream of URA3 when URA3 was approximately 20 kb from the end of chromosome VII did not alter expression of the gene. However, this internal tract of (TG1-3)<sub>n</sub> could spontaneously become telomeric, in which case expression of the URA3 gene was repressed. These data demonstrate that yeast telomeres exert a position effect on the transcription of nearby genes, an effect that is under epigenetic control.

Cell 1990 Nov 16;63(4):751-62

Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTERT.

The maintenance of chromosome termini, or telomeres, requires the action of the enzyme telomerase, as conventional DNA polymerases cannot fully replicate the ends of linear molecules. Telomerase is expressed and telomere length is maintained in human germ cells and the great majority of primary human tumors. However, telomerase is not detectable in most normal somatic cells; this corresponds to the gradual telomere loss observed with each cell division. It has been proposed that telomere erosion eventually signals entry into senescence or cell crisis and that activation of telomerase is usually required for immortal cell proliferation. In addition to the human telomerase RNA component (hTR; ref. 11), TR1/TLP1 (refs 12, 13), a protein that is homologous to the p80 protein associated with the Tetrahymena enzyme, has been identified in humans. More recently, the human telomerase reverse transcriptase (hTERT; refs 15, 16), which is homologous to the reverse transcriptase (RT)-like proteins associated with the *Euplotes aediculatus* (Ea\_p123), *Saccharomyces cerevisiae* (Est2p) and *Schizosaccharomyces pombe* (5pTrt1) telomerases, has been reported to be a telomerase protein subunit. A catalytic function has been demonstrated for Est2p in the RT-like class but not for p80 or its homologues. We now report that in vitro transcription and translation of hTERT when co-synthesized or mixed with hTR reconstitutes telomerase activity that exhibits enzymatic properties like those of the native enzyme. Single amino-acid changes in conserved telomerase-specific and RT motifs reduce or abolish activity, providing direct evidence that hTERT is the catalytic protein component of telomerase. Normal human diploid cells transiently expressing hTERT possessed telomerase activity, demonstrating that hTERT is the limiting component necessary for restoration of telomerase activity in these cells. The ability to reconstitute telomerase permits further analysis of its biochemical and biological roles in cell aging and carcinogenesis.

Nat Genet 1997 Dec;17(4):498-502

Telomerase in brain tumors.

**INTRODUCTION:** In recent years, many scientists involved in cancer research have directed their attention to telomerase, an enzymatic complex which is specifically involved in duplicating telomeres, the very ends of linear chromosomes. The discovery that most immortal cell lines in vitro and human tumor cells in vivo have telomerase activity, in contrast to telomerase-negative normal somatic cells, has made telomerase a candidate for use as a molecular marker of malignancy and even as a target for anticancer therapies. Thus, the assessment of the role of telomerase activity in neoplastic transformation has become a key issue in oncology, as stated by the exponential increase of papers on telomerase in the last five years. **OBJECT:** In this paper, we review some recent data from the literature, including our own studies, on the regulation of telomerase activity in brain tumors.

Replicative aging, telomeres, and oxidative stress.

Aging is a very complex phenomenon, both in vivo and in vitro. Free radicals and oxidative stress have been suggested for a long time to be involved in or even to be causal for the aging process. Telomeres are special structures at the end of chromosomes. They shorten during each round of replication and this has been characterized as a mitotic counting mechanism. Our experiments show that the rate of telomere shortening in vitro is modulated by oxidative stress as well as by differences in antioxidative defence capacity between cell strains. In vivo we found a strong correlation between short telomeres in blood lymphocytes and the incidence of vascular dementia. These data suggest that parameters that characterise replicative senescence in vitro offer potential for understanding of, and intervention into, the aging process in vivo.

Ann N Y Acad Sci 2002 Apr;959:24-9

Regulation of telomerase expression in human lymphocytes.

The function of lymphocytes is highly dependent on the ability of cell to divide. Among the various factors that regulate this cellular process, telomerase-dependent maintenance of telomere length has recently drawn considerable attention. Unlike most normal human somatic cells that express telomerase only during development but not after differentiation, lymphocytes express telomerase during development and retain the ability to express telomerase after maturation in response to antigenic challenge. How telomerase is regulated and its precise role in lymphocytes is not fully understood. The recent progress in characterizing regulation of telomerase expression in human lymphocytes is discussed.

Springer Semin Immunopathol 2002;24(1):23-33

Targeting assay to study the cis functions of human telomeric proteins: evidence for inhibition of telomerase by TRF1 and for activation of telomere degradation by TRF2.

We investigated the control of telomere length by the human telomeric proteins TRF1 and TRF2. To this end, we established telomerase-positive cell lines in which the targeting of these telomeric proteins to specific telomeres could be induced. We demonstrate that their targeting leads to telomere shortening. This indicates that these proteins act in cis to repress telomere elongation. Inhibition of telomerase activity by a modified oligonucleotide did not further increase the pace of telomere erosion caused by TRF1 targeting, suggesting that telomerase itself is the target of TRF1 regulation. In contrast, TRF2 targeting and telomerase inhibition have additive effects. The possibility that TRF2 can activate a telomeric degradation pathway was directly tested in human primary cells that do not express telomerase. In these cells, overexpression of full-length TRF2 leads to an increased rate of telomere shortening.

Mol Cell Biol 2002 May;22(10):3474-87

Proliferation and telomere length in acutely mobilized blood mononuclear cells in HIV infected patients.

The aim of the study was to investigate the mobilization of T cells in response to a stressful challenge (adrenalin stimulation), and to access T cells resided in the peripheral lymphoid organs in HIV infected patients. Seventeen patients and eight HIV seronegative controls received an adrenalin infusion for 1 h. Blood was sampled before, during and 1 h after adrenalin infusion. Proliferation and mean telomere restriction fragment length (telomeres) of blood mononuclear cells (BMNC) and purified CD8+ and CD4+ cells were investigated at all time points. In patients, the proliferation to pokeweed mitogens (PWM) was lower and decreased more during adrenalin infusion. After adrenalin infusion the proliferation to PWM was restored only in the controls. In all subjects telomeres in CD4+ cells declined during adrenalin infusion. Additionally, the patients had shortened telomeres in their CD8+ cells, and particularly HAART treated patients had shortened telomeres in all cell-subtypes. The finding that patients mobilized cells with an impaired proliferation to PWM during and after adrenalin infusion has possible clinical relevance for HIV infected patients during pathological stressful conditions, such as sepsis, surgery and burns. However, this study did not find a correlation between impaired proliferation and telomeres. It is concluded that physiological stress further aggravates the HIV-induced immune deficiency.

Clin Exp Immunol 2002 Mar;127(3):499-506

Effects of cisplatin on telomerase activity and telomere length in BEL-7404 human hepatoma cells.

Telomerase activity was inhibited in a dose and time-dependent manner with the treatment of cisplatin for 24, 48 or 72 h in a concentration ranged from 0.8 to 50 microM in BEL-7404 human hepatoma cells. There were no changes in expression pattern of three telomerase subunits, its catalytic reverse transcriptase subunit (hTERT), its RNA component (hTR) or the associated protein subunit (TP1), after cisplatin treated for 72 h with indicated concentrations. Mean telomere lengths were decreased by the cisplatin

treatment. Cell growth inhibition and cell cycle accumulation in G2/M phase were found to be correlated with telomerase inhibition in the present study, but percentages of cell apoptosis did not change markedly during the process.

Cell Res 2002 Mar;12(1):55-62

Increased life span of human osteoarthritic chondrocytes by exogenous expression of telomerase.

**OBJECTIVE:** To extend the life span of human osteoarthritic (OA) articular chondrocytes by introduction of the catalytic component of human telomerase while preserving the chondrocyte-specific phenotype. **METHODS:** Human articular chondrocytes were isolated from the femoral head and tibial plateau of patients undergoing knee joint replacement for OA. The chondrocytes were cultured as monolayers and infected with a retroviral telomerase expression construct followed by selection with G418 for 10 to 14 days. Telomeric-repeat amplification protocol assays and telomere terminal restriction fragment length assays were performed on pools of transduced cells in order to measure telomerase activity and telomere length. Growth kinetics and population doubling capacity were assessed by passaging the cells in monolayer culture. Redifferentiation of the monolayer chondrocyte cultures was induced by transfer to suspension culture on poly-(2-hydroxyethyl-methacrylate) (polyHEMA)-coated dishes. Induction of the chondrocyte-specific phenotype was monitored by analysis of gene expression utilizing reverse transcription-polymerase chain reaction. **RESULTS:** OA chondrocytes isolated from three different donors (ages 41, 69 and 75 years) were transduced with a retroviral construct expressing telomerase. After selection, pooled populations of cells from all donors and a clonal cell line from one donor expressed telomerase activity and exhibited lengthening of telomeres. Chondrocytes expressing telomerase showed an increase of five to nine population doublings over 234 days of culture in monolayer. The telomerase-transduced cells recovered a chondrocyte-specific gene expression pattern following culture on polyHEMA-coated dishes. **CONCLUSION:** The exogenous expression of telomerase may represent a way to expand human OA chondrocytes while allowing maintenance of the chondrocyte-specific phenotype. These cells have the potential to be used for restoration of the articular cartilage defects occurring in this disease.

Arthritis Rheum 2002 Mar;46(3):683-93

Telomeres, telomerase and stability of the plant genome.

Telomeres, the complex nucleoprotein structures at the ends of linear eukaryotic chromosomes, along with telomerase, the enzyme that synthesizes telomeric DNA, are required to maintain a stable genome. Together, the enzyme and substrate perform this essential service by protecting chromosomes from exonucleolytic degradation and end-to-end fusions and by compensating for the inability of conventional DNA replication machinery to completely duplicate the ends of linear chromosomes. Telomeres are also important for chromosome organization within the nucleus, especially during mitosis and meiosis. The contributions of telomeres and telomerases to plant genome stability have been confirmed by analysis of Arabidopsis mutants that lack telomerase activity. These mutants have unstable genomes, but manage to survive up to ten generations with increasingly shortened telomeres and cytogenetic abnormalities. Comparisons between telomerase-deficient Arabidopsis and telomerase-deficient mice reveal distinct differences in the consequences of massive genome damage, probably reflecting the greater developmental and genomic plasticity of plants.

Plant Mol Biol 2002 Mar;48(4):331-7

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