JPP

Journal of

Pharmacy and Pharmacolog

British Pharmaceutical Conference

Science Proceedings 2004

September Supplement

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Investigation of endogenous and H_2O_2-induced DNA damage in lymphocytes derived from schizophrenic patients and control subjects using the comet assay

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Schizophrenia, a mental disorder affecting approximately 1% of the population world-wide, typically occurs in the second or third decade of life, with individuals being affected throughout most of their life. Although the aetiology of the disorder remains unknown, investigation of the effects of oxidative stress in schizophrenia have largely focused on the determination of lipid oxidation products (Scottish Schizophrenia Research Group 2000). Oxidative damage to lipids and proteins can result in a number of pathophysiological processes while changes in DNA may alter gene expression or cause cell death and lead to genetic modification and mutagenesis. There is increasing evidence that oxidation is involved in the development of cancer and a recent study has reported an increased overall risk of cancer in patients with schizophrenia compared WITH that of the general population (Lichtermann et al 2001). To date, only one study has examined the effects of oxidative insult on the cellular DNA of a Greek sample of male schizophrenic patients and controls, although no significant difference was reported (Psimadas et al 2004). However, the study reported here is the first to investigate the effects of oxidative stress in a British sample of male and female schizophrenic patients and controls on the level of DNA damage. In this study a comparison of endogenous and hydrogen peroxide (H_2O_2)-induced DNA damage in schizophrenic and normal lymphocytes was undertaken. Ethical approval was obtained from the LREC and all procedures carried out in accordance with the Helsinki Declaration (1975) and the Data Protection Act (1998). Schizophrenic patients ($n = 15$) and apparently healthy controls ($n = 17$) were recruited and informed consent obtained from ward patients and staff at the New Craigs Hospital, Inverness. The trial protocol was reviewed by ward consultants at the hospital and participants matched for age, gender and smoking status. Venous blood (9 mL) was collected from 32 subjects (27 smokers, 5 non-smokers), comprising 11 male and 4 female schizophrenic patients, (average age = 37.9 ± 11.0) and 12 male and 5 female healthy controls (average age = 38.9 ± 9.2). Lymphocytes were separated by centrifugation and either treated with 0, 50 and 200 μM H_2O_2 or cryopreserved at −80°C. The single cell gel electrophoresis assay (comet assay) was used to evaluate DNA damage; cells were embedded in agarose on a microscope slide, lysed and immersed in alkaline buffer to enable DNA unwinding. Nucleoids were electrophoresed, washed and stained and scored visually using a fluorescence microscope. One-hundred random comets from each gel were scored by an examiner who was blinded to treatment group and were classified into one of five classes according to the relative intensity of fluorescence in the tail with a value of 0–4 (0 = undamaged, 4 = maximally damaged). Preliminary data suggests no significant decrease in the level of endogenous DNA damage between schizophrenic patients and the control group. The susceptibility of lymphocytic DNA to an oxidative challenge (H_2O_2) is currently under investigation. Further understanding of the role that oxidative stress may play will be valuable for developing new and innovative therapeutic strategies for schizophrenia and associated co-morbidities.

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Synthesis and effects of four novel dihydropyridines on rat atrium and colon smooth muscle

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The influx currents of calcium through L-type voltage dependent calcium channels play a crucial role in modulation of smooth muscle contractions. Therefore, the dihydropyridine compounds, as L-type calcium channel blockers, have received much attention in therapeutics. Numerous investigations are carried out to design novel dihydropyrines with more selectivity and less adverse effects. One of the adverse effects associated with nifedipine, which is a classical dihydropyridine, is its negative inotropic effects on the heart. In this study a series of novel 4-(3-benzyl-2-alkylsulfanyl-3H-imidazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dialkyl esters (**4a–d**) were synthesized in which o-nitrophenyl of nifedipine has been replaced with benzyl imidazolyl substituent. Initially 1-benzyl-2-mercapto-imidazole-5-methanol (**1**) was synthesized from benzylamine hydrochloride and dihydroxyacetone then it was alkylated to 2-alkylthio-1-benzylimidazole-5-methanol (**2**). Oxidation of **2** gave carbaldehyde (**3**). Compound **3** was converted to the novel 4-(3-benzyl-2-alkylsulfanyl-3H-imidazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dialkyl esters (**4a–d**) through classical Hantzsch method. The compounds were characterized by ^{1}H NMR and IR spectroscopy (Hadizadeh et al 2002). All test compounds (**4a–d**), which differed only at alkyl groups, showed positive inotropic effects on the isolated rat left atrium (Vogel & Vogel 1997). This was in contrast to classical dihydropridines of which nifedipine is the prototype. EC50 values were defined as concentration needed to increase percent of contraction by 50% and IC50 values were defined as concentrations needed to decrease percent of contraction by 50%. 4-(3-Benzyl-2-ethylthio-3H-imidazol-4-yl)-2,6-dimethyl-1,4-dihydro pyridine-3,5-dicarboxylic acid diethyl ester (**4a**) was the most potent and its EC50 was found to be 4×10^{-5} M (positive inotropic effect). In contrast, nifedipine decreased percent of contraction and its IC50 was found to be 5×10^{-8} M (negative inotropic effect). Test compounds (**4a–d**) decreased contractile responses of colon muscle to KCl (Vogel & Vogel 1997). Compound **4a** decreased percent of contraction of colon muscle in presence of KCl and its IC50 was 6×10^{-5} M. IC50 for nifedipine was 5×10^{-8} M. Since these compounds are analogues of nifedipine, their effects are most likely due to modulation of L-type calcium channels. It may be concluded that replacement of o-nitophenyl substituent in nifedipine with imidazolyl substituent may cause some partial calcium channel agonist properties at the heart muscle while calcium channel antagonist properties at other smooth muscles (colon muscle) persists. These compounds may be effective in patients with congestive heart failure.

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Effects of phytoestrogens on the contractile activity of rat blood vessels

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Genistein (GEN) a tyrosine kinase inhibitor and its analogue daidzein (DAID), devoid of this activity, are plant-derived isoflavonoids that possess weak, oestrogen-like relaxant effects on blood vessels. Populations consuming diets rich in these phytoestrogens appear to have reduced cardiovascular disease risks and it has been proposed that GEN could substitute for oestrogens in hormone replacement therapy. The mechanism by which phytoestrogens relax blood vessels remains controversial. Lee & Man (2003) observed genistein-induced relaxation, which was independent of the endothelium, whereas Mishra et al (2000) reported endothelium-dependent relaxation by both genistein and daidzein. This work compares relaxant actions of GEN and DAID with those of the endogenous oestrogen, 17β oestradiol (EST), in two different blood vessels of the rat. Aortic rings, intact or without endothelium, from male Hooded-Lister rats (250–350 g) were studied in Krebs' solution (37°C, 95% O_2, 5% CO_2) containing indomethacin (10 μM) (KS) under 2 g tension. Functional aortic endothelium was confirmed by relaxation (> 30%) to acetylcholine (1 μM) following contraction by KCl (60 mM). Portal veins (PV) were placed in KS under 0.5 g tension. Concentration–response curves to KCl (10–100 mM) were constructed in each tissue in the presence or absence of EST (10–20 μM), GEN (20–40 μM) or DAID (20–40 μM), N = 4–6. Genistein caused concentration-related reduction in responses to KCl in intact aorta and PV, maximum relaxation being 32 ± 3.6% ($P < 0.01$) and 51 ± 6.0% ($P < 0.001$) respectively. DAID-induced relaxation was weak in intact aorta (21 ± 2.5%, $P < 0.05$) but marked in PV, maximal relaxation was 62 ± 4.0% ($P < 0.001$). GEN and DAID were ineffective in de-endothelialised aorta. Higher phytoestrogen concentrations were not used due to vehicle effects. EST caused greater relaxation than phytoestrogens in all tissues (Table 1). The results show that actions of GEN and DAID differ from those of EST, causing weak relaxation in intact aorta but no relaxation following endothelium removal. Both agents significantly relaxed PV; DAID appeared to be more effective, suggesting that tyrosine kinase inhibition plays no part in the relaxation mechanism. EST was considerably more effective than the phytoestrogens, producing comparable relaxation in all tissues. We conclude that phytoestrogen-induced relaxation is weak and endothelium-dependent in rat aorta, but not in PV. GEN and DAID-induced relaxation in PV is substantial and, like EST, relaxation is independent of the endothelium as in PV, the longitudinal muscle studied in experiments is separated from endothelium by circular muscle.