Teratological Study of the Antioxidant Stobadine in Rats

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Abstract. The potential teratogenicity of the antioxidant stobadine (STO) was studied in Wistar rats. Daily oral doses of 5, 15 and 50 mg/kg STO were given from the 6th day of gestation up to weaning of pups – day 21 post partum. No significant differences between the STO treated groups and the control group were found in litter size, pre- and postimplantation losses and foetal body weight. External, skeletal and internal examinations of the foetuses revealed no evidence of teratogenesis. The offspring from the STO treated dams exhibited a high survival rate in their postnatal development. It can be concluded that STO had no adverse effects on the pre- and postnatal development of the offspring in rats.

Key words: Antioxidant — Stobadine — Teratogenicity — Rat

Introduction

Oxidative stress represents an important risk factor of tissue and organ injuries to mothers and especially to the developing foetus (Allen and Balin 1989; Fantel 1996). The growing frequency of injuries induced by oxidative stress acting during pre- and perinatal development (Grabowski 1977; Amiel-Tison and Ellison 1986; Fantel 1996) has resulted in the need to search for new drugs with marked preventive antioxidant effects on the developing foetus under hypoxia-ischaemia conditions. Recent extensive studies have confirmed that stobadine (STO, cis-(-)-2,3,4,4a,5,9b-hexahydro-2,8-dimethyl-1H- pyrido-[4,3b]indole (CAS No. 95751-51-2) (Chemical Abstracts 1987) is to be classified as one of the most effective synthetic antioxidants with neuro- and cardioprotective properties, exerting a high free-radical scavenging capacity (Orviský et al. 1997; Horáková and Štolc 1998). Preclinical safety evaluation of STO was performed in rats (Gajdošíková et al. 1995) and beagle dogs (Majerčík et al. 1984) and no adverse effects were detected. Single intravenous injection of STO to ICR mice and repeated oral administration produced no evidence of teratogenicity (Ujházy et al. 1994). The protective effect of STO against cyclophosphamide (CPA) mutagenicity was confirmed in foetuses of CPA treated

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female ICR mice (Chorvatovičová and Ujházy 1995). The purpose of this study was to determine the effects of STO administered during pregnancy and lactation on the developing rat foetus.

Materials and Methods

Animals

Virgin female SPF Wistar rats (No = 108, aged 3–4 months, weight 200–220 g), obtained from the Breeding Facility IEP SAS Dobrá Voda, Slovakia, were used The animals were housed under standard conditions Food and tapwater were provided *ad libitum* After 7 days of adaptation, the females were mated with males (presence of spermatozoa in vaginal smear indicated day 0 of gestation)

Drugs

STO was prepared by *Štolc et al* (1983) in the Institute of Experimental Pharmacology SAS, Bratislava, Slovakia STO in the form of dipalmitate salt DP 1031 (m w 715 2, 99 5% purity) was dissolved in 0 5% methycellulose (Methocel, MC 4000 cP, Fluka AG, Busch SG, Switzerland) at a constant dosage volume of 0 5 ml/100 g body weight The dams were treated by oral gavage with STO in single doses of 5, 15 and 50 mg/kg/d from the 6^{th} day of gestation up to weaning of pups – day 21 post partum Controls received as vehicle 0 5% MC over the same period

Teratological examination

The group of 73 pregnant rats (18 controls, 55 STO) was followed-up concerning body weight and clinical signs of toxicity till day 20 of pregnancy, when they were killed by cervical dislocation The peritoneal cavity and uterus were opened, and the number of corpora lutea, implantations, resorptions, live and dead foetuses were recorded The live foetuses were removed from the uterus and their wet weight recorded All foetuses were inspected for external malformations Two-thirds of foetuses from each litter were exsanguinated, eviscerated, stripped of most subcutaneous tissue, fixed in 96% ethanol and then cleared and stained with alizarin red S for examination of the skeleton (Lorke 1977) The remaining foetuses were fixed for soft-tissue examination by the razor section technique (Wilson 1965)

The other 35 pregnant rats (10 controls, 25 STO) were allowed to deliver spontaneously The day of delivery was designated as day 0 after birth At birth, the numbers of live and stillborn pups were recorded The offspring were followed-up till weaning (postnatal day 21), with body weight monitoring every week

Statistical evaluation

The data were analysed by means of ANOVA and Fisher's exact test (skeletal and visceral abnormalities) The data are expressed as mean ± S E M

Results

No maternal death, abortion and dead foetuses occurred either in the control or experimental groups. No adverse effects on embryo-foetal development were found (Table 1). The incidence of skeletal and visceral anomalies of rat foetuses is presented in Table 2. Significantly increased anomalies of sternebrae were found in the

Table 1. Effect of oral administration of STO on embryofoetal development in rats

Days of treatment	Dose (mg/kg)	No. of dams	Litter size[1]	Foetal loss (%)		Foetal weight (g)
				Pre-impl.[2]	Post-impl.[3]	
6 dg – 21 pp	Control	18	11.17 ± 0.54	7.36 ± 2.38	2.76 ± 0.97	3.57 ± 0.02
	5	16	10.69 ± 0.44	12.96 ± 2.30	6.68 ± 2.40	3.39 ± 0.02
	15	17	11.00 ± 0.45	12.14 ± 2.98	2.31 ± 1.12	3.17 ± 0.02
	50	22	11.45 ± 0.61	11.76 ± 3.66	6.24 ± 1.87	3.50 ± 0.02

Pre-impl. – preimplantation loss, post-impl. – postimplantation loss, dg – day of gestation, pp – post partum, [1] – term live foetuses, [2] – corpora lutea – implantation sites/corpora lutea (×100), [3] – implantation sites – viable foetuses/implantation sites (×100)

Table 2. Skeletal and visceral anomalies of foetuses after oral administration of STO

Parameters	Control	S T O		
		5	15	50 (mg/kg)
No. of examined foetuses/litter	134/18	111/16	119/17	166/22
	Skeletal anomalies			
Skull[1,a] (%)	3 (2.24)	5 (4.50)	2 (1.68)	3 (1.81)
Sternebrae[2,a] (%)	18 (13.43)	25 (22.50)	33 (27.73**)	24 (14.46)
Ribs[3,a] (%)	47 (35.07)	18 (16.22)	27 (22.69)	45 (27.11)
Pelvic[4,a] (%)	0	2 (1.80)	0	2 (1.20)
Forefoot[5,a] (%)	0	0	0	1 (0.60)
Vertebrae[6,a] (%)	0	0	1 (0.84)	0
No. of examined foetuses/litter	67/18	60/16	68/17	86/22
	Visceral anomalies			
DRP[7,b] (%)	13 (19.40)	17 (28.33)	12 (17.65)	24 (27.91)
TA[8,b] (%)	0	0	1 (1.47)	0

[1] – delayed ossification of the parietal, interparietal and supraoccipital bone, [2] – unossified and retarded sternabrae, [3] – 14th thoracolumbar rudimentary and wavy ribs, [4] – incomplete ossification of the pelvic bone, [5] – retarded ossification of the metacarpals, [6] – incomplete ossification of the caudal vertebrae, [7] – dilatation of renal pelvis, [8] – testicular anomalies. [a] – number of skeletal anomalies, [b] – number of visceral anomalies, ** Significantly different from control, $p < 0.01$ (Fisher's exact test)

group given 15 mg/kg STO. Concerning visceral anomalies, dilatation of the renal pelvis and testicular malposition were recorded, yet with no apparent relationship to STO treatment.

Table 3. Postnatal growth of offspring from STO treated dams

Parameters	Control	STO		
		5	15	50 (mg/kg)
No. of litters	10	6	9	10
Gestation period (day)	22.2 ± 0.13	22.5 ± 0.22	22.1 ± 0.11	22.2 ± 0.13
No. of live pups/litter	10.1 ± 1.27	11.17 ± 1.38	11.11 ± 0.68	10.30 ± 0.65
Viability index (%)[1]				
Day 1	100	100	100	100
Day 4	99.0	97.0	97.0	97.1
Day 7	99.0	97.0	97.0	97.1
Day 21	99.0	97.0	97.0	97.1
Body weight of offspring (g)				
Day 21 – Male	38.11 ± 1.09	37.69 ± 1.68	37.14 ± 0.80	38.87 ± 0.53
– Female	35.87 ± 0.55	37.34 ± 1.57	38.65 ± 0.77**	37.59 ± 0.59*

[1] – number of live pups on days 1, 4, 7 and 21/number of live pups on day 1 (×100), ** $p < 0.01$, * $p < 0.05$ (significant difference compared to control).

The data concerning observation of dams and postnatal growth of their offspring up to day 21 of age are shown in Table 3. No significant differences were found between the STO treated groups and controls in the number of live newborns per litter. The survival rate of the offspring during weaning expressed by means of viability index was very high and almost constant in all experimental groups. On day 21 of age, the body weight of female offspring was significantly higher in the 15 and 50 mg/kg STO group compared to controls (Table 3). The results regarding neurobehavioural development of pups and sexual maturation are presented by *Dubovický et al.* (1999).

Discussion

Administration of STO to pregnant rats from day 6 of gestation up to day 21 post partum had no unfavourable effects on the course of gestation, delivery, pre- and early postnatal development of offspring.

As for prenatal development of rat foetuses, STO did not cause any significant changes of litter size, pre- and postimplantation loss and foetal body weight. These findings suggest that STO has no adverse effects on the prenatal growth of the rat offspring. The sporadic incidence of skeletal variations, delayed ossification and dilatation of the renal pelvis, are not uncommon in teratological studies even in control groups (Palmer 1972, 1977; Kimmel and Wilson 1973; Manson and Kang 1989). Consequently, the incidence of some minor skeletal anomalies detected in our study may be regarded as common skeletal variations, representing merely transient developmental changes (Schardein 1987). Thus, STO does not appear to exert teratogenic activity in rats. The lack of teratogenicity observed in this study

is consistent with the results of our teratogenic study in mice (Ujházy et al. 1994) and chick embryos (Mihálikova et al. 1993). In the postnatal period, a high survival rate of the offspring from the dams given STO was recorded.

It can be concluded that oral administration of STO in the doses of 5, 15 and 50 mg/kg/d to female rats during the period of gestation up to weaning of pups on postnatal day 21 had no adverse effects on the prenatal and early postnatal development of the rat offspring

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