EFFECT OF MELATONIN ON BIOCHEMICAL VARIABLES INDUCED BY PHENYTOIN IN ORGANS OF MOTHERS, FOETUSES AND OFFSPRINGS OF RATS

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SUMMARY

The present pre- and postnatal study was carried out to investigate the effect of melatonin (MEL), a potent antioxidant, on biochemical variables in the in vivo model of intrauterine hypoxia in rats. Chronic hypoxia was induced pharmacologically by the administration of the anticonvulsant phenytoin (PHT) during pregnancy. Rats were orally treated by PHT (150 mg/kg) from day 7 to 18 of gestation. MEL in drinking water (40 μg/ml) was administered from day 0 to 19 of gestation. The activity of the lysosomal enzyme N-acetyl-ß-D-glucosaminidase (NAGA) and the level of glutathione (GSH) were used as markers of tissue damage. In the prenatal study PHT-induced toxic damage was associated with an increase in NAGA activity and decrease of GSH level in placenta and in maternal serum and heart. MEL partially inhibited the changes of NAGA activity given above. MEL was able to increase only the decreased level of GSH in maternal heart. PHT decreased the level of GSH and increased the activity of NAGA in foetal organs, the improvement occurred in the liver and lungs, but not in foetal brain. In the postnatal study a significant increase of liver GSH level was found in all (control, MEL, PHT, MEL+PHT) groups of 1-day-old pups, while the activity of NAGA remained unchanged. We did not observe any significant differences in NAGA activity in the lungs and heart of pups. MEL increased the GSH level in lungs and heart. We concluded that administration of MEL during pregnancy partially inhibited the biochemical changes induced by PHT.

Key words: phenytoin, intrauterinne hypoxia, NAGA, GSH, melatonin, rat

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INTRODUCTION

When administered in pregnancy, phenytoin (PHT), a widely used anticonvulsant, is thought to cause chronic intrauterine hypoxia/ischaemia and toxicity in the embryo or foetus via reactive oxygen intermediates. Free radicals or reactive oxygen species can oxidise molecular targets such as DNA, protein and lipid in a process referred to as oxidative stress, which is thought to alter cellular function, potentially resulting in *in utero* death or teratogenicity. The pathology of oxidative stress can be prevented by antioxidants (free radical scavengers), known to be effective in treating conditions associated with oxidative stress (1, 2, 3). Melatonin (MEL), a secretory product of the pineal gland, is known to have free radical scavenging and antioxidative properties in several oxidative processes (4, 5). MEL participates in the regulation of a number of important physiological and pathological processes, plays a role in the reduction of oxidative damage and functions as a cell-protective agent (6, 7). In our previous biochemical study (8), a rat model of intrauterine chronic hypoxia induced by PHT was evaluated to study possible preventive effects of antioxidants. The activity of the lysosomal enzyme N-acetyl-ß-D-glucosaminidase (NAGA) and the level of glutathione (GSH) were used as markers of cell damage. The objective of this study was to examine the potential protective properties of MEL on oxidative organ damage in our model.

MATERIAL AND METHODS

Animals: Wistar/DV pregnant rats (initial weight 200-220 g, 3–4-month-old) from the Breeding Facility Dobrá Voda, Slovakia, were used. The animals were kept under controlled conditions at 22±2 °C and 55±5% relative humidity. Food pellets and tap water were available *ad libitum*. Animals were exposed to a 12/12 h light/dark cycle.

Treatment: PHT dissolved in H_2O and adjusted to pH 11 with NaOH was administered p.o. from day 7 until day 18 of gestation in the dose of 150 mg/kg. Control groups received water with pH 11 over the same period. Sodium PHT, batch No. 0080499, was a kind gift from Slovakofarma, J. S. Co. Hlohovec, Slovakia. Dosage volume was 0.5 ml / 100 g body weight. Melatonin (MEL) was dissolved in absolute ethanol and diluted to a final concentration of 40 μg/ml with tap water. MEL solution was supplied with drinking water in the dark period, during gestation days 0 to 19. On day 20 of pregnancy the animals were sacrificed by cervical dislocation. The peritoneal cavity and uterus were opened and live foetuses were dissected. Placentas, maternal heart and foetal organs (brain, liver and lungs) were removed. Pups were sacrificed on day 1 of gestation.

Determination of biochemical variables: Tissue samples of 50-60 mg were put in ice cold phosphate buffer pH 7.4, contai-

ning 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 the lysosomal enzyme N-acetyl-ß-D-glucosaminidase (NAGA), the level of GSH and proteins were assayed according to standard methods (9, 10, 11) in supernatants and in serum. All chemicals and enzyme substrates (Sigma, USA) were of analytical grade.

Statistical evaluation: ANOVA, one way analysis of variance, with Bonferroni multiple comparisons test was used for statistical analysis, $p<0.05$ was considered significant.

RESULTS

PHT-induced toxic damage was associated with an increase in NAGA activity and decrease of GSH level in maternal serum and heart and in the placenta. MEL partially inhibited the changes of NAGA activity listed above (Table 1).

MEL was able to step up the decrease of level of GSH in maternal heart only. PHT decreased the level of GSH and increased the activity of NAGA in foetal organs, the improvement occurred in the liver and lungs, but not in foetal brain (Table 2).

A significant increase of liver GSH level was found in all groups of pups, the activity of NAGA remained unchanged. We did not observe any significant differences in NAGA activity in lungs and heart of 1-day-old pups. MEL increased the GSH level in the lungs and heart (Table 3).

DISCUSSION

The present pre- and postnatal study was carried out to investigate the antioxidant power of MEL in the *in vivo* model of intrauterine hypoxia (oxidative stress) in rats by assessing biochemical variables used in our previous studies (8, 12), i.e. NAGA and GSH. In the current study administration of MEL during pregnancy partially inhibited the biochemical changes induced by PHT.

When administered in pregnancy, PHT, a widely used anticonvulsant, 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. Understanding of the biochemical and molecular changes associated with oxidative stress may promote establishment of experimental models for testing drugs protecting tissues from injury (13).

A number of observations suggest that detoxification of a xenobiotic free radical intermediate with antioxidants may provide important embryoprotection (14). GSH may be involved in the detoxification of a teratogenic reactive intermediate of PHT and/or in cytoprotection against oxidative stress. The GSH depletors or inhibitors of GSH synthesis potentiate PHT teratogenicity in mice (15, 16). Conversely N-acetylcysteine, a precursor of cysteine,

Table 1. *Effect of PHT and MEL on NAGA activity and GSH level in rat maternal serum, heart and in placenta*

		Control	MEL	PHT	MEL+PHT
Serum	NAGA	7.72 ± 0.41	9.73 ± 0.71	10.63 ± 0.82 **	9.24 ± 0.53
	GSH	2.73 ± 0.22	1.77 ± 0.14 ***	1.69 ± 0.11 ***	1.92 ± 0.17 **
Heart	NAGA	1.55 ± 0.07	1.64 ± 0.06	1.99 ± 0.16 **	1.89 ± 0.09
	GSH	2.39 ± 0.11	2.46 ± 0.11	1.95 ± 0.14 **	2.33 ± 0.16
Placenta	NAGA	5.72 ± 0.33	6.11 ± 0.33	8.34 ± 0.42 ***	6.84 ± 0.32 ooo
	GSH	2.94 ± 0.22	1.93 ± 0.15 ***	1.96 ± 0.11 ***	1.94 ± 0.12 ***

Values are given as means ± S.E.M., (n=10). Activity of N-acetyl-ß-D-glucosaminidase (NAGA) is expressed in µg 4-nitrophenol / min / mg protein. Glutathione (GSH) is expressed in µg / mg protein. MEL – melatonin; PHT – phenytoin; ** p<0.01, *** p<0.001 versus control; ooo p<0.001 versus PHT

Table 2. *Effect of PHT and MEL on NAGA activity and GSH level in foetal organs*

		Control	MEL	PHT	MEL+PHT
Liver	NAGA	3.91 ± 0.27	4.32 ± 0.16	5.04 ± 0.26 *	4.47 ± 0.23
	GSH	5.51 ± 0.23	5.22 ± 0.21*	4.56 ± 0.11**	5.28 ± 0.17 o
Lungs	NAGA	2.43 ± 0.17	3.17 ± 0.26 **	3.33 ± 0.21**	2.61 ± 0.32
	GSH	5.29 ± 0.28	3.12 ± 0.14 ***	3.82 ± 0.34 **	3.77 ± 0.31 **
Brain	NAGA	1.64 ± 0.25	2.52 ± 0.07 **	2.74 ± 0.15***	2.69 ± 0.11 ***
	GSH	4.44 ± 0.25	4.23 ± 0.27	3.55 ± 0.29	3.99 ± 0.32

Values are given as means ± S.E.M., (n=10). Activity of N-acetyl-ß-D-glucosaminidase (NAGA) is expressed in µg 4-nitrophenol / min / mg protein. Glutathione (GSH) is expressed in µg / mg protein. MEL – melatonin; PHT – phenytoin; * p<0.05, ** p<0.01, *** p<0.001 versus control; o p<0.05 versus PHT

Table 3. *Effect of PHT and MEL on NAGA activity and GSH level in organs of pups*

		Control	MEL	PHT	MEL+PHT
Liver	NAGA	7.29 ± 0.42	6.17 ± 0.32	7.71 ± 0.33 *	6.52 ± 0.35
	GSH	2.93 ± 0.15	3.75 ± 0.13*	3.86 ± 0.23**	3.83 ± 0.19 **
Lungs	NAGA	3.35 ± 0.19	3.51 ± 0.11	3.23 ± 0.12	2.86 ± 0.18 oo
	GSH	1.53 ± 0.11	2.68 ± 0.24 ***ooo	1.32 ± 0.11	1.96 ± 0.21 * oo
Heart	NAGA	2.96 ± 0.16	3.14 ± 0.08	3.12 ± 0.17	2.86 ± 0.13
	GSH	2.03 ± 0.15	3.03 ± 0.12 ***ooo	1.72 ± 0.15	1.83 ± 0.12

Values are given as means ± S.E.M., (n=10). Activity of N-acetyl-ß-D-glucosaminidase (NAGA) is expressed in µg 4-nitrophenol / min / mg protein. Glutathione (GSH) is expressed in µg / mg protein. MEL – melatonin; PHT – phenytoin; * p<0.05, ** p<0.01, *** p<0.001 versus control; oo p<0.01, ooo p<0.001 versus PHT

a rate limiting amino acid in GSH biosynthesis, can provide partial protection against murine PHT embryotoxicity and teratogenicity (17). Winn and Wells (18) demonstrated the teratologic importance of antioxidant balance: maternal administration of the antioxidative enzyme catalase enhanced embryonic activity and inhibited PHT teratogenicity. Yet on the other hand, maternal pretreatment with another antioxidative enzyme, superoxide dismutase, increased the teratogenity of PHT.

MEL was shown to have a variety of functions, and research over the last decade has shown it to be both a direct free radical scavenger and an indirect antioxidant. Because of these actions, and possibly others that remain to be defined, MEL was reported to reduce the toxicity and increase the efficacy of a large number of drugs whose side effects are well documented. Considering the low toxicity of MEL and its ability to reduce side effects and increase the efficacy of these drugs, its use as a combination therapy with these agents seems important and worthy of pursuit (5, 19).

The results of Ujházy et al. (20), given in the present issue, showed that MEL did not protect the PHT-induced developmental toxicity in rats. Similarly the study of Dubovický et al. (21) suggests that the prenatal administration of MEL fails to protect neurobehavioural development from hypoxia induced by PHT however in the light of the results of the present study, which showed that MEL was able to improve partially the biochemical changes in maternal, foetal and pup organs, the further studies are required using different MEL and PHT concentrations, and various regimens assessed at different gestational stages. We assume that a detailed study of MEL action should contribute to the elucidation of its antioxidative activity in pregnancy and early postnatal development.

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