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Pregnancy increases ibuprofen gastrotoxicity in rats

Dynamic processes during pregnancy lead to several biochemical, morphological and functional changes in both the fetus and pregnant woman. The course of some diseases may undergo exacerbation or moderation dependent on the pregnant or non-pregnant condition of the female. Pharmacokinetics of drugs administered may also undergo changes. Despite the high risk of taking medicines during pregnancy, approximately 65% of pregnant women use medicines at their own discretion without any consultation with the physician. It poses a great danger to both mother and fetus (8). However, there are many indications justifying certain medication since several illnesses when untreated can result in more severe complications than the adverse effects of the medicine used (12, 16).

According to many studies pain and fever are dangerous both to fetus and mother (3, 16). The medicines that are often used to relieve these ailments are cyclooxygenase (COX) inhibitors, and among these the arylpropionic acid derivative – ibuprofen. The drug is characterized by a wide spectrum of therapeutic activity. In small doses (200–400 mg/dose) ibuprofen shows analgesic and antipyretic effect whereas in high doses (600–1200 mg/dose) it reveals anti-inflammatory activity. Similar to other nonselective COX inhibitors, ibuprofen tolerance depends on the level of inhibition of COX-1, which increases along with medicine blood concentration. Inhibition of COX-1 is responsible for the damage of gastric mucosa, nephrotoxicity and malfunction of thrombocytes (13, 14).

Gastrointestinal complications, especially gastrotoxicity are the most dangerous and most common accusations against COX inhibitor therapy. Cases of gastrointestinal hemorrhages should be especially emphasized in this context as they may result in not only health but also life-threatening complications. They are often determined not only by COX inhibition therapy itself, but also by individual predisposition, e.g., chronic peptic ulcer, alcohol abuse, cigarette smoking and antithrombotic prophylaxis. The level of ailments intensification accompanying therapy with COX inhibitors, e.g., lack of appetite, nausea, vomiting, meteorism, constipation, diarrhea and abdominal pain, depends on the kind and dose of medicine used (17).

Good tolerance of low doses of ibuprofen accompanied by its high analgesic activity allowed to grant ibuprofen its over-the-counter (OTC) status (9). Despite well-documented tolerance, neither its prenatal nor maternal toxicity has been investigated and recognized sufficiently.

The aim of this study was to evaluate the maternal effects of antipyretic and analgesic doses of ibuprofen when administered during the organogenesis period. To check the drug gestational tolerability non-pregnant groups were also studied. The results come from an ongoing project on the prenatal risk of COX inhibitors.

MATERIAL AND METHODS

This experiment was designed in accordance with international guidelines (7) and the guidelines #133/2001–162/2000 of the Local Bioethical Committee. Sexually mature albino rats of Wistar CRL:(WI)WUBR strain, 12–15 weeks of age, obtained from a commercial breeder (Rembertów-Warsaw, Poland) were used. They were acclimated for 2 weeks. The animals were kept under standard laboratory conditions. Filtrated municipal tap water (Lublin, Poland) and standard laboratory rat feed (LSM®; Motycz, Poland) were provided *ad libitum*.

On mating days, females of the proper weight were placed in cages with males for approximately 14 hours. The following morning, a vaginal smear was performed to determine if copulation had occurred. The day sperm was detected was designated gestation day 0. Sperm- positive females were housed in plastic cages with commercial wood bedding and assigned randomly to the pregnant experimental and control groups with 11 animals in each group.

Ibuprofen (Shaun, India – a gift from Polfa Pabianice, Poland; purity >99%) was ground with Tween-80 (Sigma Chemical Co., Saint Louis MO, USA) and diluted in distilled water. The suspension was prepared and administered intragastrically once daily in a volume of 10 ml/kg from day 8 through day 21 of gestation/experiment to pregnant and non-pregnant animals. The low dose, similar to the human antipyretic and analgesic one, was set at 5.7 mg/kg/dose. The middle dose was increased thirtyfold and set at 171.0 mg/kg. Due to 100% mortality the high dose was reduced from 570 mg/kg to 285 mg/kg (Table 2).

Animals in control groups received the Tween-80 water suspension in volumes corresponding to those given in drug-treated groups. All animals were observed at least three times a day for morbidity and mortality. In cases of morbidity animals were sacrificed by intraperitoneal injection of sodium pentobarbital.

On gestation/experiment day 21, animals were anaesthetized, and decapitated. The internal organs were carefully examined for macroscopic changes. Each stomach was removed by abdominal delivery, cut longitudinally, spread out, pinned to the board and fixed in 10% formalin. Then the samples were embedded in paraffin blocks, sectioned at 5 μm and then stained routinely with hematoxylin and eosin (H & E) and periodic acid Schiff with alcian blue. The slides were examined using Olympus BX45 without knowledge of treatment groups.

Microscopic changes were graded as: (1) superficial (increased cytoplasmic eosinophilia of surface epithelium or focal shedding of surface epithelium but with general preservation of mucosal architecture), (2) profound (coagulative necrosis with erosions or ulcerations of different extension and depth) and (3) perforations. Samples were collected from all the animals, however, some death cases were excluded from microscopic evaluation due to advanced autolytic changes.

The unit for statistical measurement was female. Quantitative continuous data were compared among experimental groups using the Kolmogorov-Smirnov test. Differences in continuous variables were evaluated by ANOVA Kruskal-Wallis, or if the data were not considered to be normally distributed, by the Mann-Whitney U-test. Differences between corresponding pregnant and non-pregnant groups were analyzed by χ^2 test. An $\alpha=0.05$ ($p<0.05$) was considered significant.

RESULTS

There was a 100% mortality incidence of non-pregnant animals exposed to the highest doses of ibuprofen (Table 1). The mortality rate was lower after 285.0 mg/kg.

Decreased activity, dehydration, stained and casted hair were the most commonly observed clinical signs.

Different signs of gastrointestinal toxicity were found during the necropsy of dead or sacrificed animals that were exposed to the middle and high doses of ibuprofen (Table 1). Isolated enlarged stomach with or without grossly visible hemorrhages was seen in drug-exposed groups. Adhesions between intestine loops and pancreas, stomach, sometimes kidney, liver and uterus as

well as enlargement of intestinal loops and peritoneal fluid were seen in some of these animals. There were no macroscopically seen pathological lesions in animals treated with the lowest doses.

Microscopic examination of both control groups found normal stomach morphology, except for two animals from non-pregnant group that showed mild superficial lesions. However, examination of all non-pregnant and pregnant study groups revealed gastric injuries of different stages and extension (Table 1). They were noted exclusively in glandular stomach; however sparse inflammatory infiltration was observed also in mucosa and submucosa of forestomach. In both non-pregnant and pregnant groups superficial lesions (Fig. 1A) were the most common (in 9 and 7 of 33 cases in non-pregnant and pregnant groups respectively), but in pregnant animals the lesions were more extensive. The profound lesions (Fig. 1B and C) were less common (in 3 and 4 of 33 cases in non-pregnant and pregnant groups respectively) but similar to above mentioned lesions in pregnant rats they were frequently multiple. Perforations were rare (in 1 and 2 of 33 cases in non-pregnant and pregnant groups respectively) and seen only in animals exposed to the high dose of ibuprofen (Fig. 1D). Dose-dependence was also observed in cases of superficial and profound lesions. In both study groups, shortening of gastric pits, focal edema of submucosa and sparse inflammatory infiltration mainly composed of lymphocytes, neutrophils and eosinophils in superficial part of mucosa and in submucosa were also observed. Erosions and ulcerations were accompanied by sparse inflammatory infiltrations and regenerative changes in epithelium.

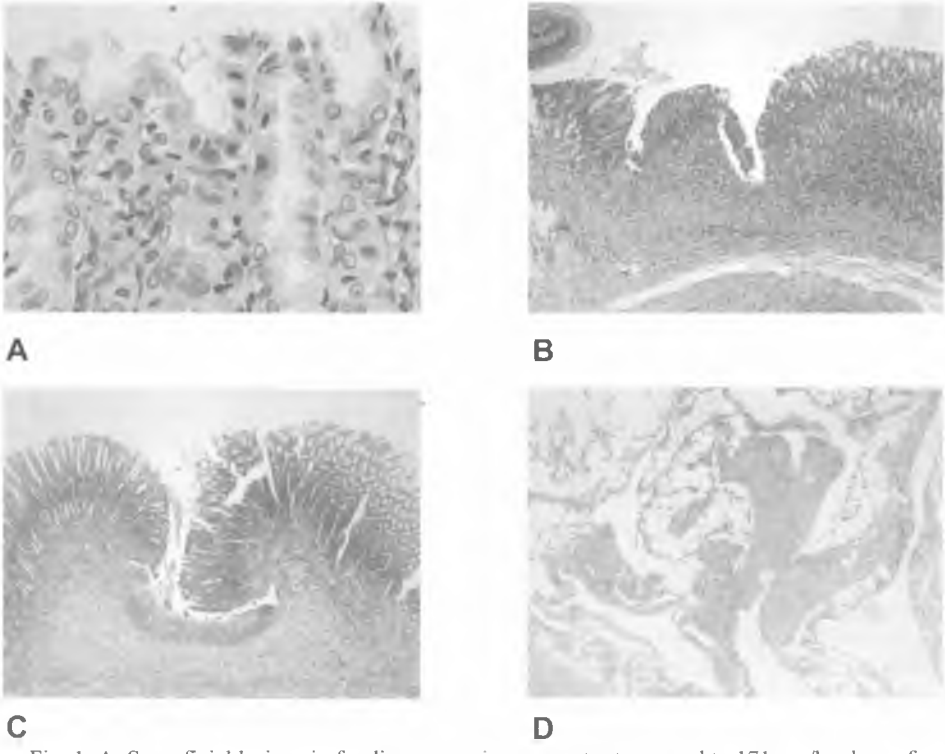

Fig. 1. A: Superficial lesions in fundic mucosa in pregnant rat exposed to 171 mg/kg dose of ibuprofen (H & E, x400); B: Erosions of fundic mucosa with acute inflammatory infiltration in non-pregnant rat exposed to 285 mg/kg dose of ibuprofen (H & E, x100); C: Ulcaration of full thickness of pyloric mucosa and superficial part of submucosa with regenerative changes in pregnant rat exposed to 171 mg/kg dose of ibuprofen (H & E, x100); D: Fibrinopurulent peritonitis due to gastric wall perforation in pregnant rat exposed to 285 mg/kg dose of ibuprofen (H & E, x 100)

Table 1. Group characteristic and histological findings in control and ibuprofen exposed non-pregnant and pregnant (C) animals

Group	Dose (mg/kg/dose)	No of dead animals	No of live animals	Gastrointestinal lesions		
				superficial	profound	perforation
Control		0	12	2	0	0
I1	5.7	0	11	1	0	0
I2	171.0	0	11	2	1	0
I3	285.0	1	10	6	2	1
Control –C		0	12	0	0	0
I1C	5.7	0	11	0	0	0
I2C	171.0	0	11	3	2	0
I3C	285.0	3	8	4	2	2
I4C	570.0	11[a]	0	-	-	-

[a]No histological examination was done

Significant decrease of body weight gain was observed in both pregnant and non-pregnant groups exposed to ibuprofen in doses 171 and 285 mg/kg, when compared with corresponding control group (Table 2). Significant increase of liver weight was noted in non-pregnant groups exposed to the middle and high doses. Lack of kidney weight change was observed.

Table 2. Body weight gain and organs weight in control and ibuprofen exposed group in pregnant and non-pregnant (C) animals

	No of animals	No of dead animals	Body weight changes from day 7 to 18	Weight (g)		
				liver	left kidney	right kidney
Control	12	0	6.3 ± 11.27	10.04 ± 1.43	0.88 ± 0.08	0.88 ± 0.09
I1	11	0	7.0 ± 3.58	11.82 ± 1.08	0.85 ± 0.11	0.90 ± 0.14
I2	11	0	-30.4 ± 4.98^	12.22 ± 0.73^	0.85 ± 0.12	0.95 ± 0.13
I3	11	1	-39.3 ± 16.16^*	12.81 ± 0.71^	0.98 ± 0.12	1.00 ± 0.09
Control-C	12	0	24.0 ± 11.61	13.29 ± 0.72	0.99 ± 0.05	1.02 ± 0.05
I1C	11	0	16.8 ± 19.32	14.60 ± 2.16	0.93 ± 0.07	0.96 ± 0.45
I2C	11	0	-15.3 ± 24.96^*	14.34 ± 1.14	1.03 ± 0.08	1.07 ± 0.12
I3C	11	3	-29.6 ± 8.17^*	14.62 ± 1.16	1.13 ± 0.26	1.89 ± 0.44

^ $P \leq 0.05$ compared to control

* $P \leq 0.05$ compared to low-dose group

DISCUSSION

The present study confirmed the harmful effect of non-selective COX inhibitors on the stomach mucus and similar to the previous reports (1) showed increased ibuprofen toxicity during pregnancy.

Similar results were reported by A d a m s et al. (1), who observed 100% mortality in rats treated with ibuprofen in the dose of 549 mg/kg. Macroscopic ulcerations of the gastrointestinal tract and simultaneous peritonitis were noted. Additionally gastric and intestinal perforations were present in dogs and rabbits. At the dose of 180 mg/kg significant decrease of red blood cells count, hemoglobin concentration and hematocrit was present in rats. As in this study, significant increase

of liver weight was observed in non-pregnant animals. Higher mortality and severity of histological changes in gastric mucosa in pregnant versus non-pregnant groups was found.

Similar toxic effect of ibuprofen was obtained by Adeyeye et al. (2) who administered ibuprofen suspension in the doses of 17 mg/kg and 44 mg/kg and continued the study after 30 minutes by inducing irritation of the stomach wall with bethanechol chloride in the dose of 5 mg/kg. Observations of the stomach 7 hours after medicine administration revealed significant increase in the number of damages compared to the control group. Mostly superficial, ibuprofen dose-dependent lesions were found. After administering a suspension containing medicine molecules covered with tough paraffin, fewer amounts of gastrointestinal tract lesions were observed compared to the administration of pure ibuprofen suspension. Such histological changes were also reported when ibuprofen were administered in triple daily doses 255.0–600 mg/kg/day (6). However, the drug tolerability was lower for pregnant than non-pregnant rat. Such observations were done also for other nonselective COX-2 inhibitors, e.g., tolmetin, piroxicam and selective one – DFU.

Suleyman et al. (18) studied the preventive effect of nimesulid and ranitidine on the rat stomach inducing at the same time gastrotoxic effect with indometacine and ibuprofen. Ulcerations of various shape and depth, mainly superficial, were observed when animals fasting for 24 hours were administered ibuprofen in the dose of 400 mg/kg. The average ulceration area was 9.5 mm^2. Ulcer niche decreased to 2.0 mm^2 when the animals were administered 150 mg/kg of ranitidine at the same time. Ulcerations of the stomach were not observed when ibuprofen and nimesulid were administered at the same time.

Another confirmation of the positive relationship between ibuprofen administration and appearance of gastrointestinal ulceration are the results of a study by Beck et al. (4). Significant increase of gastric ulcer index (number of ulcers x diameter) and intestinal damage (% of surface damaged) were noted 24 hours after intragastrical administration of 100 mg/kg of the drug. Such toxicity was not observed among animals that were fed during the time of the study. Small superficial lesions of the mucous membrane of the stomach and intestines were observed when ibuprofen was administered in the dose of 25 mg/kg orally and 100 mg intravenously. No relation to feeding the animals was observed.

In contrast to Adams (1) and our results, no toxic effect on dams' organism was observed by Golovanova et al. (10), who treated rats in various periods of pregnancy with ibuprofen in the dose of 100 mg/kg. However, higher general toxicity of COX inhibitors was previously reported also for paracetamol, propyphenazone and other drugs (5).

Unlike experimental studies clinical ones emphasize safety of large doses of ibuprofen compared to other COX inhibitors. Perez-Guthann et al. (15) analyzed data of 123,895 patients treated with naproxen, diclofenac and ibuprofen and found that the estimated hazard ratio of upper gastrointestinal tract injury amounted to respectively 2.3, 1.8, and 0.4 per 10,000 patients. Moore et al. (14) confirmed high tolerability of maximal OTC daily dose of ibuprofen – 1.2 g with acetylsalicylic acid (3.0 g) and paracetamol (3.0 g). The drug tolerability was higher than acetylsalicylic acid and comparable with paracetamol. The most frequent adverse effects reported by patients were associated with the alimentary system, including stomachache and dyspepsia (respectively 2.8% and 1.4%). Consistent results concerning maximal daily dose were presented by Doyle et al. (9), who examined a group of 833 patients treated with ibuprofen in the dose of 400 mg three times a day for at least 10 days. No increase of the gastric mucosa lesions was found during the endoscopic examination. The analysis of the data coming from 12 centers showed that the risk of gastrointestinal damage was the lowest at the analgesic doses of ibuprofen, and that drug tolerance was decreasing with the increase of the dose. The calculated risk of mucosal lesions at the anti-inflammatory dose was similar to the risk characterizing low doses of naproxen and indomethacin (11)

Due to high anti-inflammatory activity of ibuprofen and its low toxicity, the European Antirheumatological Union recognized ibuprofen as the safest among all non-steroidal anti-inflammatory drugs (16).

CONCLUSIONS

Based on the obtained results it could be stressed that the lowest dose of ibuprofen used in this study (5.7 mg/kg) is the no observed adverse effect level (NOAEL) since higher doses (171 and 285 mg/kg) caused gastric mucosal injury and decreased animal growth. Non-pregnant animals better tolerated the drug.

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SUMMARY

The aim of the study was to examine ibuprofen gastrotoxicity in pregnant and non-pregnant rats. The drug was administered on gestation/experimental day 8 through day 21, once a day, intragastrically in Tween-80 water suspension in three doses: 5.7, 171.0 and 285 mg/kg. On gestation/experiment day 21, animals were sacrificed. Each stomach was removed by abdominal delivery, examined macro- and microscopically. Dose-related superficial lesions of the gastric mucosa were found. More extensive changes were found in pregnant groups. Perforations were rare and seen only in animals exposed to the high dose of ibuprofen. The present study confirms the harmful effect of ibuprofen on the stomach mucous, especially in pregnant rats.

Ciąża nasila gastrotoksyczność ibuprofenu u szczura

Celem pracy była ocena gastrotoksyczności ibuprofenu u ciężarnych i niecięzarnych samic szczura. Lek podawany był od 8 do 21 dnia ciąży/badania, jeden raz dziennie, dożołądkowo w wodnej zawiesinie Tween'u 80, w trzech dawkach: 5,7; 171,0 i 285 mg/kg. W 21 dniu zwierzęta uśmiercano. Oceniano budowę makro- i mikroskopową żołądka. W pracy stwierdzono obecność powierzchownych, zależnych od dawki, uszkodzeń błony śluzowej. Obserwowane zmiany były bardziej nasilone wśród samic ciężarnych. Sporadycznie, w grupach narażonych na najwyższą dawkę, obserwowano perforacje ściany żołądka. Wyniki badań wskazują na gastrotoksyczność ibuprofenu, która była wyższa wśród samic ciężarnych.