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Somatic and skeleton development of rat foetuses following in-utero exposure to isopropylantipyrine (propyphenazone) during the second trimester of gestation

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Isopropylantipyrine (IPA, propyphenazone) is a pyrazolone derivative, widely used as an antipyretic and analgesic drug. The aim of the study was to evaluate the influence of propyphenazone on rat development. IPA was administered to pregnant rats from day 8 to day 14 of pregnancy once a day, orally by a stomach tube at doses of 2.10 (R1), 21.0 (R2), and 210.0 mg/kg/day (R3). The dams were sacrificed on day 21 of gestation and corpora luteum, implants, resorptions, and live foetuses were counted. The weight of foetuses and placentas, the length of foetuses and their tails were checked. The foetuses were fixed in alcohol and skeletons were stained with alizarin. There was a statistical difference in body length in R1, R2 and numbers of subcutaneous ecchymose in R1. External and skeletal examination of the foetuses revealed no evidence of teratogenesis. It can be concluded that IPA has no harmful effects on the prenatal development of the rat offspring at doses used in the present study.

key words: isopropylantipyrine, propyphenazone, teratogen, congenital-malformation, pregnancy, rat

INTRODUCTION

Embryotoxicity and teratogenicity are side effects that complicated therapy with various drugs during pregnancy. These side effects can occur in high doses — close to the toxic level for the mother. However, some of the medicines have selective development toxicity in doses that are well tolerated by the mother [3,10,13]. Amniopterin was the first known drug, which interferes with normal pregnancy [16]. This finding was unimportant until 1959 when German and Australian clinicians became aware of the epidemic of phocomelia. Epidemiological and experimental studies showed that this unusual limb reduction defect was caused by thalidomide — the drug, which was marketed as safe, even when taken in overdose [3,10].

Responding to public pressure the toxicologists of the World Health Organization (WHO) held a meeting in Geneva from 14 to 19 November 1966 to develop guidelines for evaluating drug safety and the subsequent extrapolation of animal data to man [18]. The WHO principles published after the meeting, as well as the law in associated countries, stated that each new drug be evaluated for its prenatal toxic effects. However, older drugs on the market did not need these studies [7,18]. As a consequence many widely used xenobiotics are still in use without teratology data.

One of these substances is isopropylantipyrine (IPA, propyphenazone, propyfenazone(a), 2,3-dimethyl-1-phenyl-4-isopropyl-pyrazolin-5-one, CAS 479–92–5) — a popular analgesic and antipyretic medicine,

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which has been produced in the Roche laboratory since 1933, and later by other pharmaceutical companies. Until 1977 it had been used as a monosubstance with an annual world-wide consumption exceeding 100 tons. Subsequently consumption rose dramatically when European authorities mandated the replacement of aminophenazone by IPA in combination analgesics [1,8].

To the best of my knowledge, for almost 70 years no one published data regarding prenatal effects of IPA except for the short conclusion of the company's study in IPA Product Monograph [8].

This study was taken up to evaluate prenatal toxicity of IPA, particularly any influence on bone congenital malformations in single alizarin-stained specimens, which is a sensitive and widely used marker in teratological studies.

MATERIAL AND METHODS

This experiment was based on an animal experimental model designed according to the standards and principles of WHO and the guidelines of the Bioethical Committee of the Medical University School of Lublin, Poland [9,17,18].

The experiment was conducted on Wistar breed rats, originally obtained from a commercial breeder (Warszawa-Rembertów, Poland), with initial body weight of 180 ± 15 grams. The animals were housed in standard laboratory cages (maximum of 5 rats per cage) at a room temperature of 20 ± 3°C on a daylight cycle (7 A.M.–7 P.M.). Standard laboratory fodder (Motycz, Poland) and tap water were provided ad libitum. Food and water consumption were monitored daily.

Virgin females after two weeks of acclimatisation period were mated overnight with males of the same stock. The presence of a vaginal plug or sperm in the vaginal smear examined the following morning was taken to indicate successful mating and the day was considered the first day of gestation. The inseminated animals were randomly placed in experimental or control groups with a minimum of 12 in each group. Some females were not pregnant despite the presence of spermatozoa in the smear.

Isopropylantipyrine — IPA (Hoechst, Germany; 99.4% purity) was ground with Tween 80 (Sigma, Germany) and then diluted in distilled water. The suspension was administered orally with the use of a stomach tube, once daily between 7.30 A.M. and 8 A.M., on days 8–14 of gestation. The tested substance was given in three different doses: R1 — 2.1 mg/kg body weight (p = 7 — number of pregnant female; f = 104 — number of foetuses; s = 74 — number of examined alizarin staining specimens), R2 — 21.0 mg/kg b.w. (p = 11, f = 158, s = 119), and R3 — 210.0 mg/kg b.w. (p = 7, f = 97, s = 56). The volumes that were given were proportionate to the body weight of the animals, e.g., 10 ml/kg b.w.

Two control groups were designed: T — the females received the Tween 80 solution during the whole second trimester of pregnancy (p = 10, f = 148, s = 118), K — untreated control (p = 19, f = 275, s = 176). The animals in group T received Tween 80 water suspension in volumes corresponding to those given in the treatment groups. The body weight gain of the dams was monitored on days 1, 8, 14, and 21 of pregnancy.

All the animals were killed by decapitation on the 21st day of gestation, and the uteri were delivered by laparotomy. The corpora lutea, implants, resorptions, and the live and dead foetuses were counted. Foetuses were removed, separated from placenta and examined macroscopically for external malformations. The weight of foetuses and placentas, the lengths of foetuses and their tails were checked. The pre- and postimplantation mortality factors were calculated.

At least 2/3 of the foetuses from each litter were fixed in 95% ethanol for the study of the skeleton by single alizarin red S staining and examined under a stereo-dissection microscope.

Data were statistically analysed using Mann-Whitney test and followed by ANOVA [14]. The level of significance was set at $p < 0.05$ ($\alpha = 0.05$).

RESULTS

During the whole experiment, no maternal deaths and behavioural changes were recorded in any group. The drug-treated females consumed as much food and water as the controls and gained comparable weight. There were no signs of maternal toxicity due to IPA treatment (data not shown).

Since there were no statistical differences in foetal parameters between both control groups (T, K), they were united in one pooled control group (CON) to minimise observation error [2].

The number of corpora luteum, foetuses and resorptions did not exhibit any significant difference compared with the pooled control group. This resulted in insignificant differences in the preimplantation and postimplantation mortality factors (Tab. 1). The body weight of the foetuses and the weight of placentas from the IPA-treated groups were lower than the ones in the pooled control group but with-

Table 1. Tested foetal development parameters in isopropylantipyrine-treated (R) and pooled control groups (CON)

	CON MD ± SD	R1 MD ± SD	R2 MD ± SD	R3 MD ± SD
Foetal weight [g]	3.80 ± 0.42	3.64 ± 0.08	3.74 ± 0.30	3.56 ± 0.23
Foetal length [mm]	38.55 ± 1.09	36.87 ± 0.32*	37.54 ± 1.10*	38.12 ± 0.96
Tail length [mm]	11.85 ± 0.52	11.80 ± 0.23	11,67 ± 0.28	11.74 ± 0.35
Placenta weight [g]	0.59 ± 0.06	0.57 ± 0.05	0.57 ± 0.04	0.56 ± 0.02
Number of corpora lutea	15.13 ± 0.43	15.57 ± 2.07	15.09 ± 1.70	14.66 ± 0.51
Number of foetuses	14.31 ± 2.25	14.85 ± 2.41	14.36 ± 2.61	13.83 ± 1.16
Number of resorptions	0.55 ± 0.68	0.42 ± 0.53	0.36 ± 0.80	0.50 ± 0.54
Preimplantation mortality	2.08 ± 4.37	2.02 ± 3.27	2.86 ± 5.20	2.30 ± 3.25
Postimplantation mortality	3.40 ± 4.19	2.85 ± 3.4	2.89 ± 6.37	3.58 ± 3.59
Number of subcutaneous haematomas	0.20 ± 0.41	1.00 ± 0.81	0.63 ± 0.92	1.00 ± 1.59

MD — mean deviation, SD — standard deviation, * — differ significantly ($p < 0.05$) *vs* pooled control value

out any statistical correlation. A significant decrease in the length of foetuses was found in the groups R1 and R2, when compared with the control group. The decrease in this parameter with the highest dose did not cause significant alterations. The mean tail length was not statistically different.

The external malformations were not noted in drug-treated and control groups except for subcutaneous haematomas which were found in parietal region (K), left auricular cochlea (R3), buccal region (R3), left triangle of the neck (R2, R3), right triangle of the neck (R2), collar-shaped haematoma of the neck (R2), interscapular region (R1, 2x R2, R3), right inguinal region (R3), sacral region (R1), left leg (K, T, R1), left calcaneal region (R1, R2) and on the mobile part of the tail (K). The analysis of their numbers revealed a marginal significant increase only in the group R1, when compared with the pooled control group (Fig. 1).

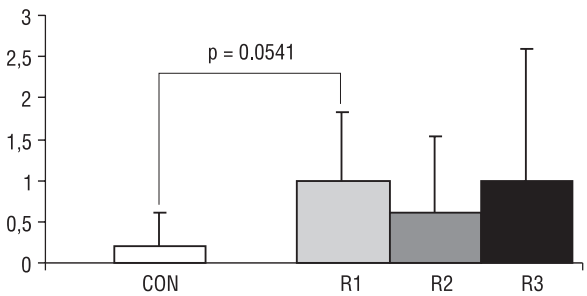

Figure 1. Incidence of subcutaneous haematomas in isopropylantipyrine-treated (R) and pooled control groups (CON).

Table 2 summarises the frequency and types of bone malformations observed in examined groups. The statistical examination showed insignificant differences between pooled control and IPA-treated groups.

The examination of 249 skeletons of IPA-exposed foetuses and 294 from both control groups showed a partial ossification of the frontal, parietal, interparietal, supraoccipital bones. In one of the foetuses from the R3 group, the interparietal bone was missing. The other bones of the skull, including basicranial ones, did not show any malformation (Fig. 2).

The missing hyoid bone was observed in one specimen from R1 group and in one specimen from R3 group. The reduction of alizarin staining of the hyoid bone was found in all experimental and controls groups.

The bilateral wavy thoracic ribs were found in only two specimens from group R2. Both foetuses came from the same litter. In one foetus the wavy ribs coexisted with bilateral, asymmetrical, multilevel reduction of vertebrae ossification (Fig. 3). The individual cases of the short 13th ribs were noted only in both control groups. The wavy 13th ribs were observed occasionally in all examined groups. The bud unilateral extra lumbar ribs (L1) were observed in all IPA-exposed and untreated control groups. The bilateral bud lumbar ribs, on the level L1, were observed only in one specimen of the foetuses exposed in-utero to the middle dose of IPA. The unilateral short L1 ribs were observed in the groups R1 and R2 as well as in one case in untreated control groups.

Table 2. Incidence of skeletal variations (%) in isopropylantipyrine-treated (R) and pooled control groups (CON)

	CON	R1	R2	R3
Number of examined alizarin staining specimens	294	74	119	56
Frontal, reduced ossification	–	–	2 (1.68)	1 (1.78)
Parietal, reduced ossification	19 (6.46)	6 (8.11)	12 (10.08)	8 (14,28)
Interparietal, missing	–	–	–	1 (1.78)
Interparietal, reduced ossification	22 (7.48)	7 (9.45)	16 (13.44)	8 (14.28)
Supraoccipital, reduced ossification	10 (3.40)	5 (6.75)	9 (7.56)	5 (8.92)
Hyoid, missing	–	1 (1.35)	–	1 (1.78)
Hyoid, reduced ossification	2 (0.68)	1 (1.35)	3 (2.52)	2 (3.57)
Vertebral arches, multilevel reduced ossification	–	–	1 (0.84)	–
Wave ribs	–	–	2 (1.68)	–
13th rib, wavy	7 (2.38)	1 (1.35)	4 (3.36)	1 (1.78)
13th rib, unilateral short	2 (0.68)	–	–	–
Supernumerary rib, bud unilateral (L1)	1 (0.34)	2 (2.70)	1 (0.84)	1 (1.78)
Supernumerary rib, bud bilateral (L1)	–	–	1 (0.84)	–
Supernumerary rib, short unilateral (L1)	1 (0.34)	1 (1.35)	1 (0.84)	–
Metacarpal, unilateral missing of one bone	3 (1.02)	1 (1.35)	1 (0.84)	–
Metacarpal, bilateral missing of one bone	4 (1.36)	3 (4.05)	3 (2.54)	4 (7.14)
Metacarpal, unilateral missing of two bones	–	–	–	1 (1.78)
Metatarsal, unilateral missing of two bones	10 (3.40)	5 (6.75)	8 (6.72)	6 (10.71)
Metatarsal, bilateral missing of two bones	–	–	1 (0.84)	–
6th sternebrae, missing	2 (0.68)	–	3 (2.54)	1 (1.78)
6th sternebrae, rudimentary	4 (1.36)	2 (2.70)	3 (2.52)	2 (3.57)
6th sternebrae, cleaved	1 (0.34)	–	–	–
6th sternebrae, bifurcated at distal end	1 (0.34)	–	2 (1.68)	–
6th sternebrae, reduced ossification	20 (6.80)	6 (8.10)	10 (8.40)	4 (7.14)
5th sternebrae, missing	1 (0.34)	1 (1.35)	3 (2.52)	2 (3.57)
5th sternebrae, rudimentary	21 (7.14)	2 (2.70)	8 (6.72)	4 (7.14)
5th sternebrae, dumbbell-shaped	14 (4.76)	3 (4.05)	6 (5.04)	1 (1.78)
5th sternebrae, reduced ossification	3 (1.02)	4 (5.40)	2 (1.68)	1 (1.78)
4th sternebrae, missing	–	1 (1.35)	–	–
4th sternebrae, rudimentary	2 (0.68)	–	1 (0.84)	1 (1.78)
3rd sternebrae, rudimentary	–	–	1 (0.84)	2 (3.57)
2nd sternebrae, missing	–	1 (1.35)	–	–
2nd sternebrae, cleaved	–	–	–	1 (1.78)
1st sternebrae, cleaved	–	–	–	1 (1.78)
1st sternebrae, reduced ossification	1 (0.34)	–	–	2 (3.57)

Unilateral absence of the 4th metacarpal bone was observed in the R1, R2 and pooled control groups. Bilateral absence of one metacarpal bone was found in all examined groups. One foetus from group R3 had bilateral missing of the 1st and the 4th metacarpal bones. The 5th metatarsal bone was not observed in a few foetuses from all examined groups. Bilateral missing of two metatarsal bones was found only in one foetus exposed prenatally to the middle doses of IPA. Also present were wavy ribs and bilateral

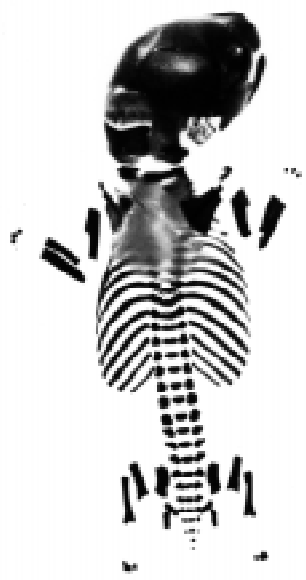

Figure 2. Well-formed skeleton of rat foetus on gestation day 21 in alizarin red-S staining.

Figure 3. Skeleton of rat foetus of gestation day 21 in alizarin red-S staining. Bilateral wavy ribs, and poorly ossified 7th, 9th, 10th, 11th, 12th, 13th thoracic vertebral arches on the left side; 6th, 8th, 12th, 13th thoracic vertebral arches on the right side, and 1st, 4th, 5th lumbar left vertebral arches. Bilateral missing of metacarpal bone, and poorly ossified phalanges.

missing of one metacarpal bone and poorly ossified phalanges (Fig. 3).

The 5th and/or 6th sternebrae was the most common malformed part of the sternum. Anomalies of the other four sternebraes were seen occasionally. The missing, rudimentary, and poorly ossified sternebrae were the most common anomalies. The other malformations such as dumbbell-shaping and bifurcating were seen in the control and IPA-treated groups. Cleavage of the 6th sternebrae was found in only one specimen from the untreated control group.

DISCUSSION

The results of this study show that the tested IPA did not cause any external and skeleton malformations and only slightly changed the foetal parameters.

The body weight, the length of the foetuses and their tails are considered sensitive indicators of an animal's response to xenobiotics [6,9]. A significant decrease in body length, which was observed in groups R1 and R2, was not confirmed in group R3 — exposed to the highest dose of IPA. Knowing that other parameters were not statistically different, the changes can be interpreted as occasional.

An increased number of subcutaneous haematomas that occurred in group R1, can be interpreted as a side effect of inhibition of cycolooxygenase and block in synthesis of thromboxan [1]. This finding as well as the decreasing of the body weight was not confirmed in the highest dose-group.

In the previous test, IPA administered at doses 30.0, 150.0, 300.0, 450.0 mg/kg b.w./day from day 9 until day 14 of rat pregnancy did not cause any development side effects [8]. Only a slight inhibition of the body weight gain was observed in the highest dose group compared with the control one. Those results come from the conclusion of the teratological study published as a short note in IPA Product Monograph [8]. However, the author did not explain the methods used to verify this conclusion.

The spectrum and frequency of bone malformation presented in this study are similar to results observed in foetuses exposed prenatally to caffeine in doses 0.7–70.0 mg/kg b.w. and to paracetamol in doses 3.5–350.0 mg/kg b.w. [2,4,5]. Only the wavy ribs found in two foetuses, and poorly ossified arches of vertebras, together with metacarpal and metatarsal missing in one of them, were related only to IPA. The other malformations were seen in caffeine — as well as paracetamol-exposed foetuses. Those results are fully comparably since the same methodology, was used for testing all three substances. It was shown that caffeine and paracetamol did not disturb the rat's skeleton development [2,4]. IPA was even better tolerated than paracetamol which lowered the foetal body weight and length, and was as

well tolerated as the experimental doses of caffeine. However, due to different chemical structure, mechanism of action, and metabolic pathways of caffeine, paracetamol and IPA this kind of analysis is purely theoretical [1,8,11,12].

Although there are difficulties in extrapolating the above results to humans, they do not suggest an influence of IPA on developing organisms. It was not possible to find any other publications describing the influence of IPA on the foetal development or the side effects of its administration on pregnant organisms. Accordingly, larger epidemiological and subsequent animal studies using other laboratory animals are needed to evaluate the potential influence of IPA on human development.

In conclusion it could be stressed that isopropylantipyrine administered for the whole second trimester of pregnancy did not cause embryotoxic effect and that no harmful effects of isopropylantipyrine at doses used in the present study on rat's skeleton development were observed.

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