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Blockade of reticular formation activity, due to carisoprodol maternal administration, and its effects on rat skeleton development

Carisoprodol (N-isopropyl-2-methyl-2-propyl-1,3-propanediol dicarbamate) is a rapid centrally acting muscle relaxant due to blockade of interneuronal activity in descending reticular formation and depressing transmission of polysynaptic neurones in the spinal cord. This mechanism of action was proved in experimental animals. However, the drug does not directly relax tense skeletal muscle in man. Because of additional sedative properties carisoprodol has been also used to treat acute musculoskeletal pain syndromes, headache and other painful discomforts (12).

Like other drugs, carisoprodol is not free of side effects. Because of its central activity drowsiness, vertigo ataxia, tremor and other central nervous system side effects are the most often reported adverse reactions, that complicate therapy with the drug. Allergic, idiosyncratic reaction, gastrointestinal reactions, especially nausea, vomiting and sporadically leucopenia were seen in carisoprodol treated patients as well. Unlike well-known adult toxicity, documented in various experimental and epidemiological studies, the prenatal and early postnatal adverse effects are still unclear, even since carisoprodol placental barrier crossing was shown. Due to these facts the drug is not labelled for use during pregnancy unless the potential benefits justify the potential risks to the fetus. To solve this difficult situation it is important to acquaint prenatal drug effects and if any one is detected to learn types of malformations caused by maternal treatment.

The experiment was designated to evaluate the influence of carisoprodol on prenatal development in the animal experimental model. The particular attention was given to skeleton examination according directives and methodology ratified by experts of World Health Organization (WHO). Since thalidomide tragedy this kind of study is referential for each of the new drug or other chemicals on the market to estimate their

gestational toxicity. In spite of positive or negative findings other studies, especially neurobehavioral, histological ones, done in the first and second offspring generations are needed to confirm or to deny toxicity of the tested substance (5).

MATERIAL AND METHODS

The experiment was based on the animal model. The guidelines for the care and use of the animals were designed according to WHO technical direction, general principles in laboratory animal research (8) and permission of the Bioethical Committee of the Medical University of Lublin, Poland.

Wistar breed rats were originally obtained from a commercial breeder (Warszawa-Rembertów, Poland) with initial body weight of 180±15 g. The animals were housed (5 animals in one cage) in suspended plastic cages with steel tops at the temperature 20±3°C on a daylight cycle 7 a.m. – 7 p.m. Laboratory food (Wytwórnia Pasz – Motycz, Poland) and tap water were provided *ad libitum*. Before experiment the animals were acclimatised for 2 weeks to reduce transport stress. For breeding, one male of the same stock was housed with five females throughout the dark cycle. The presence of vaginal plug or sperm in the vaginal smear examined the following morning was taken as an index of copulation. According to Finnel et al. method (7) pregnancy was considered to begin at the midpoint of the dark cycle (2 a.m). Afterwards animals were randomly divided in three groups (10 animals per group). Despite the presence of spermatozoa in the smear, some females were not pregnant.

Carisoprodol (Soma, Wallace Laboratories, USA) was used in the study. After dilution in distilled water the substance was administered orally with the use of stomach tube every 4 hours in the day cycle on the 7^{th} to 13^{th} day. Pregnant females were treated with carisoprodol in the following doses: T1 – 20 mg/kg body mass/24h, T2 – 200 mg/kg/24h, T3 – 400 mg/kg/24h. In control group (H) the dames were treated with distilled water. The body weight gain of the dames was monitored on the 1^{st}, 8^{th}, 15^{th}, and 21^{st} day of pregnancy.

The dames were sacrificed by decapitation and Caesarean sections were performed on 21^{st} day of gestation. After laparotomy and preparation of uterus the number of implantations, living and dead fetuses were counted. Fetuses were separated from placenta and macroscopically examined for external malformation. All fetuses were eviscerationed, washed in running tap water, then fixed in 95% ethanol for the study of skeleton by single alizarin red S staining according to Dawson red-S alizarin method. The glycerol fixed specimens were examined under a stereo-dissection microscope.

Data were statistically analyzed using Mann-Whitney test and ANOVA (14). The level of significance was set at $p<0.05$.

RESULTS

All the treated animals were alive until the day of experiment. They consumed as much food and water as controls and gained comparable weight. There were no noticeable differences in behaviour and health status between the treated animals and controls.

The macroscopic examination of 407 fetuses has not revealed any malformations in drug-treated and control groups except for an insignificant number of subcutaneous haematomas, which were found on interscapular region in group T2 and T3.

No statistical differences between carisoprodol-exposed groups and control one in fetal parameters such as body weight, body length, tail length were noted (Tab. 1).

Table 1. The group characteristic and fetal parameters (Mean±Standard Deviation) in control carisoprodol-treated groups

	H	T1	T2	T3
Dose of carisoprodol (mg/kg/d)	-	20	200	400
No. of pregnant females	7	9	7	8
No. of fetuses (alizarin specimens)	96	117	91	103
Fetal weight (g)	3.40±0.02	3.66±0.67	3.56±0.22	3.7±0.91
Fetal length (mm)	40.17±0.23	41.23±1.17	41.10±1.22	40.25±0.45
Tail length (mm)	13.27±0.1	12.23±0.45	12.76±0.65	12.2±0.98
Placental weight (g)	0.58±0.12	0.63±0.25	0.6±0.12	0.6±0.23
Preimplantation mortality (%)	3.34±0.36	3.56±2.8	4.35±35	3.28±4.0
Postimplantation mortality (%)	3.45±0.67	5.12±1.98	3.97±3.12	4.5±3.75

The examination of alizarin-stained specimens showed insignificant number of reduction of ossification in cranio-facial bones, as well as the other skeleton anomalies. The ribs, especially the last pair were the most often malformed part of the fetal skeleton. The wavy 13^{th} ribs were observed in all the examined groups, including the control one. However, the shorter 13^{th} rib was seen only in fetus from control and groups T3. Single bud or short extra lumbar unilateral ribs occurred in control group as well as in T1. Missing, rudimentary, cleaved, and bifurcated distal end form of sternebrae were seen in drug-treated and control groups. Occasionally missing and reduced alizarin staining of

metacarpal and metatarsal bones were found in carisoprodol-treated as well as control groups. Different degrees of phalanges classification were also noted (Fig. 1). No other anomalies of appendicular skeleton formation were seen.

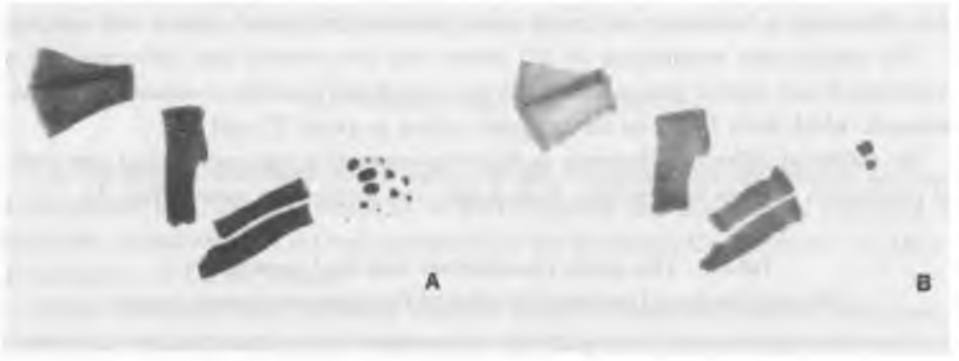

Fig. 1. Upper extremity of rat fetuses on gestational day 21 (alizarin red-S). Well–formed bones of upper extremity (A). Absence of two metacarpal bones and reduction in phalanges staining (B)

DISCUSSION

The results presented above in connection with statistic analysis show that carisoprodol is not a teratogenic factor for rat fetuses. The detected skeleton abnormality due to their low frequency could be interpreted as developmental variations. Less probable is their drug-induced cause especially that similar kind of anomalies and their insignificant rate were seen in both control groups. The above results are also similar to these obtained from rat fetuses exposed to other central active substances such as methyloxanthine derivatives – caffeine, and non-selective cyclooxygenase inhibitor paracetamol, and peripherally active – propyphenazone (2, 3, 4). The chemical and pharmacological differences between all those drugs make it difficult to fully explore these results to carisoprodol, especially that no animal data and limited number of human study regarding its prenatal toxicity have been published.

The Collaborative Perinatal Project that monitored 50,282 mother-child pairs, found 14 children exposed to carisoprodol during the first trimester of gestation (9). No teratogenic or any other toxic effects were seen among that group.

In Michigan study conducted on 229.101 pregnancies, 326 carisoprodol-exposed newborns for at least the first trimester of gestation had been noted (F. Rosa, personal communication to US Food and Drug Administration). Twenty (6.1%) major birth defects were observed (14 expected), including (observed/expected) 3/3 cardiovascular defects, 2/0.5 oral clefts, and 1/1 hypospadias (15). No other major defects were observed.

In this group only two were suspected to be involved as carisoprodol treatment. The others were probably associated with other maternal treatment or diseases.

It should be noted that meprobamate – the active carisoprodol metabolite – prescribed also as a sedative drug – is widely abused during pregnancy. It induced multiple congenital heart abnormalities. Abdominal wall and diaphragm defects, omphalocele, Down's syndrome, partial deafness, joint deformations after moprobamate *in utero* exposure were seen as well. The total rate of the drug-induced anomalies was set on level 1.9% - 12.1% (6, 11, 13).

Other muscle relaxants such as baclofen and methocarbamol show no teratogenic effects to fetus in various experimental studies where rats, mice and rabbits were used (15). Opposite to those negative findings dose-related vertebral arch widening after 30 mg/kg of abaclofen administered on day 10 of gestation was reported by Briner (1). It is interesting that higher dose (60 mg/kg) did not produce this kind of malformation effect. The human data showed no morphological abnormalities in child born from mother treated with metacarbamol for the whole gestational period. However, prolonged crying, restlessness, seizures and easy irritability were seen in those infants. This kind of adverse reactions are probably secondary central drug activity (11).

Based on the obtained results and literature review it could be concluded that carisoprodol did not cause congenital anomalies in rat fetuses. However, due to limited human data and meprobamate dependence it should be given only if the potential benefit justifies the potential risk to the fetuses.

REFERENCES

1. Briner W.: Muscimol- and baclofen-induced spina bifida in the rat. Med. Sci. Res., 24, 639, 1995.
2. Burdan F.: Somatic and skeleton development of rat fetuses following *in utero* exposure to isopropylantipyrine (propyphenazone) during the second trimester of gestation. Folia Morphol., 59, 167, 2000.
3. Burdan F.: The effects of short-time caffeine administration on skeleton development in Wistar rats. Folia Morphol., 59, 91, 2000.
4. Burdan F.: Evaluation of bone formation in fetal skeletons following prenatal paracetamol administration in single alizarin-stained specimens in Wistar rats. Folia Morphol., 59, 167, 2000.
5. Burdan F.: Teratology, 40 years' thalidomide tragedy. Gin. Pol. (in press), 2001.
6. Crombie D. L. et. al.: Fetal effects of tranquilizers in pregnancy. N. Eng. J. Med., 293, 198, 1975.

7. Finnell R. H. et. al.: Strain differences in heat-induced neural tube defects in mice. Teratology, 33, 247, 1986.
8. Górska P.: Principles in laboratory animal research for experimental purpose. Med. Sci. Monit., 6, 171, 2000.
9. Heinonen O. P. et. al.: Birth Defects and Drugs in Pregnancy. Littleton, Publishing Sciences Group, 357, 1977.
10. Koren G. et. al.: Drugs in pregnancy. N. Engl. J. Med., 338, 1128, 1998.
11. Milkovicz L., van den Berg B. J.: Effects of prenatal meprobamate and chlordiazepoxide hydrochloride on human embryotic and fetal development. N. Eng. J. Med., 291, 1268, 1974.
12. Product Information: Soma. Wallace Laboratories, 1994.
13. Ringrose C. A. D.: The hazard of neurotropic drugs in the fertile years. Can. Med. Assoc. J., 106, 1058, 1972.
14. Shein-Chung C., Jen-Pei L. eds.: Design and Analysis of Animal Studies in Pharmaceutical Development. Marcel Dekker, New York 1998.
15. Shepard T. H.: Catalog of Teratogenic Agents. 6th ed. Baltimore, Johns Hopkins University Press, 66, 1989.

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SUMMARY

The purpose of this experiment was to study the influence of reticular formation blockade, due to carisoprodol maternal administration, on rat skeleton development. The drug was administered three times a day orally by stomach tube at doses: T1 – 20mg/kg/24h, T2 – 200 mg/kg/24h, T3 – 400 mg/kg/24h. The fetuses obtained on 21st day of gestation were counted and macroscopically examined. Placental and fetal weight, fetal and tail length were checked. After fixation in 95% ethanol the fetuses were stained under single alizarin red S Dawson method and examined under a stereo-dissection microscope. Morphological examination revealed no major malformations. Insignificant number of subcutaneous ecchymose and various skeleton anomalies were observed. The experiment revealed that carisoprodol has no influence on rat skeleton development.

Blokada aktywności tworu siatkowatego po podaniu matce carisoprodolu oraz jego wpływ na rozwój szkieletu szczura

Celem eksperymentu była ocena wpływu blokady tworu siatkowatego po podaniu matce carisoprodolu oraz przebadanie wpływu na rozwój szkieletu kostnego. Lek był podawany trzy razy dziennie sondą dożołądkową w dawkach: T1 – 20mg/kg/24h, T2 – 200mg/kg/

24h, T3 – 400mg/kg/24h. W 21 dniu ciąży cesarskim cięciem wydobywano płody. Dokonano analizy makroskopowej. Płody i łożyska ważono. Mierzono długość płodu i ogona. Utrwalone w 95% alkoholu preparaty barwiono alizaryną metodą Dawsona. Oceny dokonano przy użyciu mikroskopu świetlnego. Nie wykryto znaczącego wzrostu występowania malformacji w porównaniu z kontrolą. Doświadczenie wykazało, że carisoprodol nie wpływa na rozwój szkieletu kostnego szczura.