PHARMACOLOGICAL DIGEST

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Acupuncture may aid in the treatment of cocaine dependence

Acupuncture treatments for chronic cocaine addiction appear to be effective. Dr. Arthur Margolin and colleagues randomly assigned 82 cocaine-dependent patients who were on methadone maintenance therapy to receive auricular acupuncture, a needle insertion control procedure or no-needle relaxation therapy. Patients received 5 weekly treatments over the 8 weeks of the trial, including group and individual counseling. The researchers tested the subject's urine for the presence of cocaine three times a week. Intent-to-treat analysis of the urine data showed that patients assigned to acupuncture were significantly more likely to provide cocaine-negative urine samples relative to both the relaxation control and the needle insertion control. The odds ratios were 3.41 and 2.40, respectively. In addition, the researchers found that those receiving acupuncture who completed the program provided significantly more consecutive cocaine-negative urine samples while in treatment than either control condition. Those receiving acupuncture were more likely to be cocaine abstinent during the final week of the trial, with an abstinence rate of 58.8%, compared with 23.5% for the needle control group and 9.1% for the relaxation control group. The biological mechanism is still not known.
[Arch Intern Med 2000; 160: 2305-2312]

Oxidative stress linked to hypertension in animal experiments

Induced oxidative stress, resulting in overproduction of free radicals, causes severe hypertension in normotensive rats. The researchers report that antioxidant therapy following induction of oxidative stress markedly reduces hypertension and have demonstrated an association between hypertension and oxidative stress in animals in a number of previous studies with different models, including hypertension induced by lead, chronic renal failure or genetic. In this study, oxidative stress was produced in genetically normotensive rats by depleting levels of glutathione. The rats were given buthionine sulfoximine, a glutathione synthase inhibitor, in their drinking water over a 2-week period while a control group was given drug-free water. The buthionine sulfoximine-treated group showed a threefold decrease in tissue glutathione content, a marked elevation in blood pressure, and a significant reduction in the urinary excretion of the NO metabolite nitrate plus nitrite, which suggests depressed NO availability. In addition, they found a significant accumulation in various tissues of nitrotyrosine, which is the footprint of NO inactivation by reactive oxygen species. The team gave some animals in each group vitamin E-fortified chow and vitamin C-supplemented drinking water. Among animals in the buthionine sulfoximine group that received supplements, the research team noted reduction in blood pressure, improvement in urinary nitrate-plus-nitrite excretion, and mitigation of nitrotyrosine accumulation. Supplementation had no effect on the control group. In conclusion, the researchers have suggested that an intelligent regimen of antioxidant therapy or lifestyle could be a means of preventing hypertension or, with additional therapy, ameliorating it.
[Hypertension 2000; 36: 142-146]

***H. pylori* resistant to metronidazole, tetracycline and amoxycillin**

Helicobacter pylori is resistant in vitro to metronidazole, tetracycline and amoxycillin. Wu and colleagues collected 153 clinical isolates of *H. pylori* from the gastric biopsy specimens of 81 females and 72 males. Agar plates, containing two-fold dilutions of metronidazole, tetracycline and amoxycillin, were inoculated with the *H. pylori* suspensions. The researchers also assessed β-lactamase production by acidometry. The investigators found that of the inoculated isolates, 77.8% were resistant to metronidazole (MIC > 8

mg/L), 58.8% to tetracycline (MIC > 16 mg/L) and 71.9% to amoxycillin (MIC > 0.5 mg/L). Surprisingly, 39.2% of H. pylori isolates were resistant to all three antibiotics tested. Resistance to metronidazole was more common in isolates from females than in those from males. They concluded that the mechanism of amoxycillin resistance was not linked to production of β-lactamase, as none of the isolates produced β-lactamase. The reason that so many multiresistant strains were identified may reflect extensive use of these three antibiotics in this area. They suggested that therapy regimens should be adjusted to include one of these three antibiotics combined with another agent, such as clarithromycin, for which rates of resistance were low and in vitro efficacy high. Dr. Wu's group also recommended testing for antibiotic sensitivity of bacteria before treating patients.
[J Antimicrob Chemother 2000; 46: 121-123]

NF-kappa-B inhibitor shows promise in mouse arthritis model

SP100030, a T-cell-specific inhibitor of the transcription factor NF-kappa-B, blocks cytokine expression in cell culture and decreases collagen-induced arthritis severity in mice. NF-kappa-B is active in rheumatoid arthritis synovium and plays a key role in the inflammatory processes contributing to the disease. The researchers investigated a possible therapeutic role of NF-kappa-B inhibition in arthritis by studying the effects of SP100030 in vitro and in collagen-induced arthritis in mice. SP100030 markedly diminished IL-2, TNF-alpha, and IL-8 mRNA levels in stimulated Jurkat cells compared with control cells, suggesting that SP100030 inhibited NF-kappa-B regulated cytokine production at the transcriptional level. In the murine collagen-induced arthritis (CIA) model, SP100030-treated mice had significantly lower arthritis scores than did control animals, and paws from mice treated from day 20 to day 34 showed a trend toward decreased inflammation by histology. Current anti-cytokine therapies are highly targeted to a single cytokine (e.g., anti-TNF and IL-1Ra) and can have dramatic clinical improvement in a subset of patients (perhaps 30% to 40%), By targeting pathways that regulate a number of different cytokines, one can suppress a panel of pro-inflammatory factors and potentially be more effective. SP100030 is the first-generation product and is not orally bioavailable. This is in the process of being optimized so that a clinical candidate can move forward.
[J Immunol 2000; 165: 1652-1658]

G Protein-coupled receptor structure suggests activation mechanism

The three-dimensional crystal structure of the light-activated protein rhodopsin, a member of the G-protein coupled receptor (GPCR) family, reveals its likely molecular mechanism of activation. The GPCR [proteins] are involved in many physiological processes and are attractive targets or pharmacological intervention to modify these processes in normal and pathological states. Furthermore, GPCRs share many structural features, so discoveries about rhodopsin's structure and interactions may have application for other GPCRs. The researchers determined the three-dimensional crystal structure of rhodopsin at 2.8-Angstrom resolution. As predicted from earlier models, rhodopsin includes a bundle of seven transmembrane alpha helices connected by six loops of differing lengths. Three highly organized loops in the extracellular region associate to form the basis for the compact arrangement of the transmembrane helices, the investigators note. A chromophore interacts with a cluster of residues to determine maximum-absorption wavelength, and changes in these interactions among rhodopsins facilitate color discrimination. Several regions of the cytoplasmic loops are critical to the function of rhodopsin. A conserved set of residues on the cytoplasmic surface, where G-protein activation occurs, likely undergo a conformational change upon photoactivation of the chromophore that leads to rhodopsin activation and signal transduction. Because most of the vertebrate visual pigments share similar size distributions for all of the domains, structure-function relationships deduced from the current model are likely to be directly applicable to the members of this subfamily. The relevance of rhodopsin's structure may be even broader. Elucidating the molecular mechanisms of receptor activation that are shared by the GPCR family should have far-reaching implications. New insights gained will help to understand how GPCRs transduce the signals that regulate embryonic development and control the heart, blood vessels, endocrine responses, synaptic traffic in the brain and, indeed, the functions of virtually every eukaryotic cell.
[Science 2000; 289: 739-745, 733-734]

Green tea consumption enhances plasma antioxidant capacity

Drinking as little as 300 mL (10 oz) of green tea significantly increases the total antioxidant capacity of plasma. Epidemiologic studies have reported a lower incidence of coronary heart disease and cancer among drinkers of green tea, but few studies have measured its antioxidant effects. The investigators measured the total antioxidant capacity of plasma in 10 healthy subjects 1 hour and 2 hours after they drank tea prepared from 2.5 g (in 150 mL water), 5.0 g (in 300 mL), or 7.5 g (in 450 mL) green tea leaves. Although antioxidant capacity did not increase significantly after 150 mL of green tea, plasma antioxidant capacity rose 7% at 1 h and 6.2% at 2 h after consumption of 300 mL of green tea. Similarly, consumption of 450 mL of green tea was associated with an increase in plasma antioxidant concentration of 12.0% increase at 1 h and 12.7% at 2 h. These increases are similar to those previously reported after ingesting 300 mL of red wine (18% at 1 h and 11% at 2 h). With these findings we could assume that antioxidant effect of green tea is sustained for at least 2 h, the authors conclude. Green tea and red wine are readily available drinks that contain high levels of antioxidants. Although green tea contains less antioxidant effect than red wine, green tea is considered to have high value as favorite food or drinks as it does not contain alcohol. The roles of each component of green tea in the increase in antioxidant capacity still need further investigation.
[Eur J Clin Nutr 2000; 54: 527-529]

Third-generation OCs appear not to increase risk of venous thromboembolism

Despite continued controversy about the association between third-generation oral contraceptives (OCs) and an increased risk of venous thromboembolism, concern is unfounded. Farmer and colleagues collected data from the General Practice Research Database on women between 15 and 49 years of age who took combined OCs from 1993 to 1998. The analysis showed that the use of third-generation combined oral contraceptives fell from 53% during January 1993 to October 1995 to 14% during November 1995 to December 1998. However, the investigators found that there was no significant change in the incidence of venous thromboembolism between the two periods after age was adjusted for. Given these data, the team says that the notion that third-generation OCs are associated with a 2-fold risk of venous thromboembolism compared with older OC is unfounded.
[BMJ 2000; 321: 477-479]

Novel HIV-1 entry inhibitor provides potent antiviral activity

PRO 542, a novel HIV-1 entry inhibitor incorporating four copies of the virus-binding domains of CD4, safely reduced plasma HIV-1 RNA and plasma viremia in preliminary studies. In targeting cell-free virus, PRO 542 is unique among antiretroviral agents that are either approved or in late-stage clinical development, including other entry inhibitors. Jacobson and colleagues conducted the first human study of PRO 542, a phase I safety and pharmacokinetics trial, in 15 HIV-infected volunteers. Serum concentrations of PRO 542 doses of 10 mg/kg peaked at 564 mcg/mL, well above the 20 mcg/mL concentration required to achieve a 90% reduction in viral infectivity in vitro. Because of the mean serum half-life for the 5 mg/kg dose (4.2 days) and the 10 mg/kg dose (3.3 days), serum PRO 542 concentrations above 20 mcg/mL were sustained for up to 1 week. Plasma HIV RNA fell significantly after a single 10 mg/kg dose of PRO 542, and plasma viremia disappeared for up to 4 weeks in some subjects. No patient experienced dose-limiting toxicities from PRO 542 or developed measurable levels of antibodies to PRO 542. Taken together, the virus load and HIV culture analyses indicate that PRO 542 possess antiviral activity in humans, they conclude. Multiple-dose phase II trials of PRO 542 in this patient population will be required in order to determine the dosages and serum concentrations required for sustained antiviral activity.
[J Infect Dis 2000; 182: 326-329]

Insulin may have anti-inflammatory and anti-atherosclerotic effects

By increasing nitric oxide synthase and nitric oxide production, insulin reduces the expression of intercellular adhesion molecule-1, (ICAM-1), and thereby produces anti-inflammatory and anti-atherosclerotic effects. Dandona and colleagues induced insulin into human endothelial cells from aortas. They found that insulin (100 and 1,000 microunits/mL) caused a decrease in the expression of ICAM-1 (messenger ribonucleic

acid and protein) by these cells in a dose-dependent manner after incubation for 2 days. Associated with the decrease in ICAM-1, there was an insulin-induced increase in endothelial nitric oxide synthase. The investigators treated the aortic endothelial cells with N-nitro-L-arginine to determine if the insulin-induced inhibition of ICAM-1 was mediated by nitric oxide. They found that the insulin-induced decrease in ICAM-1 expression at the messenger ribonucleic acid and protein levels was inhibited by N-nitro-L-arginine. Thus, they conclude, the inhibitory effect of insulin on ICAM-I expression is mediated by nitric oxide. This effect of insulin is suggestive of an anti-inflammatory action of this hormone. This observed insulin effect, along with its vasodilatory and antiplatelet effects, militate against a proatherogenic role for insulin.
[J Clin Endocrinol Metab 2000;85:2572-2575]

High levels of bioavailable estrogen reduce risk of cognitive decline in women

Postmenopausal women with high levels of non-protein-bound, bioavailable estrogen are less likely to develop cognitive impairment than women with low levels of bioavailable estrogen. Because women on estrogen replacement tend to be younger and more educated, and have healthier lifestyles, the role of estrogen in preventing dementia has been unclear. The researchers found that total serum estrogen levels have not been associated with cognitive function in older women, but this may be because 90% of estrogen in the blood is bound to protein and is not able to cross the blood-brain barrier. To see if the levels of free estrogen correlated with cognitive function, they measured non-protein-bound serum estrogen, bioavailable serum estrogen, and cognitive function in 425 women older than 65 years of age. Although initial cognitive scores were similar, 6 years later 17 of 106 (16%) women with the lowest levels of non-protein-bound estrogen at baseline had cognitive impairment. In contrast, only 5 of 94 (5%) women with the highest levels were impaired. After adjusting for various factors, this gave an odds ratio of 0.3 for women with the highest levels of non-protein-bound estrogen. The results for bioavailable estrogen were similar. There was a very clear relationship between level of bioavailable estrogen and risk of decline. The higher the estrogen, the less decline. It really supports the idea that estrogen might be protective against dementia. In an accompanying editorial, Dr. Mary C. Tierney, from the University of Toronto, Ontario, Canada, comments that it is critical that the study be repeated with a more representative sample of women (27% of the study subjects developed breast cancer) and with more sensitive measures of cognitive function. If cognitive decline is confirmed to be related to low concentrations of serum oestradiol but not to higher postmenopausal concentrations, she believes that the next step will be to investigate whether those women at highest risk will show the greatest benefit from low-dose hormone replacement therapy.
[Lancet 2000; 356: 694-695, 708-712]

Coffee drinking may damage blood vessels

Drinking coffee, the world's most widely consumed pharmacologically active substance, has potentially harmful effects on blood vessels, according to research presented at the 22nd World Congress of the European Society of Cardiology in Amsterdam. Dr. M. O'Rourke and colleagues from St. Vincents Hospital, Sydney, Australia, presented data that link caffeine consumption with acute deterioration of the elastic properties of the aorta. They believe that the findings have important implications for left ventricular function and coronary blood flow. In the Australian study, 18 healthy middle-aged volunteers consumed 250 mg of caffeine, the amount found in 2 to 3 cups of coffee. Carotid-femoral pulse wave velocity was used as an index of aortic elasticity. Caffeine led to an acute 8% increase in pulse wave velocity, an effect that lasted for at least 3 hours. This effect was accompanied by acute increases in systolic and diastolic pressure of 8% and 10%, respectively. In a separate study of 15 healthy volunteers, Dr. Georg Noll and colleagues from the University Hospital of Zurich, Switzerland, demonstrated for the first time that coffee drinking and caffeine infusion enhance sympathetic nerve activity, leading to a pronounced blood pressure increase in the nine non-habitual coffee drinkers. Conversely, they found no blood pressure increase, despite similar sympathetic nerve activation, in the six habitual coffee drinkers. In the study, arterial blood pressure, heart rate and muscle sympathetic nervous activity were continuously recorded before and after subjects drank triple espresso or decaffeinated triple espresso, or received an intravenous infusion of caffeine

(250 mg bolus) or placebo (saline). Coffee drinking and caffeine infusion induced similar increases in muscle sympathetic nervous activity and systolic blood pressure in non-habitual coffee drinkers.
[http://www.medscape.com/reuters/prof/2000/08/08.31/20000831clin018.html]

Physical activity may cut erectile dysfunction risk

Remaining active or becoming physically active in midlife are among lifestyle factors that may reduce the likelihood of erectile dysfunction. The researchers sought to determine the influence of smoking, alcohol consumption, obesity and a sedentary lifestyle on the risk of erectile dysfunction. They surveyed 1,709 men aged 40 to 70 years at baseline in 1987 to 1989 and followed up on 1,156 between 1995 and 1997. Data were analyzed for a total of 593 followed subjects who had been free of moderate or complete erectile dysfunction during the initial survey. None of these men had had prostate cancer and they had not been treated for heart disease or diabetes. Obesity at baseline was associated with a greater risk of erectile dysfunction regardless of follow-up weight loss. Changes in smoking or in alcohol consumption over the average follow-up period of 8.8 years were not associated with erectile dysfunction. However, there was a significant association with physical activity. The highest dysfunction risk was seen among men who remained sedentary and the lowest among those who remained active or initiated physical activity. The investigators conclude that midlife changes may be too late to reverse the effects of smoking, obesity and alcohol consumption. Conversely, physical activity may reduce the risk of erectile dysfunction even if initiated in midlife.
[Urology 2000; 56: 302-30].

Evidence for cardiovascular benefits of chocolate continues to grow

The latest research supporting the potential cardiovascular health benefits of chocolate was presented during a symposium at the 22nd congress of the European Society of Cardiology in Amsterdam. Earlier this year, it was reported in vitro and human data showing that flavonoids found in cocoa may help protect against cardiovascular disease, from studies presented at the American Association for the Advancement of Science's annual meeting. Dr. Carl Keen, of the University of California, Davis, told symposium attendees that the story had now moved forward, with new research showing significant increases in plasma prostacyclin levels and a decrease in leukotriene levels in human volunteers who consumed 37 g/d of chocolate. Prostacyclin is manufactured by the vascular endothelium and promotes vasodilatation, inhibits platelet clumping, the formation of blood clots, and the entry of LDL-cholesterol into the arterial wall. Conversely, leukotrienes are vasoconstrictive, causing a slow and persistent contraction in the smooth muscle of the blood vessels, and can be platelet aggregators. A lowering of the leukotriene/prostacyclin ratio as observed in the study may have beneficial effects on platelets and possibly inflammation and vessel dilation. We are really excited about the prostacyclin research, which will be published in the next couple of months. Dr. Gerard Hornstra, of the University of Maastricht, acknowledges that there are concerns that other chocolate ingredients, such as fat, might increase cardiovascular risk. However, he believe that this is unlikely because of the profile of fatty acids present in chocolate. Chocolate is about 30% fat, mainly from cocoa butter, which contains about 60% saturated fatty acids (35% stearic acid and 25% palmitic acids) and about 40% unsaturated fatty acids mainly oleic acid. Palmitic acid increases and oleic acid decreases plasma LDL-cholesterol with stearic acid having a negligible effect. Based on these considerations, it can be expected that the contribution of chocolate consumption to cardiovascular risk is low.
[http://www.medscape.com/reuters/prof/2000/08/08.30/20000830drgd003.html]

Metformin reverses fatty liver disease in mouse model

US-based researchers have discovered that the oral diabetes drug metformin improves fatty liver disease in genetically obese insulin-resistant mice. The researchers explained that previous studies in humans and experimental animals have demonstrated a strong relationship between liver steatosis and insulin resistance. In the present study, they determined whether fatty liver disease in obese, leptin-deficient (ob/ob) mice might be improved by treatment with metformin, which reduces hyperinsulinemia and improves hepatic insulin resistance. The authors reported that, in

ob/ob mice, metformin improved fatty liver disease, reversing hepatomegaly, steatosis and aminotransferase abnormalities. According to the paper, metformin inhibited the expression of both tumor necrosis factor (TNF)-alpha and the TNF-inducible factors that promoted hepatic steatosis and necrosis. The investigators conclude that these findings justify cautious evaluation of metformin as a treatment for fatty liver disease in patients with obesity-related insulin resistance. They say, however, that the benefits of this agent should first be assessed in other animal models.
[Nat Med 2000; 6: 998-1003]

Novel immune system stimulant shows anti-tumor potential

An immune system stimulant given subcutaneously every other week causes significant lesion regression in cancer patients whose immune systems are reasonably competent. The study results were presented in Toronto at the 28th World Congress of the International Society of Hematology. Dr. Floyd Taub, chairman, Lifetime Pharmaceuticals, College Park, Maryland, reported results from the first phase I/II study of beta-alethine, a disulfide. Every 14 days for 3 months, researchers at McGill University, Montreal, Canada, gave 2 micrograms of beta-alethine to patients with low-grade B-cell lymphoma and maximal response to therapy, or indolent disease that did not yet require therapy. He reported that, to date, eight lymphoma patients and six myeloma patients in a separate protocol, five of whom had undergone stem cell transplantation, had received beta-alethine for up to 1 year. The investigators observed virtually no adverse effects from the biweekly regimen. Prior to treatment, patients underwent delayed-type hypersensitivity testing to assess the competency of their immune system. Three out of four patients whose immune systems could be activated ended the trial with less tumor than they began. In contrast, three out of four patients who had a poor immune response on delayed hypersensitivity testing continued to progress, a difference between the two groups which did reach statistical significance. One patient who remained on the study drug for 1 year had a 50% decrease in tumor burden. He explains that beta-alethine appears to stimulate an orchestrated, coordinated cytokine response. T cells also become activated and more cytotoxic, and tumor necrosis factor on the surface of lymphocytes is increased, which takes the drug right into the cancer. He also suggests that beta-alethine might work well in patients with hepatitis C, where perhaps a small amount of immune stimulation would be all that's needed for the body to win out over the virus. In addition to the ongoing Canadian trial, the drug is now under investigation in four sites in the US for the treatment and potential prevention of various cancers.
[http://www.medscape.com/reuters/prof/2000/08/08.30/20000830drgd002.html]

Vitamin D analogue inhibits mouse skin tumorigenesis

Researchers from Johns Hopkins University, in Baltimore, Maryland, have announced the synthesis of a vitamin D analogue that has potent tumor prevention properties. The molecule, dubbed QW-1624F2-2, is still in the early stages of development and may not be ready for human trials for another 2 to 3 years. But investigators are encouraged by its ability to prevent cancerous tumors in laboratory animals without the normal toxic side effects of vitamin D. The Hopkins researchers induced cancers in four groups of rats by swabbing them with the carcinogen dimethylbenzanthracine. Each group then received a different analogue of vitamin D topically, twice a week for 20 weeks. A control group was treated with vehicle. At the end of the experiment, control animals had a mean of 13 tumors while treated animals had a mean of just 5, for a reduction of 63%. Overall, the incidence of tumors was reduced in treated animals by 28%, lead investigator Dr. Gary H. Posner said at the annual meeting of the American Chemical Society. None of the treated rats showed any signs of hypercalcemia. Also, there was no compromise in weight gain in any of the animals. Since vitamin D is absorbed by almost every organ system in the body, research groups all over the world are actively searching for analogues that are safe and might be useful against cancer, immune system disorders and skin diseases. This results represent a glimmer of hope but it should be stressed that they are preliminary.
[http://www.medscape.com/reuters/prof/2000/08/08.24/20000824drgd004.html]