PHARMACOLOGICAL DIGEST

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Estrogen complements cardiovascular risk reduction of statins in postmenopausal women

Estrogen therapy improves markers of fibrinolysis and vascular inflammation when added to simvastatin in hypercholesterolemic postmenopausal women. In a prospective study, 28 hypercholesterolemic postmenopausal women were randomized to receive either 0.625 mg conjugated equine estrogen, q.d., 10 mg simvastatin, q.d. or a combination of the two for 6 weeks. Levels of plasminogen activator inhibitor-1 and cell adhesion molecule E-selectin, markers of fibrinolysis, inflammation and platelet aggregability, and levels of total cholesterol, HDL and LDL were measured. Estrogen therapy alone or in combination with simvastatin significantly raised HDL level to a greater extent than the statin alone. Estrogen alone or in combination with simvastatin lowered plasma levels of plasminogen activator inhibitor-1 and cell adhesion molecule E-selectin. A combination of estrogen and simvastatin lowered LDL cholesterol to a greater degree than either therapy alone. The findings suggest that estrogen therapy may provide vasculoprotective benefit to hypercholesterolemic postmenopausal women, even if they are already on statin therapy.

[Circulation 1999; 99: 354-360]

DNA vaccination activates dendritic cells to initiate immune response in mice

DNA vaccination transfects only a few dendritic cells, but this leads to general activation of dendritic cells and to long-lasting CD4 T cell responses in mice. The vaccine coded for the fifth component of complement, C5, a protein that is not secreted by transfected cells. The investigators detected C5 in keratinocytes in the ears of mice and in approximately 2% of dendritic cells within draining lymph nodes of 5.4-vaccinated mice. In mice vaccinated with empty vector, no dendritic cells expressed C5. The antigen-expressing dendritic cells presumably migrated from the site of DNA application to the draining lymph nodes. In the spleen of 5.4-vaccinated mice, frequencies of C5-specific T cells peaked 4 weeks after vaccination and at 40 weeks were stiil greater than in untreated controls. Although DNA vaccination results in direct transfection of only a very small proportion of dendritic cells, it leads to general activation of all found in the draining lymph nodes, thus providing optimal conditions for effective T cell activation. In 5.4-vaccinated mice, the scientists detected C5 in keratinocytes for up to 12 weeks, but in dendritic cells from draining lymph nodes they detected C5 for no longer than 2 weeks. When they injected transgenic lymph node T cells into mice that had been immunized with the 5.4 vaccine 20 days before, the T cells remained unactivated. These latter findings propose that there is no long-lived source of antigenic material present. It seems that DNA vaccination can induce memory CD4 T cells that can be long-lived without repeated antigenic stimulation. These may lie in optimal activation and expansion of most antigen-specific T cell precursors, rather than in long-term storage of antigen leading to periodic restimulation.

[J Exp Med 1999; 189: 169-177]

Gene linked to smoking behavior

Researchers have found a gene that appears to influence an individual's risk of starting to smoke and of becoming addicted to nicotine. The findings may lead to more effective

smoking cessation therapies tailored to a smoker's genetic makeup. Study of 289 smokers and 233 nonsmokers showed that people who had the dopamine transporter gene SLC6A3-9 were less likely to smoke, or if they did, they tended to start later and found it easier to give up than those who lacked the gene. People with this particular gene were 1.5 times more likely to quit smoking than people who did not carry it. This gene was associated with lower levels of a personality trait called "novelty seeking", which was thought to be modulated by dopamine and had been linked to cigarette smoking behavior. Those with low levels of novelty seeking had an easier time giving up cigarettes than those with high levels of novelty seeking. The study authors concluded that a fuller understanding of the genetics of cigarette smoking behavior could lead to more effective, targeted pharmacological and psychoeducational cessation strategies that took such individual differences into account.

[Health Psychology 1999; 18: 7-13,14-20]

Adding etanercept to methotrexate improves rheumatoid arthritis

In patients with rheumatoid arthritis whose signs and symptoms are not controlled with methotrexate, the addition of etanercept, a genetically engineered receptor-fusion protein, binding and inhibiting tumor necrosis factor, and blocking the inflammatory process in rheumatoid arthritis, is safe and results in clinically significant reductions in disease activity. The study involved 75 women and 14 men who had active rheumatoid arthritis, average duration 13 years, despite treatment with oral or subcutaneous methotrexate for at least 6 months. For the 4 weeks prior to the study, the patients had been receiving a stable dose of 10 to 25 mg per week. In a double-blind protocol, the investigators randomly assigned 59 patients to receive twice-weekly injections of etanercept, 25 mg per dose, and 30 patients to receive placebo injections. All patients continued to receive the same dose of methotrexate. After 6 months of treatment, at least a 20% reduction in various measures of disease activity, specified by the American College of Rheumatology (ACR), in 71% of the patients who received combination therapy, versus 27% of those who received monotherapy. The study group observed a 50% reduction in disease activity in 39% and 3% of the groups, respectively, and a 70% reduction in 15% and 0%, respectively. The FDA has specified that improvement of at least 70% according to the ACR criteria constitutes a "major clinical response". Toxicity in the short-term study was minimal, and except for mild injection-site reactions, there were no significant differences between the groups with regard to adverse events. Specifically, gastro-intestinal side effects, hematologic effects, and headaches were not increased by the addition of etanercept. Additional research is needed to determine whether etanercept is an appropriate initial therapy for rheumatoid arthritis and to identify the patients who will benefit most.

[N Engl J Med 1999; 340: 253-259, 310-312]

Vaccine reduces colon cancer recurrence

An experimental vaccine reduced the risk of tumor recurrence in some patients who had undergone surgery for colon cancer. The study involved 254 patients with stage II (the cancer had spread through the bowel wall but not further) and stage III (the cancer had spread through the bowel wall to local lymph nodes) colon cancer. Following surgery to remove their tumors, half of the patients were given the experimental vaccine as part of a procedure called "active specific immunotherapy" because it was made using cells from a patient's own tumor. The other half of the group of patients did not receive the vaccine. The vaccine was administered once a week for three weeks shortly after surgery, and once more six months later. The researchers reported that after a median follow-up of 5.3 years, patients who received the vaccine had an overall 44% reduction in risk of recurrence of the cancer. The greatest reduction in recurrence rate was seen in patients with stage II cancers. In these patients, those who received the vaccine had a significantly longer recurrence-free period, and 61% risk reduction for recurrences and showed a trend towards improved overall survival. The vaccine had little effect on the recurrence rate in patients whose disease had progressed to stage III. In addition, it did not have an effect on overall survival in patients who received it, but this

might have been partly due to deaths from other diseases, such as heart disease, in the group studied. The side effects of the vaccine were minimal, and appeared to contribute to a better quality of life in the patients who received it. The investigators estimated that use of the vaccine in addition to adjuvant chemotherapy could provide benefit over surgery alone in more than 90% of colon cancer patients (64% stage II, 30% stage III). One drawback to the procedure was the requirement for a high-quality vaccine-manufacturing center that could make the vaccine from the individual's tumor.

[Lancet 1999; 353: 345-350]

AHA: too early to recommend antioxidant supplements

Although epidemiologic evidence suggests that diets rich in antioxidants can reduce heart disease risk, it is still too early to recommend the use of antioxidant supplements for the general public, according to American Heart Association (AHA) officials. Primary prevention trials examining beta carotene, vitamin E, and vitamin C supplements have produced mixed results about reduction in heart disease risk. In the Nurses' Health Study, more than 85,000 women followed for approximately 8 years revealed a relative risk of major coronary disease of 0.66 for women with the highest vitamin E consumption compared with those with the lowest vitamin E consumption. Lower risk was associated with levels of vitamin E intake that were achievable only by supplementation. Subsequent analyses revealed a 43% lower risk in vitamin E supplement users versus nonusers and an inverse relationship between risk and duration of supplement use. Primary prevention trials have not uncovered similar benefits. In the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study, researchers found no reduction in risk of lung cancer or major coronary events in more than 29,000 male smokers who took daily doses of vitamin E, beta-carotene, or both. Similarly, investigators found no reduction in cancer or heart disease risks with beta-carotene supplementation in the Physician's Health Study, which followed more than 22,000 US physicians for 12 years. The evidence was much stronger among patients with established heart disease. In the Cambridge Heart Antioxidant Study, the risk of myocardial infarction was reduced by 77% and the risk of all cardiovascular events was reduced by 47% among patients who received high doses of alpha-tocopherol. In view of these findings, the most prudent and scientifically supportable recommendation for the general population is to consume a balanced diet with emphasis on antioxidant-rich fruits and vegetables and whole grains. Although diet alone may not provide the levels of vitamin E intake that have been associated with the lowest risk in a few observational studies, the absence of efficacy and safety data from randomized trials precludes the establishment of population wide recommendation regarding vitamin E supplementation.

[Circulation 1999; 99: 591-595]

BRCA1/2 gene mutations unrelated to survival in women with breast or ovarian cancers

New evidence adds to the controversy surrounding the effects of BRCA1 and BRCA2 mutations on survival in women with breast and ovarian cancers, and suggests that survival is similar in women with and without the mutations. The researchers examined survival after breast or ovarian cancers among BRCA1 and BRCA2 mutation carriers and noncarriers in Jewish women in a community of Ashkenazi. They tested more than 5,300 women for three specific mutations and interviewed them regarding carrier status and survival of first-degree relatives who had been diagnosed with one of the two cancers. Among 50 mutation carriers, 58 of their first-degree relatives had been diagnosed with breast cancer and 10 with ovarian cancer, and among 907 noncarriers in the population, the corresponding numbers were 979 and 116. Median survivals with breast cancer among the relatives of carriers and noncarriers were 16 years and 18 years, respectively, a difference that did not reach statistical significance. Survival with ovarian cancer also did not appear to be affected by BRCA1 or BRCA2 mutation carrier status. The findings suggested that BRCA1 and/or BRCA2 mutation carrier status did not have a major impact on overall survival time among patients with breast or ovarian cancer. They concluded that screening for the mutations would not contribute

prognostic information about survival among these patients.

[Journal of the National Cancer Institute 1999; 91: 201-203, 259-263]

Hepatocyte growth factor gene therapy treats liver cirrhosis in rats

Repeated transfections of human hepatocyte growth factor (HGF) gene into the skeletal muscles of rats, using the hemagglutinating virus of Japan (HVJ) as a carrier, can produce complete resolution of liver cirrhosis. Rats were induced pathological changes similar to those of human liver cirrhosis by injection of dimethylnitrosamine. Beginning 4 weeks later, the rats were injected weekly with saline or with 10 or 20 mg of HGF-HVJ liposomes. Gene therapy increased the plasma levels of human HGF as well as endogenous rat HGF. It inhibited plasma levels of transforming growth factor beta-1, which according to the research team induced liver fibrosis, inhibited the growth of hepatocytes and induced apoptosis. Quantitative analysis of fibrosis by image analysis techniques showed a more than 70% reduction of fibrosis after HGF-HVJ liposome treatment. Hepatocyte apoptosis was suppressed in HGF-transfected rats, and immunohistochemical staining showed that 47% of hepatocytes were mitotic. Transfection also improved survival from 34 days of untreated rats to 43 days of transfected rats with 20 mg of HGF DNA. In nine rats that were transfected with 40 mg of HGF DNA, liver cirrhosis resolved completely within 50 days after the start of the dimethylnitrosamine injection.

[Nat Med 1999; 5: 226-230]

Digoxin plus atenolol offers best control of chronic atrial fibrillation

The combination of digoxin and atenolol provides better stabilization of heart rate in patients with chronic atrial fibrillation than either drug alone, diltiazem alone or diltiazem plus digoxin. The finding comes from a crossover comparison of the five regimens in 12 patients with chronic atrial fibrillation. For single-drug therapy, diltiazem provided the worst control of ventricular heart rate, while atenolol offered the best control. Atenolol plus digoxin offered the best overall results, reducing the average daily ventricular rate to only 65 compared with 75.9 and 78.9, respectively, for atenolol alone and digoxin alone. The two drugs appeared to work synergistically. Digoxin favorably modulated the parasympathetic nervous system, while atenolol blocked the effect of the sympathetic nervous system, which drived the heart rate. This synergism resulted in almost ideal control of the heart. The study showed that, while digoxin remained the first-line therapy for chronic atrial fibrillation, it was among the least effective drugs for this indication when used alone. Data provided a rational basis for the choice of a pharmacologic regimen for rate control in atrial fibrillation and an approach for defining the significance of various regimens for preventing the deterioration of ventricular and left atrial function.

[J Am Coll Cardiol 1999; 33: 304-310]

Possible new vaccine fights malaria throughout its life cycle

A team of researchers has discovered a possible new way to vaccinate against malaria, one of the world's biggest killers. Creating a vaccine is crucial because the parasite has begun developing resistance to drugs used to treat malaria, and even mosquitos that spread the disease are withstanding pesticides. But finding a malaria vaccine has proved exceptionally difficult and failed because most vaccines focused on just one part of the malaria parasite's complex life cycle. The investigators have created a multipronged vaccine designed to make the immune system fight the parasite at many stages, i.e., when the mosquito bite sends it into the body, when it invades the liver and when infection moves into the bloodstream. Antigens corresponding to the sporozoite, liver, erythrocytic asexual, and sexual stages were constructed as a synthetic gene that encoded for 12 B cell, 6 T cell proliferative, and 3 cytotoxic T lymphocyte epitopes derived from 9 stage-specific P. falciparum. Immunization in rabbits with the purified protein in the presence of different adjuvants generated antibody response that recognized vaccine antigen, linear peptide contained in the vaccine, and all stages of P. falciparum. A multicomponent, multistage

malaria vaccine can induce immune response that inhibits parasite development at multiple stages. The rational and approach used in the development of a multicomponent P. falciparum vaccine will be useful in the development of a multispecies human malaria vaccine and vaccine against other infectious diseases.

[Proc Natl Acad USA 1999; 96: 1615-1620]