VARIABLES OF OXIDATIVE STRESS IN PRE- AND PERINATAL DEVELOPMENT OF THE RAT

[1]Jana NAVAROVÁ, [1]Ružena SOTNÍKOVÁ, [1]Mojmír MACH, [1]Eduard UJHÁZY, [1]Vladimír KNEZL, [2]Pavel BLAŽÍČEK, [1]Michal DUBOVICKÝ
[1]Institute of Experimental Pharmacology SASc, Bratislava, Slovak Republic
[2]Hospital of Ministry of Defence, Bratislava, Slovak Republic

Abstract

The aim of the study was to evaluate models for studying possible preventive effects of selective antioxidants on biochemical changes induced by chronic intrauterine hypoxia and neonatal anoxia in Wistar/ /DV rats. Anoxia of newborn rats and chronic intrauterine hypoxia induced by phenytoin caused oxidative stress followed by organ ischaemia/reperfusion and reactive oxygen species generation. In biochemical quantification of oxidative stress we used the activity of the lysosomal enzyme N-acetyl-ß-D--glucosaminidase (NAGA) and the level of glutathione (GSH) in foetal liver and brain, and in the placenta and brain, lungs and liver of newborn rats as markers of tissue damage. Neonatal anoxia (25 min) in 2-day-old pups induced significant increase in NAGA activity in lungs and liver and GSH increase in brain and liver. The activity of NAGA in brain and the GSH level in lungs remained unchanged. On day 20 of gestation, PHT-induced toxic damage (150 mg/kg) was associated with an increase in NAGA activity and decrease in GSH level in placenta and foetal liver. NAGA activity and GSH level in foetal brain remained unchanged. The neonatal anoxia and PHT-induced biochemical changes established in the models used provide the possibility to assess potential preventive effects of antioxidants.

Introduction

Oxidative stress represents an important risk factor of tissue and organ injuries to mothers and especially to the developing foetus, which is insufficiently protected by antioxidative enzyme systems. Intrauterine hypoxia and anoxia of newborn and oxidative stress associated with it caused organ ischaemia following reperfusion and reactive oxygen species generation. There is a need for effective prevention, proper diagnosis, and search for new drugs with preventive antioxidant effects. Animal models are important for the study of basic mechanisms leading to hypoxic/ischaemic organ damage, as well as for the study and the evaluation of effects and mechanisms of protective drugs. Phenytoin (PHT), a widely used anticonvulsant, when administered in pregnancy, is thought to cause toxicity in the embryo or foetus via reactive intermediates. Free radicals or reactive oxygen species can oxidise molecular targets such as DNA, proteins and lipids in a process referred to as oxidative stress, which is thought to alter cellular function, potentially resulting in *in utero* death or teratogenicity (1). Hypoxic/ischaemic complications can lead not only to structural and functional changes but also to injuries on biochemical level. Free oxygen radicals generated in the conditions of oxidative stress damage the cell membranes, cell organelles as well as lysosomes. Disruption of lysosomal membrane integrity and leakage of lysosomal enzymes to the surrounding space is one of the underlying processes of cell disintegration in oxidative stress (2). The cellular antioxidant glutathione is the key molecule in the defence against oxidative stress by participating in the detoxification of free radicals (3). Its decline in plasma or tissues is an important parameter of oxidative stress.

The aim of the present study was to evaluate biochemical parameters in two models of oxidative stress. Modulations in the activity of the lysosomal enzyme N-acetyl-ß-D-glucosaminidase (NAGA) and in the level of glutathione (GSH) in embryonic tissues and placenta were used as markers of cell damage caused by oxidative stress.

Material and Methods

Wistar/DV pregnant rats (initial weight 200-220 g, 3-4 months old) from the Breeding Facility Dobrá Voda, Slovakia, were used. The animals were kept under controlled conditions. Food and tap water were available *ad libitum*.

Neonatal anoxia: On day 2 of age, the offspring were placed in a chamber with controlled temperature (36.0°C). In each experiment pups were remo-

ved from their cages 15 min before anoxia was induced, to allow their body temperature to adjust to the chamber temperature. Pups were placed into the chamber for 25 min and the air inside was removed by stream of nitrogen gas (100% N_2 and 0% O_2). The surviving pups (mortality rate 40%) were divided into two groups (8-10 pups). One group was sacrificed 10 min and the second group 120 min after anoxia. Nonhypoxic animals (controls) were placed in the chamber and exposed to room air for the same period of time (4). The organs (brain, lungs and liver) were removed from the pups, samples (50–60 mg) were put in ice-cold phosphate buffer, pH 7.4, containing Triton X-100 (0.1%) and homogenised in a hand glass homogeniser. Homogenates were centrifuged at 15 000 x g for 20 min. The activity of NAGA, the level of GSH and proteins were assayed according to standard methods (5, 6, 7) in supernatants after 15 000 x g.

Chronic intrauterine hypoxia: PHT dissolved in H_2O and adjusted to pH 11.5 with NaOH was administered p.o. from day 2 until day 19 of gestation in the dose of 150 mg/kg. Control groups received water with pH 11.5 over the same period. On day 20 of pregnancy the animals were sacrificed. The peritoneal cavity and uterus were opened and live foetuses were dissected. Placenta, foetal brain and liver were removed and processed in the same way as in the former model.

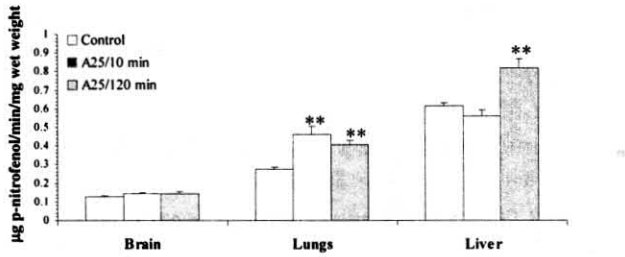
Sodium PHT, batch No. 0080499, was a kind gift from Slovakofarma, J. S. Comp. Hlohovec, Slovakia. The dosage volume was 0.5 ml/100 g body weight. All other chemicals and enzyme substrates (Sigma, USA) were of analytical grade.

Statistical evaluation: Student's t-test was used for statistical analysis, $p < 0.05$ was considered significant.

Results

Neonatal anoxia (25 min) in 2 day old pups induced significant increase in NAGA activity in lungs, as assessed both 10 min and 120 min after anoxia. The activity of NAGA in liver was increased only 120 min after anoxia. The activity of NAGA in brain changed neither 10 nor 120 min after anoxia (Fig. 1). The level of GSH in brain increased significantly 10 and 120 min after anoxia but in liver only 120 min after anoxia. The GSH level in lungs remained unchanged (Fig. 2).

PHT (150 mg/kg, p.o.) induced toxic damage was associated with an increase in the activity of NAGA in placenta and foetal liver (Fig. 3) and with a decrease of GSH in placenta and in foetal liver (Fig. 4). The activity of NAGA and the level of GSH in foetal brain remained unchanged (data not shown).



*p <0.05; ** p < 0.01; ***p < 0.001 – versus control (Student t-test)

Fig. 1: Activity of NAGA in 2 day rat

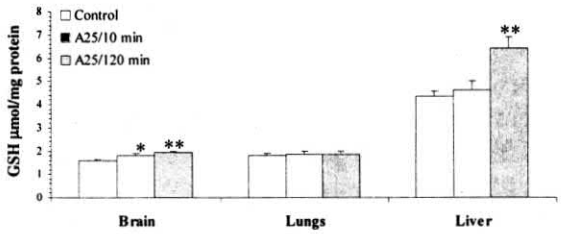
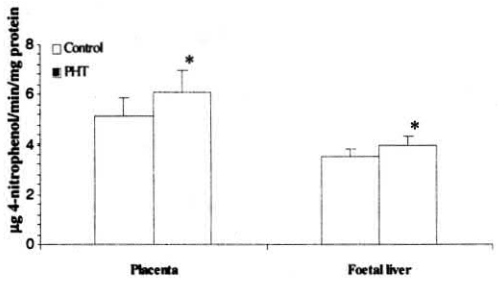


Fig. 2: Level of GSH in 2 day rat pups



*p <0.05; ** p < 0.01; ***p < 0.001 – versus control (Student t-test)

Fig. 3: Activity of NAGA in 20-day-old fetuses after

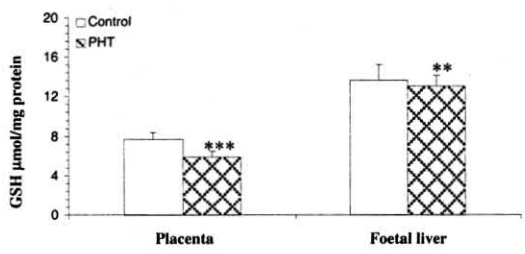


Fig. 4: Level of GSH in 20-day-old fetuses after PHT

Discussion

Perinatal hypoxic/ischaemic organ damage and oxidative stress associated with it remains a major cause of acute mortality and chronic morbidity in infants and children. Oxidative stress results in oxidative damage of biological active molecules, cells and tissues. Animal models are important in providing information regarding underlying mechanisms of perinatal hypoxic/ischaemic damage and how tissues can be protected through therapeutic intervention (8). Complications caused by oxidative stress could lead not only to structural and functional changes but also to injuries on biochemical level. TBARS, malondyaldehyde, lipofuscin, and oxidative products of cholesterol belong to the biochemical indicators of oxidative stress (9).

In the present study we wanted to quantify the oxidative stress induced by perinatal asphyxia and anoxia of pups by assessing biochemical variables that we used in our previous studies (10, 11, 12), i.e. lysosomal enzymes and GSH.

Free oxygen radicals generated in the conditions of oxidative stress damage cell membranes, cell organelles as well as lysosomes. Disruption of lysosomal membrane integrity and leakage of lysosomal enzymes to the surrounding space is one of the underlying processes of cell disintegration in oxidative stress. Increased intracellular concentration of reactive oxygen metabolites may be harmful to the integrity of secondary lysosomes since they constitute a compartment where iron would exist as a low-molecular weight complex with the ability to support peroxidation and fragmentation reactions (13). Lysosomal destabilisation may be prevented either by inhibition of cellular peroxidation or by prevention of iron-catalysed oxidative reactions, which involve peroxidation of cellular membranes, energy depletion, and leakage of lysosomal content. Understanding of the biochemical and molecular changes associated with oxidative stress may promote establishment of experimental models for testing drugs, which may protect tissues from injury (14).

GSH is an important cofactor/substrate for many physiological processes and for detoxification of xenobiotic reactive intermediates. GSH may be involved in the detoxification of a teratogenic reactive intermediate of PHT and/or in cytoprotection against oxidative stress. GSH depletors or inhibitors of GSH synthesis were found to potentiate PHT teratogenicity in mice. Conversely, N-acetylcysteine, a precursor of cysteine, the rate limiting amino acid in GSH biosynthesis, can provide partial protection against murine PHT embryotoxicity and teratogenicity (1).

In summary, in two models presented in this study, modulations were found in the activity of lysosomal enzyme NAGA and in the level of GSH in embryonic tissues and placenta. These results, along with our previous findings (13), suggest that the presented models would be suitable also for other studies on elimination of the adverse effects of oxidative stress induced by chronic perinatal hypoxia and anoxia of the newborn.

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Correspondence: Jana Navarová
Institute of Experimental Pharmacology
Slovak Academy of Sciences
Dúbravská cesta 9
842 16 Bratislava
Slovak Republic

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