DRUGS EXPTL. CLIN. RES. XVIII (SUPPL.) 23-29 (1992)

TERATOLOGICAL EVALUATION OF UKRAIN IN HAMSTERS AND RATS

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Summary: *The compound Ukrain, containing thiophosphoric acid alkaloid derivatives from the plant* Chelidonium majus L., *was given intramusculary (i.m.) on days 6-11 of gestation to hamsters and on days 6-15 of gestation to rats in doses of 0.1, 1.67 and 28 mg/kg daily. No clinical signs of toxicity were found in treated animals and no teratogenic effect could be noted in either species. Such parameters as the number of* corpora lutea, *implantation sites, pre and post-implantation losses, number of live foetuses per litter, placental weight, foetal weight and crown-rump length were not significantly different between the Ukrain treated rats and the controls. Slight embryotoxic effects (increased post-implantation losses) and in consequence decreased number of average litter size were noted in hamsters exposed to Ukrain at doses which were otherwise not embryotoxic to rats.*

Introduction

Greater Celandine (*Chelidonium majus L.*) was used in traditional medicine to treat papillomas, tuberculosis verrucosa and carcinomas (1). Nowicky et al. recently announced that the preparation named Ukrain, containing thiophosphoric acid alkaloid derivatives from *C.majus*, was found to possess immunomodulating and anticancerogenic properties without original plant toxicity (2, 3, 4). It is, however, well known that in general antineoplastic agents are embryotoxic to mammals (5, 6). For that reason the aim of this study was to evaluate the effects of Ukrain on prenatal development in golden Syrian hamsters and albino Wistar rats.

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Material and methods

Ukrain was received from Dr J.W. Nowicky, Ukrainian Anti-Cancer Institute, Vienna, Austria, in the concentration of 33.3 mg/ml. Dosing solutions for animals were all prepared by diluting Ukrain in sterile water to apply any dose in a volume of 0.1 ml per 100 g of body weight.

Animals. Adult nulliparous female hamsters of the Polish National Veterinary Institute's colony, 10-16 weeks old, weighing 90 ± 15 g, and white Wistar rats, 12-18 weeks old, weighing 180 ± 20 g, were used in this study. The animals were housed under standard laboratory conditions of lighting (10 h dark/14 h light), temperature (20 ± 2°C) and relative humidity (50-60%) with free access to commercial feed (Murigran) and water.

Hamsters. A female hamster was placed with a male in the evening. When copulation was observed,

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the pair was left together overnight. The following morning the animals were separated and the day of separation was considered to be the first day of gestation. Eighty five litters were obtained from 102 mated females and 701 live foetuses of 766 implantation sites were evaluated.

After two weeks of acclimatization, female rats were mated with males overnight and checked the next morning for the presence of sperm. The day on which spermatoza were detected was designated as day one of pregnancy. Out of 100 mated females 81 litters were obtained and 750 live foetuses on 773 implantation sites were evaluated.

Treatment schedule. Hamsters were given Ukrain by intramuscular injection (i.m.), daily from day 6 to 11 of gestation in the following doses: *group II*: 0.1 mg/kg, *group III*: 1.67 mg/kg, and *group IV*: 28 mg/kg. The intermediate dose level was calculated as the geometric mean between the low and high dose levels. Control animals (*group I*) were injected i.m. with 0.1 ml of sterile water/100 g body weight. Ukrain was administered to rats i.m. from day 6 to 15 of gestation in similar daily doses, i.e., *group I*:0, *group II*:0.1 mg/kg, *group III*:1.67 mg/kg and *group IV*: 28 mg/kg in a volume of 0.1 ml/100g b.w..

Maternal and foetal examination. Each mated female was under clinical veterinary observation and weighed weekly. Every seventh day food consumption was also recorded. Hamsters were sacrificed on day 15 and rats on day 21 of gestation. The liver, kidneys, spleen and litter with uterus of each dam were removed, weighed and examined. The uteruses were then opened to record post-implantation deaths and the position of dead and live foetuses. The numbers of implantation sites and *corpora lutea* in the ovaries were also counted for calculation of pre-implantation losses. The weight of each foetus, placental weight and crown-rump foetal length were recorded. All foetuses were examined for external malformations. Approximately one third of each litter was preserved in Bouin's fixative and examined for visceral alterations by microdissection according to the Wilson method (7). The remaining two thirds of foetuses were preserved in 95% ethanol, stained with Alizarin Red S (8) and examined for the presence of skeletal deviations and malformations (9). Foetuses with body weight less than so-called "normal range", i.e., mean of the control minus three-fold standard deviation, were classified as runts.

Statistical evaluation. Student's t- and chi-square tests were applied for evaluation of the results, when appropriate. The sampling unit was the dam.

Results and discussion

Hamsters. Animals treated with Ukrain did not show any clinical signs of toxicity, regardless of the dose applied. The lower food consumption in groups II-IV was evidently due to lower weight of mated females than to the influence of Ukrain (Table I). This seemed to be confirmed also by the percentage of body weight gain during pregnancy, which was similar in experimental and control groups. Besides that, maternal body weight gain (without uterus and litter weight) showed only small, insignificant intergroup variations (Table I). However, the relative mean liver weight of animals treated with Ukrain, as well as kidney weights (group III) were higher than those in control animals. The lower mean litter weight (plus uterus) in groups II and IV was a result of higher incidence of post-implantation losses than in control animals (Tables I and II). Consequent to this, the smaller number of live foetuses per litter in those groups was recorded (Table II). It may indicate some embryopathic effects of the compound on the hamster foetuses.

On the other hand, such parameters as pre-implantation losses, number of implantation sites, mean placental weight and foetal crown-rump length did not differ from controls. Moreover, live foetuses were well developed, even better than controls. In groups II and IV the mean foetal weights were significantly higher ($p < 0.05$) than in the controls. The number of runts was low in all groups (I–IV) (Table II). No foetuses with gross malformations were found, either in experimental or in control groups.

Table I *Maternal parameters in hamsters injected with Ukrain*

Maternal parameters	Group (dose mg/kg, daily 6–11 day of gestation)			
	I (0)	II (0.1)	III (1.67)	IV (28.0)
Females mated/pregnant	24/22	26/21	26/22	26/20
Fertility index	0.92	0.81	0.85	0.83
Food consumption, g*				
days 1–7	40.9 ± 7.6	31.6 ± 5.7***	35.3 ± 7.2***	30.2 ± 6.1***
8–14	42.5 ± 8.1	33.3 ± 7.1***	32.7 ± 6.8***	34.5 ± 8.8***
Maternal weight, g*				
day 1 of gestation	98 ± 17.3	87 ± 7.9***	89 ± 8.6***	84 ± 8.9***
7	104 ± 18.1	91 ± 7.6	96 ± 8.6	88 ± 9.7
14	123 ± 17.1	108 ± 8.9	112 ± 9.9	106 ± 10.0
Maternal wt. gain (1–15)**, g*	1.7 ± 8.1	−2.3 ± 6.2	−2.8 ± 7.4	0.3 ± 6.3
Uterus + litter weight, g*	29.5 ± 5.9	25.7 ± 4.8***	28.2 ± 5.4	24.5 ± 6.2***
Liver weight, g/100 g*	6.09 ± 0.86	6.61 ± 0.64***	6.99 ± 0.51***	6.40 ± 0.68
Kidneys weight, g/100 g*	1.03 ± 0.25	1.10 ± 0.15	1.22 ± 0.09***	1.05 ± 0.16
Spleen weight, g/100 g*	0.31 ± 0.10	0.32 ± 0.14	0.44 ± 0.12***	0.34 ± 0.17

* mean ± s.d.
** without uterus + litter
*** $p < 0.05$ *vs* control I, Student's t-test

Table II *Reproduction data for hamsters injected with Ukrain*

Reproduction data	Group (dose mg/kg, daily 6–11 day of gestation)			
	I (0)	II (0.1)	III (1.67)	IV (28.0)
Litters, n	22	21	22	20
Corpora lutea, n	220	199	210	177
mean ± s.d.	10.0 ± 1.8	9.5 ± 1.5	9.5 ± 1.4	8.8 ± 1.8*
Implantation sites, n	210	184	201	171
mean ± s.d.	9.5 ± 1.8	8.8 ± 1.4	9.1 ± 1.6	8.5 ± 1.6
Pre-implantation losses, n (%)	13 (5.7)	15 (7.5)	11 (5.2)	7 (3.9)
Post-implantation losses, n (%)	12 (5.7)	31 (16.8)**	15 (7.1)	27 (15.8)**
early	9	23	12	24
late	3	8	3	3
Live foetuses, n	198	153	186	144
mean ± s.d.	9.0 ± 1.9	7.3 ± 1.7*	8.4 ± 2.0	7.2 ± 2.1*
Male/Female ratio	1.0	1.0	0.8	1.8
Mean placental weight, g ± s.d.	0.41 ± 0.11	0.39 ± 0.06	0.36 ± 0.05	0.37 ± 0.08
Mean foetal length, mm ± s.d.	27.2 ± 1.22	27.8 ± 0.99	27.3 ± 0.94	28.3 ± 1.16
Mean foetal weight, g ± s.d	1.99 ± 0.14	2.11 ± 0.17*	2.02 ± 0.14	2.11 ± 1.17*
Runts, n (%)	9 (4.5)	5 (3.3)	6 (3.2)	2 (1.4)

$p < 0.05$ *vs* control I; *Student's t-test, **Chi^2 test

Table III *Developmental alterations (foetuses per litter) in hamsters treated with Ukrain*

Alterations*	Group (dose mg/kg)			
	I (0)	II (0.1)	III (1.67)	IV (28.0)
External examination	198/22	153/21	186/22	144/20
Visceral examination	60/22	50/21	57/22	42/20
haematoma				
pleural cavity	1/1	0	0	1/1
perineal cavity	0	0	0	1/1
Skeletal examination	137/22	102/31	128/22	102/20
Retarded ossification				
Skull:				
Braincase	1/1	1/1	3/3	2/2
Hyoid body	1/1	0	3/3	0
Axial skeleton:				
< 3 (sternebrae)	26/13	13/10	12/8	17/9
< 2 (tail)	0	0	1/1	0
Pectoral girdle:				
< 3 (metacarpals)	3/3	0	2/2	1/1
Pelvic girdle:				
< 3 (metetarsals)	26/15	13/8	29/14	21/12

* no gross malformations found

There were also no inter-group differences in development of ossification of foetal skeletons. Minor alterations seen upon skeletal examination, e.g., retarded ossification of sternal elements and metatarsals, were observed in both control and experimental groups (Table III).

Rats. No clinical signs attributable to the treatment were observed in any group of pregnant rats given different doses of Ukrain. Food consumption by dams treated with doses of 0.1 mg/kg and 28 mg/kg did not differ significantly from that in the control group (Table IV). The only exception was higher food consumption throughout the second trimester of gestation in group III receiving 1.67 mg of Ukrain per kg. The mean liver weight in this group was higher than in control animals. Rats given the highest dose of the drug, i.e., 28 mg/kg, were found to have heavier kidneys as compared to the controls.

At any time of gestation total maternal weight gain as well as corrected gain did not differ significantly between groups. There were no differences between other indices such as litter weights and mean numbers of live foetuses per litter. The results summarized in Table V indicate that not a single parameter of reproduction in rats was affected by injected doses of Ukrain.

No significant gross malformation was found in any rat foetus in the experimental groups treated with Ukrain. On visceral examination some haematomas were found in single foetuses both in experimental and control groups. In skeletons, minor alterations to the sternum (split) occurred in experimental foetuses, with no relation to the dose applied. The other skeletal changes, such as retarded ossification of the skull, sternal elements, metacarpals, metatarsals, etc., were found in single foetuses of both control and experimental groups (Table VI).

Conclusion

On the basis of the collected data it is concluded that Ukrain did not exert a teratogenic effect that

Table IV *Maternal parameters in rats injected with Ukrain*

Maternal parameters	Group (dose mg/kg, daily 6–15 day of gestation)			
	I (0)	II (0.1)	III (1.67)	IV (28.0)
Females mated/pregnant	24/20	28/20	23/21	25/20
Fertility index	0.83	0.71	0.91	0.80
Food consumption, g*				
days 1–7	126 ± 23.1	119 ± 23.1	123 ± 6.4	126 ± 19.1
8–14	130 ± 11.2	139 ± 24.4	153 ± 21.4***	128 ± 21.5
15–21	153 ± 15.6	140 ± 18.4	158 ± 21.8	147 ± 29.1
Maternal weight, g*				
day 1 of gestation	185 ± 27.6	189 ± 24.7	191 ± 22.9	181 ± 17.0
7	212 ± 28.3	209 ± 29.2	216 ± 23.0	208 ± 20.4
14	227 ± 27.0	227 ± 29.7	234 ± 26.2	223 ± 29.4
21	266 ± 29.7	267 ± 36.0	283 ± 30.7	263 ± 29.4
Maternal wt. gain (1–15)**, g*	34.9 ± 9.5	28.5 ± 14.0	41.6 ± 11.4	33.6 ± 13.5
Uterus + litter weight, g*	45.2 ± 13.5	49.8 ± 14.6	51.2 ± 12.3	48.4 ± 18.7
Liver weight, g/100 g*	5.04 ± 0.38	5.06 ± 0.60	5.39 ± 0.32***	5.21 ± 0.58
Kidneys weight, g/100 g*	0.66 ± 0.07	0.68 ± 0.06	0.70 ± 0.06	0.72 ± 0.08***
Spleen weight, g/100 g*	0.37 ± 0.13	0.32 ± 0.12	0.35 ± 0.10	0.37 ± 0.17

* mean ± s.d.

** without uterus + litter

*** $p < 0.05$ *vs* control I, Student's t-test

Table V *Reproduction data for rats injected with Ukrain*

Reproduction data	Group (dose mg/kg, daily 6–15 day of gestation)			
	I (0)	II (0.1)	III (1.67)	IV (28.0)
Litters, n	20	20	21	20
Corpora lutea, n	222	224	226	225
mean ± s.d.	11.1 ± 1.9	11.2 ± 2.5	10.8 ± 1.3	11.2 ± 2.9
Implantation sites, n	182	201	203	187
mean ± s.d.	9.1 ± 2.9	10.0 ± 2.9	9.7 ± 1.9	9.3 ± 3.8
Pre-implantation losses, n (%)	40 (18.0)	23 (10.3)	23 (10.2)	38 (16.9)
Post-implantation losses, n (%)	9 (4.9)	5 (2.5)	8 (3.9)	1 (0.5)
early	9	5	6	1
late	0	0	2	0
Live foetuses, n	173	196	195	186
mean ± s.d.	8.6 ± 2.8	9.8 ± 3.1	9.3 ± 2.3	9.3 ± 3.8
Male/Female ratio	0.86	0.68	0.65	0.73
Mean placental weight, g ± s.d.	0.55 ± 0.10	0.62 ± 0.30	0.56 ±0.10	0.60 ± 0.28
Mean foetal length, mm ± s.d.	33.5 ± 1.77	33.3 ± 1.63	34.1 ± 1.49	34.0 ± 1.28
Mean foetal weight, g ± s.d.	3.48 ± 0.36	3.31 ± 0.32	3.71 ±0.19	3.49 ± 0.36
Runts, n (%)	6 (3.5)	15 (7.6)	2 (1.0)	12 (6.4)

$p < 0.05$ *vs* control I; *Student's t-test, **Chi² test

Table VI *Developmental alterations (foetuses per litter) in rats treated with Ukrain*

	Group (dose mg/kg)			
Alterations*	I (0)	II (0.1)	III (1.67)	IV (28.0)
External examination	173/20	196/20	195/21	186/20
haematoma	1/1	7/5	7/6	6/4
Visceral examination	52/20	57/20	61/21	57/20
haematoma	1/1	0	1/1	1/1
Skeletal examination	120/20	139/20	134/21	129/20
Minor alterations				
Sternebrae (irregular)	0	0	1/1	0
Sternebrae (split)	0	15/9	1/1	22/7
Retarded ossification				
Skull:				
Braincase	15/7	16/5	10/4	8/2
Tympanic bulla	7/2	0	0	4/2
Hyoid body	6/1	14/5	20/6	7/1
Axial skeleton:				
< 6 (sternebrae)	12/5	19/10	8/6	10/4
< 2 (tail)	2/2	5/4	1/1	3/3
Pectoral girdle:				
< 3 (metacarpals)	1/1	1/1	1/1	3/2
Pelvic girdle:				
< 4 (meletarsals)	2/2	4/3	0	2/2
hypoplastic (ischium)	0	3/1	0	1/1

* no gross malformations found

could be detected under the conditions of this study. There was no indication of embryotoxicity of Ukrain in rats. In hamsters the noted incidents of higher embryolethality and lower number of live foetuses per litter may suggest some embryotoxic response.

Acknowledgements

The authors wish to express their gratitude to Professor Z. Kleinrok, Head of the Department of Pharmacology, Medical Academy, Lublin, Poland, for the suggestion which stimulated this investigation and to Dr J. Nowicky, Ukrainian Anti-Cancer Institute, Vienna, Austria, for his generosity in supplying Ukrain used in these studies.

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