PHARMACOLOGY AND TOXICOLOGY

Effect of Afobazole on Teratogenic Activity of Cyclophosphamide in Rats

O. V. Shreder, N. M. Smolnikova, A. D. Durnev, and S. B. Seredenin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 4, pp. 414-417, April, 2008
Original article submitted December 19, 2007

A new anxiolytic afobazole (1-100 mg/kg perorally, Russia) dose-dependently abolished the embryotoxic and teratogenic effects of cyclophosphamide and reduced the range of induced malformations in outbred albino rats. Our results suggest that afobazole possesses antiteratogenic activity.

Key Words: *teratogenesis; rats; cyclophosphamide; afobazole*

Much attention is paid to screening for teratogens and studying of the teratogenic effect of large-scale factors, including alcohol, tobacco smoke, and drugs [2,7,10,12]. However, little is known about pharmacological modification of teratogenesis. There are only individual studies on this problem [11].

A possible mechanism of teratogenesis is oxidative stress and associated cytotoxic, mutational, and genotoxic effects [1,10,12]. Hence, a variety of drugs with antimutagenic and antioxidant properties are screened for their ability to modulate teratogenesis.

A new anxiolytic afobazole (Russia) exhibits antimutagenic properties. Afobazole in various doses reduces or prevents the cytogenetic and genotoxic effects of medicinal mutagens dioxidine and cyclophosphamide (CPA) [7,8]. Moreover, this preparation possesses cytoprotective properties [6].

Here we studied the effect of afobazole on teratogenesis in rats induced by cytostatic CPA.

V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** adurnev@aport.ru. A. D. Durnev

MATERIALS AND METHODS

Experiments were performed on outbred albino rats weighing 200-250 g. The animals were obtained from Stolbovaya nursery. The day of spermatozoon detection in vaginal smears was considered as the 1st day of pregnancy. The rats received pelleted food, vegetables, and water and were maintained under natural light/dark cycle.

Afobazole (2-[2-(morpholino)ethylthio-5-ethoxybenzimidazole hydrochloride) was synthesized at the V. V. Zakusov Institute of Pharmacology. Afobazole in doses of 1, 10, and 100 mg/kg was given perorally. These doses were selected taking into account the fact that afobazole in a dose range of 1-10 mg/kg has maximum anxiolytic and antimutagenic activity. Afobazole was also administered in high dose (100 mg/kg) to exclude inversion of the effect due to antimutagen overdosage and to evaluate the dose—effect relationship [4,5].

CPA in a dose of 20 mg/kg was injected intraperitoneally. The dose of CPA was selected from published data [8] and results of our previous experiments. Fetal death after administration of the teratogen in this dose (100% teratogenic effect) did not exceed that in the control group.

The animals were treated with afobazole and teratogen at a 15-min interval.

Pregnant rats were divided into 5 groups. CPA was injected to group 1 animals on day 14 of pregnancy. Group 2, 3, and 4 rats perorally received afobazole in doses of 1, 10, and 100 mg/kg, respectively, on day 14 of pregnancy. These rats received intraperitoneal injection of CPA 15 min after administration of afobazole. Physiological saline was given perorally to control animals on day 14 of pregnancy.

The rats were killed by cervical dislocation on day 20 of pregnancy. The uterus was opened. The number of corpora lutea in the ovaries and the number of dead, live, and abnormal fetuses were determined. Preimplantation death was evaluated from the difference between the numbers of corpora lutea and implantation sites (percentage of the total number of corpora lutea). Postimplantation death was estimated from the difference between the numbers of implantation sites and live fetuses (percentage of implanted embryos). The fetuses were thoroughly examined. External abnormalities were recorded. The fetuses were weighted. The craniocaudal size was estimated. Some embryos were fixed in Bouin's fluid and subjected to a microanatomical study for the state of internal organs (method of Wilson—Dyban). Other embryos were fixed in 96° alcohol, stained with alizarin, and studied by a modified Dawson method [3]. Abnormalities of the skeleton, mean number of ossification centers in the metacarpus, metatarsus, spinal column, and sternum (per embryo), and skull abnormalities were recorded.

The significance of intergroup differences was evaluated by the parametric analysis of variance (Newman—Keuls test, normal distribution) and Student's *t* test (paired comparison of embryotoxic parameters). All embryos were included in the study. The individual litter was considered as a statistical unit.

RESULTS

Table 1 shows the effect of afobazole on embryotoxic activity of CPA. Administration of afobazole to pregnant females with CPA-induced injury was followed by a significant dose-dependent increase in the length and weight of fetuses (compared to teratogen-treated animals). Fetal death after CPA injection did not exceed the control level, which corresponded to the experimental model of teratogenicity [8] (Table 1). Hence, we studied the effect of afobazole on teratogenicity of CPA.

Macroscopic study showed that nearly all embryos of CPA-treated females have malformations. Multiple abnormalities were revealed, including cranioschisis, meningoencephalocele, exophthalmos, protrusion of the tongue, eventration, teratomas, and severe anomalies of the forelimbs and hindlimbs (acheiria, apodia, and hypodactyly). CPA-induced abnormalities (Fig. 1) are similar to those described previously [8,9].

The incidence of external malformations was lower in embryos from females of the afobazole+ teratogen group (Table 2). Afobazole in doses of 1 and 100 mg/kg completely abolished the development of several abnormalities (*e.g.*, microcephaly, micromyelia, acheiria, apodia, eventration, and hypodactyly of forelimbs). Besides this, afobazole reduced the severity of several serious malformations. For example, the number of fetuses with acheiria (absence of hands) and apodia (absence of feet) decreased in all groups of afobazole-treated animals.

The number of serious abnormalities in animals decreased by 2, 4, or more times after administration of afobazole.

Studying the state of internal organs in fetuses of CPA-treated rats revealed abnormalities of the brain, palatine processes, kidneys, and heart. The number of embryos with developmental abnormalities of internal organs decreased after admini-

TABLE 1. Effect of Afobazole (AF) on Embryotoxic Activity of CPA

Group	Number of corpora lutea per female	Number of implantation sites per female	Preimplantation death, %	Number of live fetuses per female	Postimplantation death, %	Mean weight of fetuses, g	Mean length of fetuses, cm
Physiological saline	11.25±0.62	10.00±0.57	11.11	9.50±0.57	5.00	2.53±0.24^{+}	3.26±0.14^{+}
CPA, 20 mg/kg	11.40±0.51	10.60±0.68	7.02	10.20±0.86	7.55	1.57±0.04*	2.56±0.03*
CPA+AF, 1 mg/kg	13.00±0.68	11.0±0.65	10.77	11.10±0.75	4.31	1.76±0.03*$^{+}$	2.70±0.03*$^{+}$
CPA+AF, 10 mg/kg	11.56±0.27	10.38±0.41	10.27	9.88±0.34	4.82	1.93±0.04*$^{+}$	2.82±0.03*$^{+}$
CPA+AF, 100 mg/kg	12.14±0.44	10.79±0.61	12.35	10.29±0.57	4.64	2.09±0.03*$^{+}$	2.99±0.02*$^{+}$

Note. $p<0.05$: *compared to the control; $^{+}$compared to the teratogenic group (CPA, 20 mg/kg).

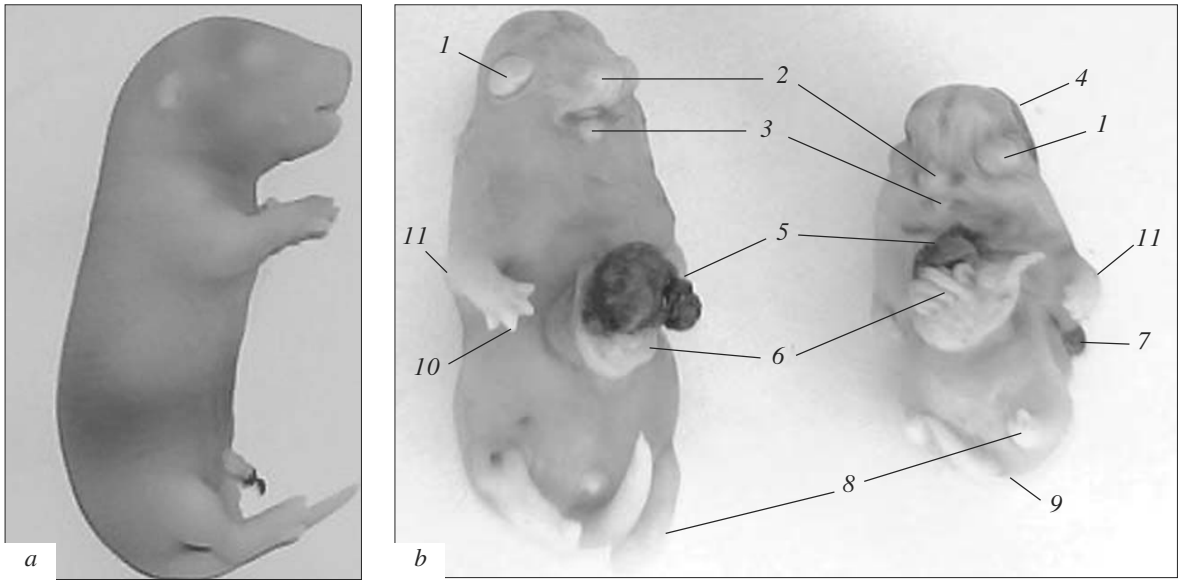

Fig. 1. Pathological effect of CPA on rat embryos. Control (*a*) and CPA-treated embryos (*b*). Exophthalmos (*1*); hypognathus (*2*); micrognathia (*3*); meningoencephalocele (*4*); eventration (extrusion of viscera outside the body through an abdominal wall defect, *5*); intestinal loops (*6*); teratoma (*7*); apodia (*8*); abnormal shape of the tail (*9*); hypodactyly and brachydactyly (*10*); and curvature of the limbs (*11*).

stration of afobazole. Afobazole in doses of 1 and 100 mg/kg was most effective.

Afobazole dose-dependently decreased the incidence of serious abnormalities of the skeletal system (Table 3). Afobazole in doses of 1 and 10 mg/kg significantly decreased the number of fetuses with such serious defects as acrania, dysmorphism of the jaw, scoliosis, and spina bifida (by 4-8 times compared to the teratogenic group). Afobazole in a dose of 100 mg/kg decreased the number of embryos with acrania and dysmorphism of the jaw to 1.4%. Scoliosis and spina bifida were not observed in the afobazole group.

Our results indicate that afobazole in doses of 1-100 mg/kg dose-dependently decreases the incidence of embryotoxic injuries, abolishes the terato-

TABLE 2. Effect of AF on Teratogenic Activity of CPA (Developmental Abnormalities)

Abnormality	CPA, 20 mg/kg (*n*=51)		CPA+AF, 1 mg/kg (*n*=111)		CPA+AF, 10 mg/kg (*n*=158)		CPA+AF, 100 mg/kg (*n*=144)	
	abs.	%	abs.	%	abs.	%	abs.	%
Cerebral hemorrhage	38	75	66*	59	35*	22	14*	10
Meningoencephalocele	51	100	30*	27	63*	40	26*	18
Cranioschisis	51	100	62	56	101	64	69	48
Microcephaly	29	57	0*	0	44*	28	0*	0
Exophthalmos	36	71	6*	5	41*	26	15*	10
Micrognathia	13	25	7*	6	25	16	1*	1
Hypognathus	21	41	6*	5	24*	15	9*	6
Protrusion of the tongue	15	29	4*	4	16*	10	19*	13
Micromyelia	41	80	0*	0	15*	9	0*	0
Eventration	14	27	5*	5	25	16	0*	0
Teratoma	45	88	23*	21	54*	34	30*	21
Forelimb injury	16	31	14*	13	5*	3	0*	0
Hindlimb injury	44	86	79*	71	78*	49	34*	24

Note. *n*, number of examined fetuses. Here and in Table 3: *$p<0.05$ compared to the teratogenic group (CPA, 20 mg/kg).

TABLE 3. Effect of AF on CPA-Induced Abnormal Development of the Skeletal System

Parameter	CPA, 20 mg/kg (*n*=28)	CPA+AF, 1 mg/kg (*n*=46)	CPA+AF, 10 mg/kg (*n*=69)	CPA+AF, 100 mg/kg (*n*=74)
Acrania,	100	13*	26*	1.4*
Dysmorphism of the jaw, %	92.7	15*	26*	1.4*
Absence of the occipital bones, %	100	15*	16*	26*
Scoliosis, %	71.4	19.8*	16*	0*
Spina bifida, %	78.6	28*	11.6*	0*
Bifurcation of parasternal sites	42.7	32.6	5.8*	1.4*

genic effect of CPA, and reduces the range of induced malformations, i. e. possesses antiteratogenic activity, which is consistent with cytoprotective [6] and antimutagenic properties of this compound [4,5].

REFERENCES

1. A. D. Durnev and S. B. Seredenin, *Muta\gens: Screening and Pharmacological Prevention of the Influence* [in Russian], Moscow (1998).
2. A. D. Durnev, N. M. Smolnikova, E. P. Nemova, *et al*., *Eksper. Klin. Farmakol*., **66**, No. 6, 32-35 (2003).
3. A. P. Dyban, V. S. Baranov, and I. M. Akimova, *Arkh. Anat*., **10**, 89-100 (1970).
4. A. K. Zhanataev, A. D. Durnev, and S. B. Seredenin, *Eksper. Klin. Farmakol.*, **63**, No. 2, 57-59 (2000).
5. A. K. Zhanataev, A. D. Durnev, and S. B. Seredenin, *Byull. Eksp. Biol. Med.*, **130**, No. 11, 539-542 (2000).
6. T. A. Zenina, I. V. Gavrish, D. S. Melkumyan, *et al*., *Ibid*., **140**, No. 8, 161-163 (2005).
7. N. M. Smol'nikova, B. I. Lyubimov, A. D. Durnev, *et al*., *Manual on Experimental (Preclinical) Study of New Pharmacological Agents*, Ed. R. U. Khabriev [in Russian], Moscow (2005), pp. 87-100.
8. N. A. Chebotar' and A. M. Kotin, *Farmakol. Toksikol*., No. 3, 374-384 (1984).
9. A. M. Chernukh and P. N. Aleksandrov, *On the Teratogenic Effect of Chemical (Medicinal) Agents* [in Russian], Moscow (1969).
10. S. Gupta, A. Agarwal, J. Banerjee, and J. G. Alvarez, *Obstet. Gynecol. Surv.*, **62**, No. 5, 335-347 (2007).
11. C. M. Nash, F. Ibram, H. C. Dringenberg, *et al*., *Alcohol*, **41**, No. 8, 577-586 (2007).
12. A. Ornoy, *Reprod. Toxicol*., **24**, No. 1, 31-41 (2007).