Neuro-Immuno-Teratogenicity of Drugs Used in Neonatal Pharmacotherapy in Relation to the Ontogenic Stage at the Time of Their Administration

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Abstract. The risk of functional teratogenicity of two drugs used in neonatal pharmacotherapy was studied indomethacin (INDO) and dexamethasone (DEX) Model experiments were carried out in Wistar strain rats, breed Konárovice, which received single subcutaneous drug injection (INDO 2 mg/kg, DEX 1 mg/kg) on postnatal day 4 (PD 4, model of human fetus/preterm newborn of 6-7-month-gestational age) or on postnatal day 9 (PD 9, model of full-term human neonate) The rats were followed up during development (body weight, maturation) till late adulthood (age 6-8 months) using tests of cognition, immune reactivity and biochemical brain analysis The results evaluated by comparing treated and control litter-mates indicated that the functional teratogenic risk was significantly higher in DEX than in INDO DEX-rats revealed disorganization of developmental processes retardation of body growth, but acceleration of sensory development (pinna and eye opening), retarded male sexual maturation Adult DEX-rats (age 6 months) of both series (PD 4, PD 9) had deficit of short-term memory (social recognition test) Disturbances of immune reactivity (decrease of humoral and rise of cell-mediated immune response) appeared both in adult INDO and DEX-rats (age 7 months), but only in the PD 9 series i e when the drugs were administered at a higher stage of the ontogenic development simulating neonatal period in humans This finding may be warning from the clinical point of view for the neonatological practice

Key words: Drug teratogenicity — Perinatal pharmacotherapy — Indomethacin — Dexamethazone — Immunotoxicity

Introduction

Drugs administered for perinatal treatment of pregnancies-at-risk and neonates-at-risk may interfere with the gene controlled, time and space determined programme

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of developmental processes and initiate disorganization of perinatal ontogenesis which is characterized by intensive cytodifferentiation and receptor formation in the already shaped organs (e.g. brain, immune and endocrine system). These disturbances on the cellular/subcellular level are not evident at birth, but form the basis for functional defects of mentioned organs (functional teratogenicity) which become manifest gradually during further development and maturation as various neuro-psycho-behavioural deviations or disorders of immunocompetence (Benešová 1995). The present study evaluates – in model experiments in rats – the risk of functional teratogenicity of two drugs used in obstetrics and neonatology: **indomethacin** given to newborns-at-risk to prevent intraventricular hemorrhage or to accelerate the closure of patent ductus arteriosus (Gersony et al. 1983; Hanigan et al. 1988; Ment et al. 1988) and **dexamethazone** prescribed to induce surfactant production in immature fetal/neonatal lung and thus to prevent/treat the neonatal respiratory distress syndrome (Liggins and Howie 1972; Soll and McQueen 1993).

Since the tested drugs are applied clinically both to full-term neonates and to prematures of various gestational age the study focused on the question whether drug administration at different stages of perinatal ontogenesis could change the quality or quantity of the functional teratogenic risk. Comparative developmental studies in several species of laboratory animals and man (Dobbing 1973, Romijn et al.1991) have shown that – concerning brain ontogenesis – neonatal rat is comparable with the human fetus at month 6 of gestation or with the preterm newborn of the same gestational age, whereas only 9–12-day-old rat pups may represent the model for the full-term human neonate. Consequently, in the presented model experiments in rats, the tested drugs were administered to rat pups as a single subcutaneous injection of a clinically relevant dose either on the fourth or on the nineth postnatal day The animals were followed up during their development and maturation till late adulthood (age 6–8 months) using tests of behaviour, immune reactivity and brain biochemical analysis.

Materials and Methods

Experiments were carried out in Wistar strain rats, breed Konárovice Drugs were administered in clinically relevant doses (indomethacin – INDO 2 mg/kg, dexamethazone – DEX 1 mg/kg) as a single subcutaneous injection to neonatal rat pups on postnatal day 4 (PD 4) or postnatal day 9 (PD 9) One half of every 10-member litter received the tested drug, the other half received saline (control group) The pups were weaned on day 28, divided according to gender, tattooed, housed in plastic cages in groups of 5, later on of 2 animals, and maintained under standard laboratory conditions in naturally lit room with detailed records of parentage, age, health condition etc till the age of 8 months In the course of the first seven weeks, following developmental landmarks were registered body weight, incisor eruption, pinna and eye opening, sexual maturation in females (vagina opening) and males (preputium loosening) In adult male rats at the age of 6-8 months, cognitive behaviour, immunoreactivity and brain biochemical analysis were evaluated

Social recognition test ("social memory")

Modified method Thor and Holloway 1982 Three 5-min-sessions (interval 45 min) with

the exposition of one rat pup (age 20-26 days) in first two sessions and with an other pup in the third session Elements of approach behaviour (sniffing, touching, following the pup) based predominatly on the olfactory discrimination (the main sensory analyzer in rats) were checked and the difference between the social interest for the familiar and for the novel pup was evaluated The behaviour of rats was videotaped The duration of the mentioned behavioural elements were registered using "ACTIVITY" software for PC and evaluated by statistical programs BMDP

Immune function tests

a) Humoral immune response – antibody production against ovalbumin
b) Cell-mediated immune response – delayed type of hypersensitivity (DTH) to bovine serum albumin

Brain biochemical analysis

After decapitation, brains were quickly removed and – using an ice cooled plate – dissected in 4 parts (cortex, hippocampus, hypothalamus, striatum) The following biochemical markers were determined

a) Markers for monoaminergic transmission – concentration of noradrenaline (NA), dopamine (DA), serotonin (5-HT) and their metabolites (dihydroxyphenylacetic – DOPAC, homovanilic – HVA, 5-hydroxyindoacetic acids – 5-HIAA) in the striatum and the hypothalamus (high performance liquid chromatography asssay with electrochemical detection)

b) Marker for cholinergic neuronal activity – high affinity choline uptake (HACU) in the hippocampus (method according to Krištofiková et al 1992)

c) Marker for free radical induced neuronal damage – lipid peroxidation (LPO) in the cortex and the hippocampus (method according to Ohkawa et al 1979)

Results

The preweaning development and maturation was monitored in 210 rat pups from PD 4 series and in 160 from PD.9 series. The comparison of body growth in control pups and their treated litter-mates indicated no differences in INDO-rats of both series DEX-pups revealed significant growth retardation in the first two weeks after the drug injection, especially in PD·9 series, but, at weaning, their body weight attained normal values In contrast to this body growth deceleration, the sensory development (pinna and eye opening) in DEX-pups of both series was significantly accelerated. On the other hand, male sexual maturation was retarded (with significant difference in PD.9 series), thus indicating gross disorganization of developmental processes induced by DEX administration at the neonatal age (Table 1) The impact of INDO on the development and maturation was slight, evident only in preweaning rats as minor retardation of eye opening.

In adult animals at the age of 6 months, disturbance of cognitive behaviour (social memory test) was found in DEX-rats of both series, whereas in INDO-rats, only some insignificant deficit appeared in PD:9 series (Table 2). Administration of both INDO and DEX in the neonatal period resulted in disturbances of adult immune reactivity (decrease of humoral and increase of cell-mediated immune response), but only in PD:9 series (Table 3) Neurobiochemical analysis of adult

Table 1. Development and maturation of rats administered INDO, DEX or saline (control) on postnatal day 4 (PD 4) or postnatal day 9 (PD 9)

Series		PD:4			PD:9		
Age postnatal day	Developmental landmark	CONTROL	INDO	DEX	CONTROL	INDO	DEX
14	eye opening (m+f)	26/97	6/52	53/60	8/62	3/52	14/44
		27%	**12%**	**88%**	**13%**	**6%**	**32%**
			**			*	
40	preputium loosening (m)	49/61	25/35	25/37	29/30	32/32	15/30
		80%	**71%**	**68%**	**97%**	**100%**	**50%**
						**	

The results are expressed as the ratio [No of rats with the landmark/total No of rats] or as percentages of positive events m=males, f=females * $p < 0\ 05$, ** $p < 0\ 01$

Table 2. Social memory in adult male rats (age 6 months)

PD:4			PD:9		
CONTROL	INDO	DEX	CONTROL	INDO	DEX
4/8	3/6	0/6	5/7	3/7	1/7
50%	**50%**	**0%**	**71%**	**43%**	**14%**
	*			*	

The results are expressed as the ratio [No of rats with correct reaction/total No of rats] or as percentages of correct reactions

animal brains (Table 4) indicated no differences from controls when INDO or DEX were applied on PD:4. At the higher stage of ontogenic development (i.e PD:9), DEX induced several deviations which might signify impaired functioning: rise of lipid peroxidation in the cortex (+20%, $p < 0.01$), increased dopamine turnover in the hypothalamus (+46%, $p < 0.01$), lower cholinergic neuronal activity in the hippocampus. On the other hand, hippocampal cholinergic activity (HACU) in INDO-rats was slightly increased.

Table 3. Immunoreactivity in adult male rats (age 7 months)

Series	PD:4		PD:9	
Immune response	INDO	DEX	INDO	DEX
Humoral (antibody production)	(=)	(=)	−34 %**	−35 %*
Cell-mediated (DTH)	+25 %	(=)	+64 %•	+108 %*

N = 13–14 (per drug group) The results are expressed as percentages of increase (+) or decrease (−) or no difference (=) in comparison with controls • $p < 0.07$, *$p < 0.05$, **$p < 0.01$

Table 4. Brain biochemical analysis in adult male rats (age 7–8 months)

Series	PD:4		PD:9	
Assay	INDO	DEX	INDO	DEX
CORTEX				
lipid peroxidation	(=)	+17 %	(=)	+20 %**
HIPPOCAMPUS HACU	(=)	(=)	+26 %	−25 %
HYPOTHALAMUS				
dopamine (DA)	(=)	(=)	(=)	−21 %
DOPAC	(=)	(=)	(=)	(=)
homovanilic acid (HVA)			+24 %	+21 %•
HVA+DOPAC / DA			+21 %	+46 %**
STRIATUM				
serotonin (5-HT)	(=)	(=)	+9 %	+9 %
5-HIAA	(=)	(=)	+11 %*	+10 %*
5-HIAA / 5-HT	(=)	(=)	(=)	(=)

For further description, see Table 3

Discussion

Model experiments in rats simulating the perinatal administration of INDO or DEX in the treatment of high-risk neonates of different gestational age indicated significant differences in the functional teratogenic risk not only between both tested drugs, but also in relation to the ontogenic stage at the time of the drug application.

As far as differences between both drugs are concerned, experimental results from the follow-up of development and maturation, as well as from the tests of adult behaviour and neurobiochemical brain analysis, showed weaker functional teratogenic effects of neonatal INDO administration. DEX induced significant disturbances already in preweaning development and maturation of rat pups showing a contrast between body growth retardation on one hand and acceleration of the sensory development (pinna and eye opening) on the other hand, in both series of experiments (i.e. PD:4 and PD:9). It is worth of mentioning that this early developmental discrepancy was described already in our previous study when DEX was applied on PD:7 (Benešová and Pavlík 1989). Additionally, the present eperiments showed that sexual maturation of male DEX-rats was significantly retarded. This mozaic of developmental acceleration as well as retardation seems to be typical for neonatal use of DEX since inadequate activation of glucocorticoid receptors may result both in precocious induction and premature termination of important ontogenic processes (e.g. cell proliferation and differentiation).

Late behavioural consequences of single neonatal administration of tested drugs were evaluated in adult male rats (age 6 months). Significant deficit of memory (social recognition test) was found in adult DEX-rats of both experimental series i.e. PD:4 and PD:9. This finding is in agreement with our previous results (Benešová and Pavlík 1989) which indicated cognitive impairment in rats after DEX administration on PD:7, using various tests of learning and memory (active and passive avoidance, food rewarded appetitive learning). In adult INDO-rats, only a slight insignificant decrease of social memory appeared in PD:9 series.

The most interesting results were found in testing immunoreactivity of adult INDO and DEX animals. Both drugs induced significant deviations of immune reactivity (in terms of a decreased humoral and increased cell-mediated immune response), but only after drug administration at the higher ontogenic stage (i.e. PD:9). A similar dependence of late immunological disturbances on the stage of ontogeny at the time of drug administration was found in our previous study with diazepam (Benešová et al. 1997). Diazepam (10 kg/kg) applied on PD:9-10 induced significant decrease of cell-mediated immune response in adult male rats, but the same diazepam dose applied on PD:4-5 resulted in no late immune defects. These results with three different drugs may lead to the conclusion that the risk of immunoteratogenicity is higher when the drug is applied in more advanced ontogenic phase, corresponding in rats to postnatal days 9–10 (model of full-term human neonate).

Disturbances of brain biochemical markers in adult rats administered neonatally with the tested drugs appeared – similarly as in tests of immunoreactivity – only in PD:9 series i.e. when the drugs were applied at the higher ontogenic stage. In adult DEX-rats, several significant deviations were found the character of which may be interpreted as impaired functioning (rise of lipid peroxidation in the cortex, increased dopamine turnover in the hypothalamus, lower cholinergic activity in the hippocampus). On the other hand, INDO-rats revealed an increase of hippocampal cholinergic activity which might be considered as a positive impact.

Conclusion

Model experiments with rats showed that the functional teratogenic impact of perinatal pharmacotherapy is highly dependent on the ontogenic stage at the time of the drug administration. The finding concerning the risk of significantly disturbed adult immunoreactivity when the tested drugs (indomethacin, dexamethazone, diazepam) were applied at a higher stage of ontogeny (PD:9 – model of human neonatal period) may be warning from the clinical point of view for neonatal therapy practice.

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The Influence of α-Lipoic Acid on the Toxicity of Cadmium

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Abstract. α-Lipoic acid (α-LA) is an important antioxidant drug with chelating properties In experiments performed in male mice (CD-1, Charles River) the effects of cadmium on lipid peroxidation (LP), GSH level, the activity of catalase and glutathione peroxidase (GSH-Px) in liver homogenates were studied Mice were injected with $CdCl_2$ 2 5 H_2O at a dose of 40 μmol kg^{-1} s c α-LA was administered simultaneously i p at the dose corresponding to α-LA-to-Cd molar ratio of 5 1 The experiments were completed at 24h Cadmium increased LP to 200 7% of controls This effect was prevented by α-LA treatment ($p \leq 0$ 05) GSH level was decreased to 81 7% of controls and it was not affected by α-LA GSH-Px activity diminished by Cd administration was corrected by α-LA ($p < 0$ 001) Catalase activity decreased by Cd remained unaffected The administration of α-LA alone enhanced LP and the activity of catalase As estimated by AAS, Cd content in the liver, the kidneys, the brain and the testes remained unaffected by α-LA treatment In the acute toxicity experiment, the mortality associated with cadmium was decreased by α-LA administration The results suggest that the toxicity of Cd was decreased mainly by the antioxidant activity of α-LA rather than by cadmium removal from tissues

Key words: Cadmium — α-Lipoic acid — Oxidative status

Introduction

α-Lipoic acid (α-LA) [6,8-thioctic acid, 1,2-dithiolane-3-pentanoic acid] is a naturally occurring substance It is present in all kinds of prokaryotic and eukaryotic cells and functions as a cofactor in the multienzyme complexes that catalyze the oxidative decarboxylation of α-keto acids α-LA is a potent antioxidant, and it has been shown to be an efficient chelator of several metals Lipoic acid β-oxidation products (bisnorlipoate and tetranorlipoate) and dihydrolipoic acid (DHLA), a reduced form of lipoic acid, contribute to the antioxidant activity of α-LA DHLA is a potent antioxidant but by virtue of its ability to chelate and reduce iron it also shows pro-oxidant activity (Biewenga et al 1997, Packer et al 1996, 1997)

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