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Embryofetotoxicity of acetaminophen (paracetamol) in experimental in vivo model

Various drugs as well as other xenobiotics can produce prenatal toxic effect that may be functional or structural, temporary or permanent. According to the principles of World Health Organisation prenatal toxicity studies are needed to establish the risk.

The study reported here was undertaken to evaluate the influence of acetaminophen on fetal organism, particularly with respect to the hepatotoxic effect as assessed by histological and histochemical studies.

Acetaminophen (APAP, APA, paracetamol N-acetyl-p-aminophenol, 4-hydroxyacetanilide) is known as a safe, widely used analgesic and antipyretic medication. However, it is not free of the adverse reaction. The most common reported side effects are the liver and renal injuries (3).

Hepatotoxicity is mediated by the activation of APAP to the reactive intermediate, N-acetyl-p-quineimine (NAPQI), by cytochrome P_{450}-dependent mixed-function oxidases. This metabolite increases concentration of intracellular free ions of calcium and increases glycogen phosphorylase activity, resulting in a cellular edema. The increase of the Ca^{++} concentration in the cytosol activates phospolipases A_2 which releases free radicals and OH^- groups. Cells die as a consequence of nuclear, ribosomal and nucleic acid disintegration (6).

MATERIAL AND METHODS

This experiment was based on an animal experimental model designed according to the standards and principles of World Health Organization and the guidelines of the Bioethical Committee of the Medical University of Lublin.

Female and male Wistar rats were obtained from commercial breeders (Warsaw-Rembertów, Poland) and were held in quarantine for two weeks in the quarantine/breeding room. Individual weight variation did not exceed ± 20% of mean value at the beginning of the acclimatisation period. The in-life phase of this study was carried out under standard laboratory conditions. All the animals had free access to certified LSH formula diet (Motycz, Poland) and fresh drinking water at all times during the entire experiment. During quarantine, all the rats were singly housed in standard laboratory cages (max. 5 per cage).

The rats were mated and the day on which sperm was found in vaginal smears was considered the first day of gestation. The females, considered being pregnant, were pooled in groups of minimum 12 animals. Some females were not pregnant despite the presence of spermatozoa in the smear.

Food and water consumption and body weight gain were monitored on day 1st, 8th, 14th, 21st of gestation.

Acetaminophen (purity>99% Sigma, USA) was grounded with Tween 80 (Sigma, USA), then diluted in distilled water. The suspension was administered once a day, between 7.30 and 8.00 a.m. from day 8th to 14th of gestation, using a stomach tube, in three doses. For group P1, the dose was 3.5 mg/kg body weight (b.w.). The dose for group P2 was increased 10 times and 100 times in group P3 when compared with the initial dose P1. There were two control groups. In group T – the females received water solution of Tween 80 during the whole second trimester of pregnancy. All the animals received volumes corresponding to those given in the treatment groups – i.e. 10 ml/kg b.w. In group K – the females did not receive any substances (untreated control).

The dames were terminated by decapitation on day 21st of the study. The litters were removed by Caesarean section. Ovaries were removed, and corpora lutea were counted. Fetuses were excised from the uterine horns, were weighed individually. The length of the fetuses and their tails were recorded. Each fetus received a gross external morphologic examination. The fetuses were sacrificed under ether anaesthesia. $^1/_4$ of the fetuses of each litter were sectioned *in situ* and their livers were taken for histological investigation.

The liver tissue was fixed in 10% buffered formalin or in alcohol and processed routinely. Each liver was examined using light microscopy (Axioskop by Zeiss) after using four stains: hematoxylin and eosin, silver Gomorii, van Giesson and histochemical stain by periodic acid-Schiff method (paS).

The experiment was done with approval of the University Ethics Commission of Medical University of Lublin.

Means and standard errors of the means were calculated for all numerical parameters and were analyzed by two-tailed Student's test or by Mann-Whitney test and followed by ANOVA test. A minimum significance level of $p<0.05$ was used in the comparisons.

RESULTS

No maternal deaths and behavioural changes were recorded in any of the groups. Throughout the experiment, the acetaminophen treated animals consumed as much food and water as the control and gained comparable weight. There were no other signs of maternal toxicity due to drug treatment (data not shown).

Insignificant differences between both control groups (T, K) were noted. Because of this we decided to unite them into one common control group (CON) to minimize observation error.

The fetal body weight exhibited evident dose-related decrease but without any significant correlation (Table 1). However, in group P3 the fetal weight was significantly lower when compared with the lowest dose (group P1). A significant decrease in the length of fetuses was found in group P3 when compared with the common control group. A mean tail length and mean placental weight were not statistically different. The number of corpora lutea, fetuses and resorptions did not exhibit any significant difference. This resulted in insignificant differences in preimplantation and postimplantation mortality factors. The fetal dead was not observed in any experimental and control groups.

Table 1. Tested parameters (Mean ± Standard Deviation/litter) in the common control (CON) and acetaminophen-treated (P) groups

	CON	P1	P2	P3
Fetal weight (g)	3.80 ±0.42	3.83 ± 0.24	3.67 ± 0.39	3.46 ± 0.27**
Fetal length (mm)	38.55 ± 1.09	38.10 ± 0.91	37.62 ± 1.50	37.53 ± 0.99*
Tail length (mm)	11.85 ± 0.52	11.82 ± 0.42	11,55 ± 0.43	11.60 ± 0.29
Placental weight (g)	0.59 ± 0.06	0.57 ± 0.04	0.62 ± 0.05	0.61 ± 0.04
Number of luteum corpuscle	15.13 ± 0.43	14.62 ± 1.59	15.87 ± 2.47	15.14 ± 2.96
Number of fetuses	14.31 ± 2.25	13.87 ± 2.23	14.37 ± 4.13	13.57 ± 3.50
Number of resorptions	0,55 ± 0.68	0.50 ± 1.06	1.12 ± 1.12	1.14 ± 1.77
Preimplantation mortality	2.08 ± 4.37	1.85 ± 3.27	3.16 ± 6.14	3.70 ± 6.95
Postimplantation mortality	3.40 ± 4.19	3.62 ± 7.61	9.13 ± 10.58	7.30 ± 10.29
Number of ecchymosis	0.20 ± 0.41	0.12 ± 0.35	0.50 ± 0.53	0.28 ± 0.48

* Differ significantly from the common control value, ** Differ significantly from group P1.

Subcutaneous ecchymoses were the only macroscopic changes found in the litters. The analysis of their numbers revealed a non-significant increase in fetuses exposed *in utero* to APAP.

The histological investigation showed that in 5 of 27 examined livers of the common control groups giant hepatocytes were observed. The giant hepatocytes were also observed in 6 of 29 livers from group P1. In one case they were accompaining local congestion (Fig. 1). In the other one the size of congestion was bigger. The intensive liver erythropoesis and giant hepatocytes were found in 4 of 21 examined livers from group P2. In other 3 livers the big congestion was observed. Giant hepatocytes and other circulatory disturbances (congestion, erytopoesis foci) were also observed in group P3 (Fig. 2). However, all of them were found in 2 foetuses of the same three litters. The mean increment of all histological changes was not statistically different, when compared with both control groups and between drug-treatment groups.

DISCUSSION

The results of this study show that the APAP did not cause any external malformation except for non-statistical number of subcutaneous ecchymoses. The highest doses of APAP decreased the fetal length when compared with the control group. Histological changes of the fetal liver were observed occasionally in all the groups, including the control ones. It is worth to mention that insignificant numbers of skeleton malformation in examined fetuses were found (2).

Numerous studies have shown maternal and fetal toxicities associated with the administration of APAP to the laboratory animals and in humans (3).

T h o m a s et al. (14) observed an increase of ALT, AST, LDH, and urea in male and female adult rat livers after 30 hours of APAP administration at doses 3 x 175 mg/kg b.w. or higher. They were accompanied with necrosis in the central lobular zone with inflammatory infiltrates as well as eosinophilic hepatocytes in the necrosis-free zones.

E m e i g h -H a r t et al. (4) studied the localisation of selective protein arylation by APAP after administration of 600 mg/kg of APAP by gavage. Covalent binding of APAP to intracellular proteins

was observed only in male mice liver that sustained cellular injury. The APAP-protein adducts were not found in the liver that did not show necrosis. Those results were confirmed by B i r g e et al. (1). They detected some protein in culture of human hepatocytes exposured to 2.5 or 10.0 mmol APAP for a day.

During 14 weeks' study, APAP administered to Swiss CD-1 mice on the diet at 375.0-1430.0 mg/kg b.w. caused a significant decrease in the number of the litters per pair and reduced the number of live pups per litter (12). The authors observed a significant decrease in liver weight, without any treatment-related gross or histopathological changes.

In *in vitro* study, APAP (0.1-1.0 mmol) caused neutral tube defects and decreased embryonic length and viability in rat conceptuses (13).

In *in vivo,* APAP administered intraperitoneally at the dose 300 mg/kg, potentiated phenytoin-induced clef plate in offspring mice, via a mechanism involving depletion of tissue glutathione levels (9).

APAP administered 3 times a day at the doses 50.0 – 200.0 mg/kg b.w. to pregnant Wistar rats, during organogenesis period, caused a significant decrease in the fetal body weight and the fetal length without any differences in pre- or postnatal mortality factors (15). In the highest dose the statistically significant increase in the placental weight and tail length were observed.

In contrast to those positive dates, APAP administered at the dose 125.0 and 250.0 mg/kg b.w. on days 8 to19 of pregnancy to Sprague-Dawley rats did not affect fetal length and weight (8). The number of resorptions was at the same level as in control groups.

Human case reports of nonmalforming toxicity of APAP also exist. H a i b a c h et al. (5) reported a toxic liver injury in human stillbirth (27^{th}-28^{th} week of pregnancy) after maternal ingestion of 29.5 g over less than 24 hours APAP. The high concentration of APAP in fetal liver tissue was observed. In the other case acute maternal overdose occurred at 15.5 weeks of pregnancy after 64 g (7). Marked hepatic necrosis and adult respiratory distress syndrome, accompanied with high APAP in mother blood concentration, were observed. The woman delivered a healthy infant at 32^{nd} week pregnancy. However, in several other cases the overdose of APAP during gestational period fatal fetal liver injury were apparently not observed (10,11). A good review of the literature that evaluated prenatal effects of APAP in numbers of animals and human studies are summarised by B u r d a n (3).

CONCLUSIONS

Acetaminophen administered over the whole second trimester of gestation caused toxic effect, lowered the fetal length and slightly impaired the morphology of the fetal rat liver.

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EXPLANATION TO FIGURES

Fig. 1. The fetal liver with giant hepatocytes, many mononuclear cells, probably erythropoietic ones. H+E. Magn. x 420 (group P1).

Fig. 2. The fetal liver with congestion and many myelopoietic foci. H+E. Magn. x 420 (group P3).

SUMMARY

The aim of the study was to evaluate the toxic effect of acetaminophen on rat fetuses. Acetaminophen, suspended in Tween 80 solution, was administered once a day, orally by a stomach tube to pregnant Wistar rats from day 8^{th} to 14^{th} of pregnancy at the dose: 3.5 (P1), 35.0 (P2), 350.0 mg/kg (P3). The pregnant females were terminated on day 21^{st} of pregnancy and the number of corpora lutea, implants, resorptions, and fetuses was counted. The fetuses and the placentas were weighed and the length of the fetuses and their tails were checked. The slides of the fetal liver were examined via light microscopy in four stains: hematoxylin and eosin, silver Gomorii, van Giesson, and periodic acid-Schiff. There was a statistical ($p<0.05$) difference in fetal body length in group P3

without any macroscopic malformation, except for the non-statistical number of subcutaneous ecchymoses. Histological adaptive changes of the fetal liver were observed occasionally in all the studied groups. In conclusion, the oral administration of acetaminophen caused an embryotoxic effect in the highest doses without any macroscopic malformation, and only slightly impaired morphology of the rat fetal liver.

Toksyczność embrionalna i płodowa acetaminophenu (paracetamol) w doświadczalnym modelu *in vivo*

Celem pracy była ocena toksyczności prenatalnej acetaminophenu (paracetamol). Acetaminophen podawany był jeden raz dziennie, dożołądkowo w wodnej zawiesinie Tweenu 80 ciężarnym samicom szczura białego szczepu Wistar w okresie od 8 do 14 dnia ciąży. W pracy użyto trzech dawek: 3,5 (P1) 35,0 (P2), 350,0 mg/kg (P3). W 21 dniu ciąży cesarskim cięciem wydobywano płody. Liczono ciałka żółte, miejsca implantacji, resorpcje i płody. Obliczano wskaźnik śmiertelności przed- i poimplantacyjnej. Płody i łożyska ważono. Mierzono długość płodu i ogona. Skrawki wątrób płodowych oceniano w mikroskopie świetlnym po uprzednim wybarwieniu hematoksyliną i eozyną, metodą van Gieson, paS i srebrzeniu siateczki wg Gomorii. Wykazano znamienne ($p<0,05$) obniżenie długości ciała płodów w grupie P3. Zmiany adaptacyjne w wątrobach płodowych, podobnie jak i wylewy podskórne, występowały ($p>0,05$) we wszystkich grupach badanych.

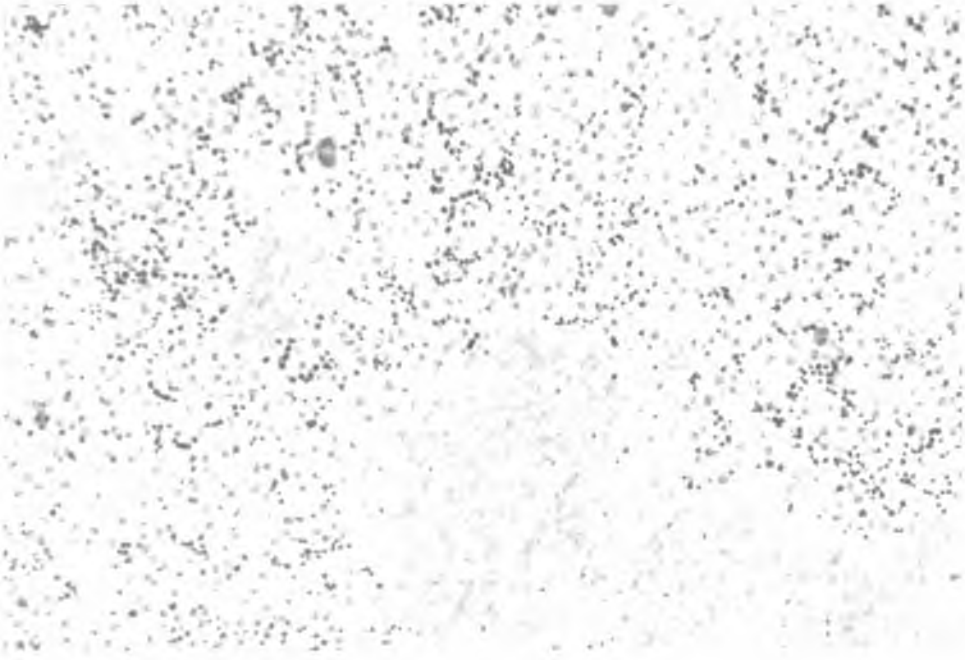

Fig. 1

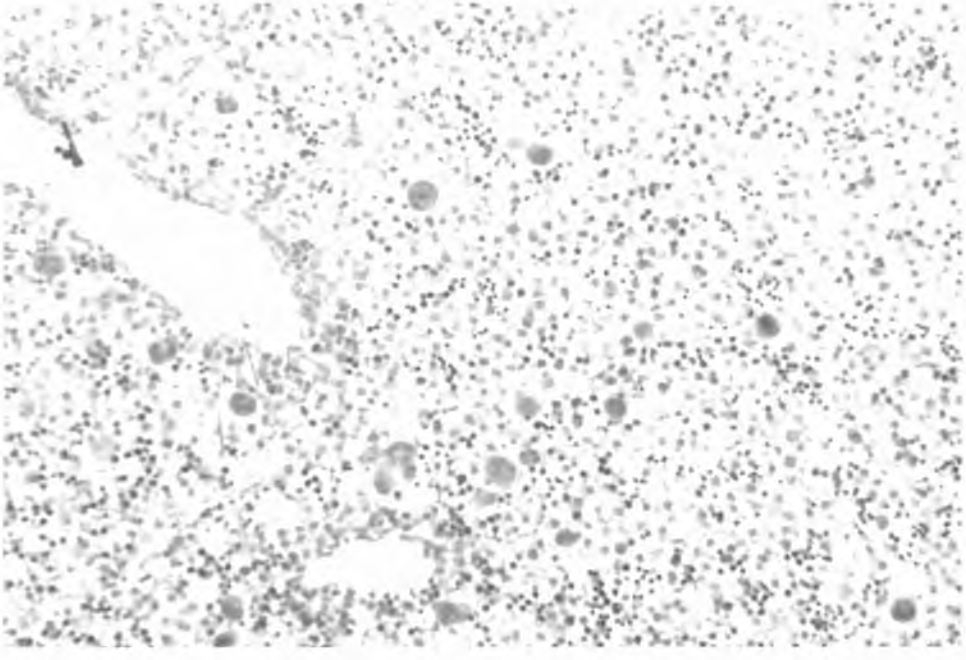

Fig. 2

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