PROTECTIVE EFFECT OF STOBADINE AGAINST ISOPROTERE-NOL INDUCED OXIDATIVE STRESS IN RATS

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Summary

This study was carried out to evaluate whether the pyridoindole stobadine (STO), which is an effective cardioprotective drug and a potent antioxidant, had any specific role in changes of lysosomal enzyme (LE) activity in the rat heart during oxidative stress induced by a high dose of a synthetic catecholamine, isoproterenol (IPN). Oxidative stress induced by catecholamines is a well recognized toxic event. This effect has been extensively observed in the heart, where high levels of catecholamines cause lipid peroxidation, energy depletion and myocardial necrosis accompanied with leakage of lysosomal content and subsequent LE activity changes in most mammals. The activities of the LE acid phosphatase and N-acetyl-β-D-glucosaminidase were studied in the rat heart as markers of cell damage. IPN-induced toxic damage in male Wistar rats (9 h after IPN hydrochloride administration, 50 mg/kg s.c.) was manifested by marked alterations in the activities of the LE in the sedimentable fraction of the rat myocardium. STO administered in various dosage regimens reduced or diminished the IPN-induced biochemical changes in the rat myocardium. The results suggest that STO is able to protect rats against IPN-induced oxidative stress.

Introduction

The synthetic catecholamine isoproterenol (IPN) is capable of inducing massive myo-cardial necrosis in most mammals when administered in high doses (20, 11). Under the condition of IPN administration, an increased production of reactive oxygen species (ROS) is to be expected, originating from several sources. Catecholamine overstimulation impairs the balance between oxygen demand and oxygen supply and hence leads to hypoxia. Kalra and Prasad (5) reported that it is oxygen free radicals that through the release of lysosomal enzymes (LE) damage the myocardium. In our previous study (7), we reported that 9 h after its administration IPN induced a statistically significant decrease of the specific activity of LE in the sedimentable fraction of rat myocardium homogenates. This reduction was the highest of the IPN-induced damage observed during the 1–18 hour period. Rathore et al. (15) showed increased lipid peroxidation and altered antioxidant system in erythro-cytes in response to IPN-induced oxidative stress. Oxidative stress is defined as an imbalance between the production and inactivation of oxygen free radicals in cells with an accumulation of highly aggressive reactive oxygen species, which may lead to lipid peroxidation and protein crosslinking in cell membranes as well as to DNA damage resulting in cell destruction (2). This pathology can be contained by free radical scavengers (antioxidants), known to be effective against oxidative stress. Stobadine (STO), a singlet molecular oxygen quencher and a potent scavenger of hydroxyl, peroxyl and alkoxyl radicals, was shown to exibit protective activities in various *in vivo* and *in vitro* conditions under pathological situations involving ROS, (4, 8). Preclinical safety evaluation of STO was performed in rats and no adverse effects were detected (3). As disruption of lysosomal membrane integrity and leakage of LE is one of the underlying processes of cell disintegration in oxidative stress (13), the aim of the present paper was to investigate the effect of STO on the activities of the LE in the rat myocardium as markers of cellular damage.

Material and methods

Animals: Male Wistar rats weighing 280–300 g from the Breeding Facility, Dobrá Voda, Slovakia, were used. The animals were housed in standard laboratory conditions, were fed standard pellet and had free access to food and water.

Drugs: Stobadine (STO), (-)-cis-2,8-dimethyl--2,3,4,4a,5,9b-hexahydro-1H-pyrido(4,3b-indole) dihydrochloride (in some papers referred to as DH 1011) was developed in the IEP SASc, Bratislava, Slovakia by (23). Its chemico-physical and pharmacological properties were described by (4). DL-isoproterenol hydrochloride (IPN) was obtained from Sigma, USA. All chemicals and enzyme substrates

used (Lachema, Czech Republic; Serva, Germany; Sigma, USA) were of analytical grade.

Experimental: The animals were divided into five groups as follows: group IPN of animals were treated with a large single dose of IPN (50 mg/kg) subcutaneously (s.c.) in saline solution (0.1 ml/100g body weight). Group C received only the same volume of saline solution subcutaneously without IPN and was used as control. Group 1 of animals were treated with a single dose of STO (30 mg/kg s.c.) 30 min prior to IPN, group 2 with the same dose of STO but 60 min after IPN and group 3 of rats were treated with a double dose of STO (2x15 mg/kg s.c.) 30 min prior to and 60 min after IPN administration. After 9 hours of IPN administration, non-fasted animals were sacrificed by decapitation and the hearts were removed rapidly, washed with ice-cold saline (0.25 mol/l KCl), dried and weighed. Each sample consisted of hearts from two animals. The myocardium was homogenised in buffer D solution, pH 7.4, containing 0.6 mol/l KCl, 10 mmol/l imidazole, 40 mmol/l EDTA and 1 mmol/l $MgCl_2$, in a Potter-Elvehjem homogeniser. The homogenates were processed according to (24). After centrifugation the pellet was suspended in ice-cold buffer D and Triton X-100 was added so as to obtain the final concentration of 0.1% and then homogenised. LE activities were determined in the pellet after 40 000 xg. The whole experiment was carried out at 4 °C.

Biochemical analysis: The LE were assayed according to the standard methods (1).

Statistical analysis: One way analysis of variances NOVA followed by Bonferroni multiple comparison test was used for statistical evaluation, with $p < 0.05$ considered significant.

Results

Fig. 1-2 demonstrate the changes in the activity of two LE, APH and NAGA, after the nine-hour effect of IPN (50mg/kg s.c.), and the effect of STO. The effect of IPN was manifested by significant decrease of activity of both enzymes in the sedimentable fraction of the rat myocardium homogenate (pellet after 40 000 x g), by 28 % of APH and 43 % of NAGA. In this study we examined three modes of STO treatment, at the total dose 30 mg/kg. The best results were observed in group 3 when STO was administered in two doses (2x15 mg/kg s.c.), 30 min prior to and 60 min after IPN administration. In this group the values of the variables studied in the myocardium protected by STO were comparable to those in the control samples and were significantly different from the IPN group, which received only IPN (Fig. 1-2). Pretreatment with STO (30 mg/kg s.c.) 30 min prior to IPN in group 1 also significantly inhibited the IPN-induced decrease of LE activities in the rat myocardium. STO, administered 60 min after IPN (group 2) was less effective.

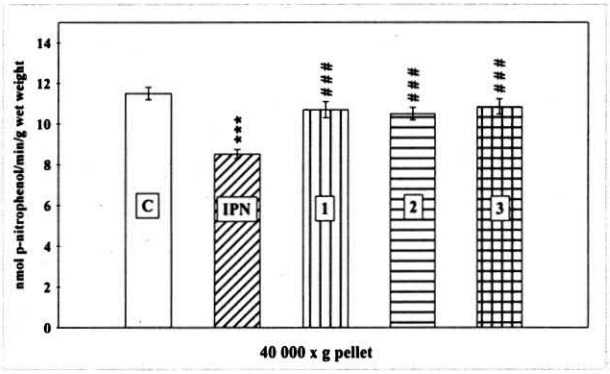

Fig. 1: Effect of stobadine on isoproterenol-induced changes in acid phosphatase activity in the sedimentable fraction of heart homogenates
*Acid phosphatase (APH); group C - control; group IPN – isoproterenol (50 mg/kg s.c.) 9 hours; group 1 - single dose of stobadine (STO) 30 mg/kg s.c. 30 min prior to IPN administration; group 2 - single dose of STO (30 mg/kg s.c.) 60 min after IPN; group 3 - double dose of STO (2x15 mg/kg s.c.) 30 min prior to and 60 min after IPN. Activity of acid phosphatase is expressed in – nmol p-nitrophenol/min/g wet weight. Values are means ± S.E.M.. Number of animals: 12–16 in each group; *p < 0.05 versus control; # p < 0.05 versus IPN*

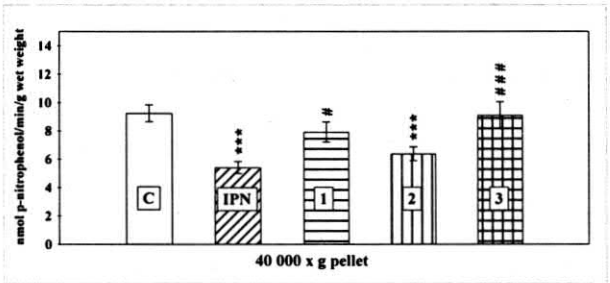

Fig. 2: Effect of stobadine on isoproterenol-induced changes in NAGA activity in the sedimentable fraction of heart homogenates
NAGA – N-acetyl-beta-D-glucosaminidase;
Activity of NAGA is expressed in - nmol p-nitrophenol/min/g wet weight;
For other abbreviations see Fig. 1.

Discussion

ROS have been implicated as mediators of tissue injury in cardiovascular pathology (6). One of their myocardial damaging effects may be operative through the release of lysosomal enzymes (5). The phospholipid-rich lysosomal membrane is a potential site of attack of free radicals. Disruption of lysosomal membrane integrity and leakage of LE is one of the underlying processes of cell disintegration in oxidative stress (13). Oxidative stress induced by catecholamines is a well recognized toxic event. This effect has been extensively observed in the heart, where high levels of catecholamines cause lipid peroxidation, energy depletion and myocardial necrosis (16). Administration of large doses of a synthetic catecholamine, IPN, causes massive myocardial necrosis in rats without coronary ligation (18). Such an IPN-induced "infarct-like" lesion is morphologically similar to a lesion described in acute myocardial infarction and sudden death in man (19). Nirmala and Puvanakrishnan (10) observed an increase in the levels of lipid peroxides in heart tissue during IPN administration. Ondrejičková et al. (12) showed the effect of STO to protect proteins by preventing oxidation of SH-groups in the rat myocardium where oxidative stress was induced by IPN. The protective effect of STO was confirmed in the isolated rat heart exposed to total ischaemia followed by reperfusion (21). Navarová et al. (9) demonstrated that STO was an efficient inhibitor of LE release *in vivo* and *in vitro* on HeLa cells. In the present study, we showed that IPN caused marked changes in the activity of LE in the sedimentable fraction of the rat myocardium. ATP and NAGA were shown to be suitable marker enzymes for assessing myocardial lysosomal integrity. We investigated whether STO had a protective influence on the rat myocardium with IPN provoked oxidative stress. In our experiment STO was able to reduce or diminish the IPN-induced biochemical changes. The results demonstrated that optimal protective effects were obtained when STO was administered in two doses, or when animals were pretreated. STO may conceivably be most effective in the initial step of the IPN-induced biochemical changes. An early change in the integrity of the lysosomal membrane may crucially affect the moment when a potentially reversible ischaemic stress turns to be irreversible. Ridout et al. (17) reported that stabilisation of the lysosomal membrane preserved cellular function in the ischaemic myocardium. The oxidative metabolism of IPN, like of other catecholamines, produces quinones, which react with oxygen to produce superoxide anions and H_2O_2. ROS are involved as causative factors in many diseases, thus the generation of ROS by catecholamines may contribute to this process (19, 14). STO was shown to be able to scavenge hydroxyl, peroxyl and alkoxyl radicals and to quench singlet oxygen (22,4). The ability of STO to scavenge free radicals may contribute to its cardioprotective properties. In conclusion, it may be postulated that STO is able to protect rats against IPN-induced oxidative stress.

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