EFFECT OF MELATONIN AND STOBADINE ON MATERNAL AND EMBRYOFOETAL TOXICITY IN RATS DUE TO INTRAUTERINE HYPOXIA INDUCED BY PHENYTOIN ADMINISTRATION

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SUMMARY

The aim of the present study was to test the hypothesis that the natural antioxidant melatonin (MEL) and the synthetic antioxidant stobadine (STO) could reduce the incidence of maternal and embryofoetal toxicity in rats due to intrauterine hypoxia. Chronic hypoxia was induced pharmacologically by the administration of the anticonvulsant phenytoin (PHT) during the entire period of pregnancy. PHT disturbed the normal course of pregnancy, affected reproductive parameters and increased the incidence of skeletal anomalies. MEL did not protect the PHT-induced development toxicity in rat. On the other hand, STO partially prevented PHT-induced reduction of foetal and placental weights. Administration of STO also decreased the frequency of pre- and post-implantation loss and resorptions in the PHT group. We concluded that pretreatment of pregnant rats with STO prevented to a certain extent reproductive and foetal development alterations caused by chronic intrauterine hypoxia.

Key words: intrauterine hypoxia, phenytoin, developmental toxicity, rat, melatonin, stobadine

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INTRODUCTION

Oxidative stress associated with various stages of pregnancy may be counteracted by administration of antioxidants (1). Since the developing organism may be poorly prepared to protect itself against oxidative stress (2, 3), administration of antioxidants via maternal organism could be a rational approach to protect embryo and foetus. Phenytoin (PHT) has been reported to cause intrauterine hypoxia affecting prenatal development (4, 5). PHT was shown to be teratogenic in rats (6). Indeed, in our previous study we found that prenatal administration of PHT to rats resulted in morphological and neurobehavioural alterations (7). The antioxidant melatonin (MEL) is an endogenous substance produced by the mammalian pineal gland and other organs, especially the enterochromaffin cells of the gastrointestinal tract and retina (8). MEL had no effect on prenatal survival, foetal body weight, or incidences of foetal malformations/variations (9). Stobadine (STO) is a potential neuro- and cardioprotective drug with high antioxidant properties (10). Results of reproductive toxicity of STO (11) and of teratological study in rats (12) showed no teratogenic potential. The aim of our study was to evaluate the possible preventive effects of selected antioxidants, i.e. natural melatonin and synthetic stobadine, on PHT toxicity development in rats.

MATERIAL AND METHODS

Female Wistar/DV rats (220–240 g body weight, 3–4 months old) had free access to water and food pellets and were kept on a 12/12 h light-dark cycle. After 7 days of adaptation, the females were mated with males (presence of spermatozoa in vaginal smear indicated day 0 of gestation).

Sodium salt of phenytoin (PHT, Slovakofarma, Hlohovec; batch No. 0080499, 99% purity) was dissolved in deionised distilled water and pH was adjusted to 11.5 with NaOH. Buffer water with pH 11.5 was used as a control vehicle. The dams were treated by oral lavage with PHT (150 mg/kg) daily from day 2 to 19 of gestation. Melatonin (MEL, Sigma-Aldrich; batch No. 13961-081) was dissolved in absolute ethanol and diluted to a final concentration of 40 μg/ml with tap water, yielding the final concentration of 0.4% ethanol. Animals were exposed to a 12/12 h light/dark cycle. MEL solution was supplied with drinking water in the dark period, during gestation days 0 to 19. Dipalmitate salt of stobadine (STO), prepared by Štolc et al. (13), was dissolved in 0.5% methylcellulose. STO (50 mg/kg/day) was administered orally (0.5 ml/100 g) from day 2 to day 19 of gestation. The females were sacrificed on day 20 of gestation and uterine contents were inspected. All foetuses were examined for external and skeletal malformations. The data were evaluated by ANOVA and Fisher´s test. Values of $p \leq 0.05$ were considered significant.

RESULTS

No maternal death, abortions or dead foetuses occurred either in control and PHT groups, or in groups treated with MEL and STO. Drug administration resulted in a significant decrease of maternal body weight during gestation from day 15 to 20 for PHT and PHT+MEL groups, and from day 10 to 20 for PHT and PHT+STO groups (data not shown). A summary of the reproductive variables evaluated in pregnant rats is presented in Table 1 and 2. No external malformations appeared in the foetuses of any experimental group.

Pretreatment with MEL did not prevent decrease in foetal and placental weight. MEL significantly reduced post-implantation loss in both control and PHT groups. MEL also significantly decreased frequency of late resorptions in both control and PHT groups (Table 1). Types and frequency of skeletal anomalies are described in Table 2. However, MEL significantly increased the incidence of cervical vertebra anomalies in the control group, as well as anomalies in cervical vertebrae, ribs and sacral vertebrae in the PHT group.

STO, on the other hand, partially prevented PHT-induced significant reduction of foetal and placental weights (Table 3). Moreover, administration of STO increased the mean number of live foetuses and decreased the frequency of pre- and post-implantation loss and resorptions in the PHT group. STO also exerted a tendency to prevent the occurrence of skeletal anomalies in sternebrae, ribs and tail (Table 4), yet these findings were not significant.

DISCUSSION

The results of our study showed maternal and embryofoetal toxicity of PHT manifested by declined body weight gain of the dams and decreased foetal and placental weight as well as increased incidence of skeletal anomalies. MEL failed to have any effect on PHT induced maternal and embryofoetal toxicity. Administration of MEL alone, however, increased the incidence of cervical vertebrae anomalies. STO administration resulted in protective

***Table 1**. Effect of PHT and MEL administration on reproductive variables in rats*

Variables	Control (n = 17)	PHT (n = 17)	PHT+MEL (n = 20)	MEL (n = 15)
Corpora lutea	12.00 ± 0.98	13.29 ± 0.47	12.60 ± 0.41	14.47 ± 0.71
Implantation	10.53 ± 0.54	11.59 ± 0.69	12.05 ± 0.52	11.33 ± 073**
Live foetuses	9.47 ± 0.54	9.18 ± 0.84	11.20 ± 0.60	10.87 ± 0.79
Dead foetuses	0	0.29 ± 0.29	0	0
Sex ratio (M/F)	81/80	83/73	125/97	74/89
Preimplant. loss 1	1.47 ± 0.37	1.71 ± 0.65	0.55 ± 0.23	2.87 ± 0.96
Early resorptions	0.76 ± 0.29	1.41 ± 0.54	0.65 ± 0.31	0.47 ± 0.19
Late resorptions	0.29 ± 0.17	0.71 ± 0.37	0.30 ± 0.13	0.07 ± 0.07*
Postimplant. loss 2	1.06 ± 0.43	2.24 ± 0.54	0.95 ± 0.37*	0.47 ± 0.22*++
Foetal weight (mg)	3.45 ± 0.03	2.74 ± 0.03**	2.68 ± 0.03**++	3.16 ± 0.03**
Placental weight (mg)	0.48 ± 0.01	0.39 ± 0.01**	0.37 ± 0.01**++	0.46 ± 0.01**

n = number of dams, M - males, F - femalles, [1] - corpora lutea - implantation sites / corpora lutea (x 100), [2] - implantation sites - viable foetuses / implantation sites (x100) *p < 0.05, **p < 0.01 - compared to control, ++p < 0.01 - compared to PHT group (ANOVA).

***Table 2**. Effect of PHT and MEL administration on skeletal anomalies in rats*

Localisation	Control (n = 98)	PHT (n = 106)	PHT+MEL (n = 147)	MEL (n = 109)
Skull	0/98	0/106	2/147	0/109
Cervical vertebrae	7/98	2/106	22/147++	22/109**
Sternebrae	45/98	70/106	101/147	61/109
Ribs	17/98	15/106	11/147**	25/109
Vertebrae	1/98	8/106**	19/147**	2/109
Pelvis	0/98	1/106	1/147	3/109

n - number of foetuses inspected, *p < 0.05, **p < 0.01 - compared to control, ++p < 0.01 - compared to PHT group (Fisher´s exact test).

***Table 3**. Effect of PHT and STO administration on reproductive variables in rats*

Variables	Control (n = 17)	PHT (n = 17)	PHT+STO (n = 17)
Corpora lutea	12.65 ± 0.56	13.71 ± 0.64	13.18 ± 0.38
Implantation	11.47 ± 0.84	12.41 ± 0.75	11.82 ± 0.81
Live foetuses	10.12 ± 0.94	8.76 ± 0.89	9.65 ± 1.02
Dead foetuses	0	0	0
Sex ratio (M/F)	88/84	75/74	89/75
Preimplant. loss 1	1.18 ± 0.38	1.53 ± 0.46	1.35 ± 0.62
Early resorptions	0.29 ± 0.19	1.29 ± 0.46	0.88 ± 0.48
Late resorptions	1.06 ± 0.39	1.53 ± 0.72	0.65 ± 0.30
Postimplant. loss 2	1.35 ± 0.42	3.65 ± 1.12	2.18 ± 0.87
Foetal weight (mg)	3.26 ± 0.03	2.49 ± 0.03**	2.61 ± 0.04**+
Placental weight (mg)	0.52 ± 0.01	0.39 ± 0.004**	0.41 ± 0.01**+

n = number of dams, M - males, F – femalles[1] - corpora lutea - implantation sites / corpora lutea (x 100) [2] - implantation sites - viable foetuses / implantation sites (x100) **p < 0.01 compared to control, +p < 0.05 - compared to PHT group (ANOVA).

***Table 4.** Effect of PHT and STO administration on skeletal anomalies in rats*

Localisation	Control (n = 108)	PHT (n = 93)	PHT+STO (n = 103)
Skull	3/108	2/93	8/103
Sternebrae	53/108	80/93**	75/103*
Ribs	29/108	11/93*	7/103**
Vertebrae	1/108	8/93*	10/103*
Hind limbs	1/108	1/93	4/103
Tail	1/108	6/93	1/103

n - number of foetuses inspected, *p < 0.05, **p < 0.01 - compared to control (Fisher´s exact test).

effects manifested by increased foetal and placental weight and by beneficial influence on some reproductive variables. PHT is known as a human and animal teratogen affecting embryos by intrauterine hypoxia (4). The adverse effects of PHT are manifested, e.g. by hydrocephalus, hydronephrosis, cleft palate, peritoneal and renal haemorrhage (6, 14). The results of this study are consistent with our previous findings (7, 15) and are in good agreement with the PHT studies of other investigators (6).

Oxidative stress was found to be a risk factor for pregnant women (5, 16). Some antioxidants, e.g. allopurinol, α-tocopherol, SOD, were reported to be protective in preterm and term animals as well as in infants (17). Animal studies showed that supplementation of vitamin E and C to diabetic rats reduced foetal malformations and diminished oxygen radical level in the foetal liver (18). Finnel et al. (19) reported that cytochrome P-450, with ability to inhibit antiepileptic stiripentol, reduced the incidence of PHT induced congenital malformations in mice. On the other hand, Harbison and Becker (20) observed that concurrent administration of SKF525A intensified the adverse foetal effects of PHT in a mouse model.

In the present study, MEL failed to protect rat foetuses from PHT teratogenicity. Interestingly, MEL administration alone caused an improvement in some reproductive variables compared to controls (Table 1). However, MEL treatment resulted in skeletal anomalies in some regions. Yet, these anomalies are considered to be retarded skeletal ossification (21). Unlike MEL, STO had a positive effect on PHT induced embryofoetal toxicity (Table 3). STO has also been reported to protect HeLa cells from PHT cytotoxic effect (22). We concluded that pretreatment of pregnant rats with STO prevented to a certain extent embryofoetal toxicity caused by chronic intrauterine hypoxia evoked by PHT.

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