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REPRODUCTION STUDIES WITH PRANOPROFEN, A NONSTEROIDAL ANTI-INFLAMMATORY AGENT IN RATS-TERATOLOGY TEST

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ABSTRACT: *Teratological study on pranoprofen, as antiinflammatory agent, was conducted by oral intubation in Sprague-Dawley rats. Pranoprofen was administered doses of 1.0, 2.5 and 5.0mg/kg/day and doze of 5.0mg/kg/day of Indomethacin was used as positive control. The rats were dosed from day 7 to 17 of gestation. At necropsy on day 20 of gestation, pathologically changes of gastrointestinal system, liver and adrenal gland were examined at the high dose administered group.*

There were no differences between control and treated group on the number of implantations, the number of alive and dead fetuses, tail length, and external, visceral and skeletal malformations.

But indomethacin treated group showed significant differences in resorption rate, maternal mortality, body weight of fetus and retarded fetal ossification. Pranoprofen seems to have no apparant risk of teratogenicity and the maximum no effect dose level was estimated to be 2.5mg/kg/day.

Keywords:*Teratological study of pranoprofen*

INTRODUCTION

Pranoprofen, a propionic acid derivative with three cyclic structure, is used as analgesic, antipyretic and anti-inflammatory agent(Drugs, 1983). It was newly developed in 1981 by Gil Boo Pharmacy Company in Japan. This non-steroidal antiinflammatory agent, like durgs of this kind, is used clinically in the treatment of rheumatoid and other types of arthritis(Gaut Z.N. 1975). Pranoprofen, salicylates and pharmacologically similar antiinflammatory agents such as indomethancin blocks the biosynthesis of prostaglandin by inhibiting the enzyme, prostaglandin synthetase (Piper and Vane, 1969; Vane, 1971), and subsequent reduction of peripheral prostaglandin level occurrs.

Generally non-steroidal anti-inflammatory analgesic agent is known to have some side-effects on the gastrointestinal systems and the reproduction system (Aiken, 1972; Wanka 1964; Lövgren, 1964; Hucker 1966; Rotemich, 1966; Menguy, 1967; Selye, 1969; Menasse-Gdynia, 1974).

In case of indomethacin, several defects were reported that the maternal death and decreased body weight, the fetal death and decreased body weight, decreased fetal ossification, decreased uterine contraction and delayed initiation during animal experiments (Chester, 1972). And pranoprofen is used only in Japan nowdays.

So that this studies were conducted for the evaluation of teratogenic effect on prano-

profen in rats.

These studies were conducted by the NIH guideline No. 267 (1985. 4. 1), guidelines for the test method of specific toxicity test on durgs. Last year, we examined the effect of fertility of pranoprofen. The present studies were conducted to investigate the effects of pranoprofen on fetal teratogenicity.

MATERIALS AND METHODS

Experimental Animal and Administration Methods.

Sprague-Dawley rats were bred in NIH rat breeding room. The animal rooms were maintained at 23±2°C and 55±10% humidity with a 12hr light-dark cycle. Animals were fed Hanil Chow diet and tap water *ad libitum*. Good conditioned and normal body weighed 12 weeks old female rats without pregnancy history and 13 weeks old male rats were used in the experiment. Estrus cycle of female rats were investigated by Long and Evance methods. Primiparous female rats were mated with males rats from 5 P.M. to 9 A.M. next day and then confirmed sperm from vaginal fluid by microscoping. This is day zero of gestation when sperms were obsered. The period of mating was from day zero of gestation to the second estrus.

The test compound, pranoprofen, is 2(5H-[1] benzopyrano (2,3-b) pyridine-7-yl) propionic acid with slightly bitter taste, white or yellowish white colored crystal compound.

The appropriate amount of pranoprofen was suspended in 100 ml of 0.5% carboxy methyl cellulose (CMC) with 2 drops of Tween 80 added the suspensions were prepared freshly each day just prior to use. Pranoprofen was administered daily by oral intubation for a period of 11 days from Day 7 to Day 17 of gestation with a dose volume of 10ml/kg. The pregnant rats were divided into 6 groups and each group consisted of 20 rats; non-treated control group, CMC vehicle control group, indomethacin group as positive, 1.0, 2.5, 5.0mg/kg of pranoprofen treat groups. Pranoprofen dosages were selected on the base of maximum tolerance level and non-toxic effect level derived from chronic toxicity test(浜田佑二, 今村, 1976). Indomethacin was used as a comparative compound on the base of reported teratogenicity test (Physician's, 1985).

A. Ⅰ Group: non-treated group
B. Ⅱ Group: CMC vehicle control group
C. Ⅲ Group: indomethacin treated group
D. Ⅳ Group: 1.0mg/kg pranoprofen group
E. Ⅴ Group: 2.5mg/kg pranoprofen group
F. Ⅵ Group: 5.0mg/kg pranoprofen group

General conditions, body weight, food and water consumptions were observed every day.

Effect of Fetuses

On the 20th day of gestation, the fetuses were removed by Cesarean Section (C.S.) from dams under ether anesthesia. Visceral anomalities were observed first and then all the uteri were grossly examined and the number of corpora lutea and fetuses alive or dead fetuses, implantation sites, resorption sites, and sex were recorded.

Live fetuses were weighed, body and tail lengths, placenta weight were measured and external malformations were examined grossly.

Using Salewski's method (Salewski, 1964), number of implantation sites were investigated from vague uteri.

Then one forth of live fetuses were chosen by random sampling from each dam and were fixed in Bouin's solution for 1 week.

They were examined for cerebral, eye, visceral malformations by Wilson's free hand razor

blade section method (Wilson, 1965), with the optical microscope. The remaining three–forths were fixed in 70% ethanol's solution for 2 days and were prepared for skeletal examination using a KOH–alizarin red S staining procedure by Dawson's method (Dawson, 1926).

The experimental results were compared with control group using X^2–test, (佐久間昭, 1975) t–test (Weil, 1970) and ranks and sum of ranks (佐久間昭, 1965).

RESULTS

Effect of Dam

The body weight change of the pranoprofen–treated rats were shown in Fig 1 and autopsy results of dams were summarized in Table 1.

Table 1. Results of teratology study in rats I. (Observations of dams).

Compound (mg/kg)	non–treated	C.M.C	Pranoprofen			Indomethacin
			1.0	2.5	5.0	5.0
• No. of dams	20	20	20	20	20	20
• No. of dead dams	0	0	0	0	2	10
• No. of nonpregnant dams	0	0	0	0	0	4
• No. of live dams	20	20	20	20	18	6
• Total implants (Litter size)	227 (11.4)	250 (12.5)	238 (11.9)	235 (11.8)	221 (12.3)	74 (12.3)
• No. of corporal lutea	11.9	12.2	12.33	11.8	12.3	14.0
• No. of dead fetuses (%)	16 (7.0)	25 (10.0)	23 (9.6)	12 (5.1)	11 (5.0)	27 (36.5)
• Resorption (%)	10 (4.4)	22 (8.8)	12 (5.0)	10 (4.3)	9 (4.0)	3 (4.1)
• Placental remnants(%)	2 (0.8)	3 (1.2)	1 (0.4)	1 (0.4)	0 (0)	0 (0)
Macerated fetuses (%)	4 (1.7)	0 (0)	10 (4.2)	1 (0.4)	2 (1.0)	24 (32.4)
• No. of live fetuses	211	225	215	223	210	47
• Gross observation in autopsy						
• Liver:congestion, discoloration	1	2	1	2	2	4
• Lung:interstitial pneumonia	1	1	0	0	1	0
• Small intestine:						
jejunum and ileum ulcer	0	0	0	1	2	3
perforation and adhesion	1	2	2	2	4	1
• Spleen:discoloration & hypertrophy	0	0	0	2	5	1
• Adrenal: hypertrophy	0	0	2	1	2	3

There were no special change of body weight between pranoprofen 1, 2.5mg/kg/day treated groups and control groups (non–treated, CMC vehicle) and also no change in general condition and behavior.

In pranoprofen 5mg/kg/day treated gorup 2 of 20 dams were died on day 18 and 19 of gestation respectively and a marked depression in the body weight gain from day 16 of gestation. Intestinal toxicity such as: adhesions, ulcerations were observed from 2 dead cases. After day 18 of gestation the body weights were increased and there were no significant differences as compared to controls. In the indomethacin group 10 female rats were died from 11th to 18th day of gestation. They didn't drink water and intake feeds for 1 day or 2 days before dying with decolored hair neck and decreased body weight. In

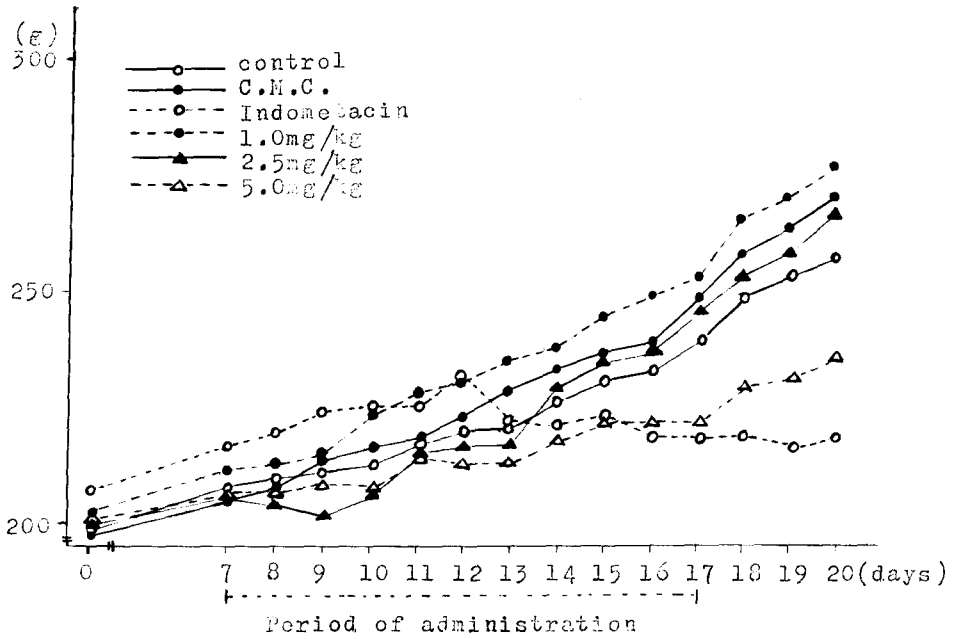

Fig. 1. Body weight changes of pregnant rats administered orally with pranoprofen.

postmortem examination, ascites, gastrointestinal ulcerations, decoloration and enlargement of spleen, congestion of liver, gassed stomach and intestinal adhesions were observed. In the indomethacin group, there was an evidence of decreased body weight from day 13 of gestation and especially on day 19 of gestation high body weight decrease were observed. There were no statistically significant differences between controls and 5mg/kg/day of pranoprofen treat group. In the indomethacin group, neither live fetuses nor implantation sites were obtained in 4 out of 20 female rats despite confirmed pregnancy. And a depression in the body weight gain were observed from day 10 to day 20 of gestation. At necropsy, there were not observed remarkable pregnancy conditions, the lesions of ovary and other organs.

Female rats were sacrificed on day 20 of gestation and the results were summarized in Table 1. There were no significant differences in the number of corpora lutea and implants between the treated and control groups.

For gross observation of major organs, one case of decolored and congestion of the liver, one case of abnormal lung and one case of perforative and adhesion small intestine were observed in non-treated group.

Also, similar symptoms were noted several cases in the CMC vehicle, 1.0 and 2.5mg/kg/day pranoprofen treated groups. In the 5.0mg/kg/day pranoprofen treated group, especially lesions of liver, small intestine and spleen were observed. In the indomethacin treated group, lesions of liver, small intestine and remarkably in adrenal gland were observed. The lesion incidence was 7.4% in the pranoprofen treated group and 25% in the indomethacin treated group, the lesion incidence in the indomethacin treated group was significantly higher than the control group.

Effect on Fetuses

The results obtained from the examination of gross observation and skeletal systems of fetuses were given in table. 2,3.

Table 2. Results of teratology study in rats II. (Gross observation of fetuses).

Compound (mg/kg)	0	C.M.C	Pronoprofen			Indomethacin
			1.0	2.5	5.0	5.0
No. of fetuses examined	211	225	215	223	210	47
boby weight of survivors (g±S.E)	3.83 ±0.24	3.84 ±0.37	3.82 ±0.14	3.95 ±0.32	3.86 ±0.17	3.39 ±0.41
Body length of survivors (cm±S.E)	3.90 ±0.04	3.86 ±0.03	3.84 ±0.04	4.23 ±0.03	3.92 ±0.04	3.52 ±0.04
Tail length of survivors (cm±S.E)	1.25 ±0.01	1.26 ±0.04	1.26 ±0.01	1.27 ±0.03	1.26 ±0.02	1.25 ±0.05
Sex ratio M/F	129/98 (1.31)	118/107 (1.10)	105/110 (0.95)	118/105 (1.12)	111/99 (1.12)	25/22 (1.14)
Weight of placenta (ζ ±S.E)	0.52 ±0.06	0.48 ±0.07	0.48 ±0.04	0.53 ±0.07	0.52 ±0.03	0.45 ±0.13
Gross observation						
• Micrognathia (Mandibula)	0	0	0	0	1	0
• Hydrops anasarca	0	0	0	0	1	0
• Club foot	0	0	0	0	0	1
Visceral anomalies						
• No. of fetuses examined	54	60	54	60	55	12
• No. of visceral anomalies	0	0	0	0	0	0

Table 3. Results of teratology study in rats III. (Skeletal observation of fetuses)

Compound (mg/kg)	0	C.M.C	Pranoprofen			Indomethacin
			1.0	2.5	5.0	5.0
No. of fetuses examined	157	165	161	163	155	35
No. of fetuses with skeletal anomalies	0	0	0	0	1	4
No. of fetuses with skeletal variation	0	0	10	2	2	0
Deficinet ossificantion of sternebra	2	2	12	4	11	9
Anomalies						
• Hypoplasia of cranial bone	0	0	0	0	0	4
• Absence of mandibula	0	0	0	0	1	0
Variations						
• Shortening of the 12th rib	0	0	0	1	0	0
• Absence of the 13th rib	0	0	0		0	0
• Absence of ossification centers in vertebral bone	0	0		0	2	0
• Absence of lumber, caudal	0	0	10	0	0	0
• Absence of tibia	0	0		1	0	0
Eye ossification	0	0	4	0	5	5

The number of mean live fetuses and body weight change were not significant compared with controls, but indomethacin treated group was shown a little lower than the control.

The death rate of fetuses in 1.0, 2.5 and 5.0mg/kg/day of pranoprofen treated groups were shown equal to that of control groups. Indomethacin treated groups were significantly higher than the control groups maceration embryo cases were observed from 24 among 27 still birth.

There were 50% of implantation absorption site in 1, 2.5, 5.0mg/kg/day pranoprofen treated and control groups.

Fig. 2. A fetus with absence of mandibula, from a dam at Pranoprofen 5.0mg/kg treated group (right) and a normal fetus (left)

Fig. 3. A fetus with hydrops anasarca, from a dam at Pranoprofen 5.0mg/kg treated group

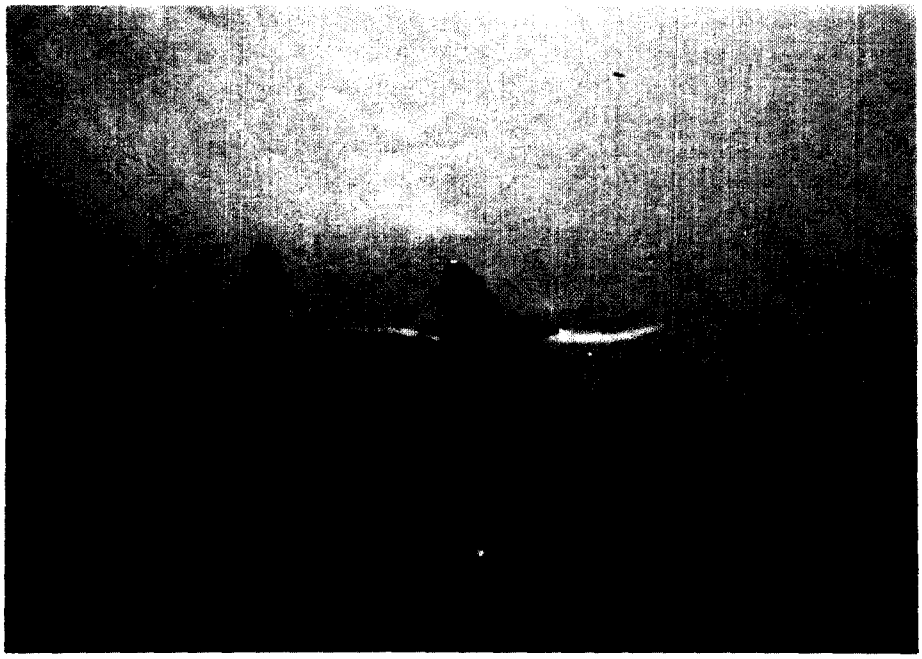

Fig. 4. A fetus with club-foot, from a dam at Indomethacin 5.0mg/kg treated group

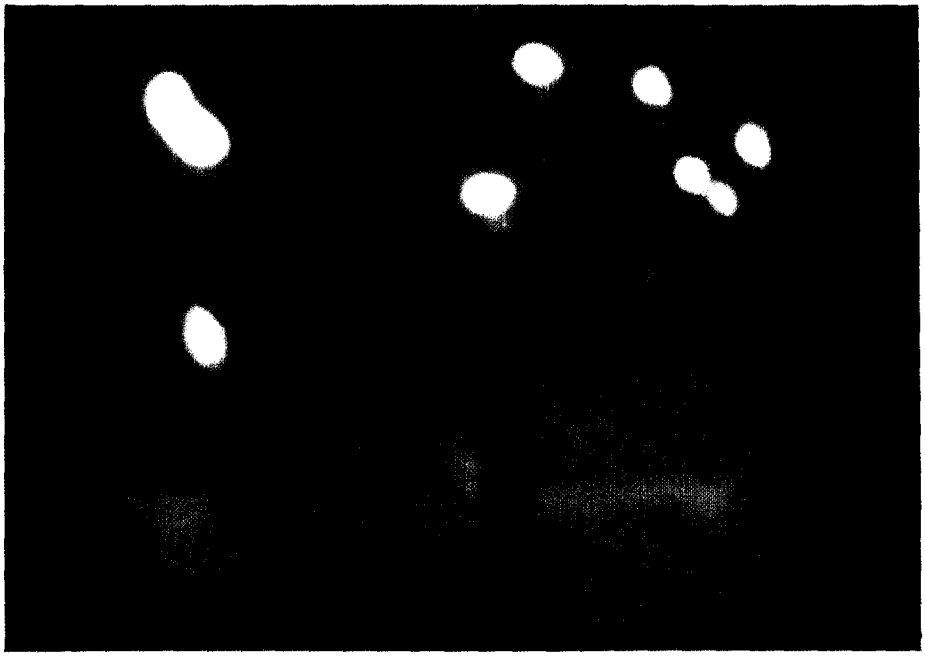

Fig. 5. Deficient ossification of sternebra

Body weight, tail length, placenta weight of live fetuses of treated groups were not significantly to those controls. (Table 2,3) From the external investigation of fetuses different on case of mandibula absence (Fig. 2) and one case of hydrops anasarca (Fig. 3) were noted in the 5.0mg/kg/day pranoprofen. One case of clubfoot was observed in the indomethacin treated group (Fig. 4).

Skeletal anomalies were observed one case in the 5.0mg/kg/day pranoprofen treated group and four case in the indomethacin treated group. Skeletal variation were observed several cases in the 1, 2.5 and 5.0mg/kg/day pranoprofen treated groups.

Deficient ossifiction of sternebra were observed all groups include control groups. (Fig. 5): 25.7% of indomethacin treated group, 1.3% of control group and from 1.2% to 7.5% of others. The reseult of skeletal observation of fetuses were summarized in Table 3.

The indomethacin treated group was significantly different those of control groups. From the 1.0mg/kg/day pranoprofen treated group one case of mandibula absence was observed and the case was previously proven it at external investigation.

Skeletal anomalies of parietal bone were observed four case (11.4%) only in the indomethacin treated group. (Fig. 6.) The absence of the 13th rib and shortening of the 12th rib were observed one case in 2.5mg/kg/day pranoprofen treated group (Fig. 7) and absence of ossification centers in vertebral bone and the absence of lumber, caudal (Fig. 8) and the absence of tibia were observed ten cases at the 1.0mg/kg/day pranoprofen treated

Fig. 6. Hypoplasia of cranial bone

group. One case of tibia absence was observed at the 2.5mg/kg/day pranoprofen treated group. Two case of ossification absence center in vertebral bone were observed at 5.0mg/kg/day pranoprofen treated group. But such incidences of abnormalities were not significant in all groups compared with controls. Eye ossification cases were observed four and five cases at the 1 (2.5%), 5.0 (3.2%)mg/kg/day pranoprofen treated groups, respectively, and five cases at the indomethacin treated group (14.3%) (Fig. 9). There were several reports on

Fig. 7. Shortening of the 12th rib and absence of the 13th rib.

Fig. 8. Absence of lumber, caudal

Fig. 9. Eye ossification

the effect of indomethacin such as corneal deposite, retinal disturbance in man but no case was observed in this animal experiment.

DISCUSSION

This study was followed by the guidlines for the method of experiment of specific toxicity on drugs. As a anti-inflammatory and analgesic agent, pranoprofen, was administered in pregnant rats during the period of organogenesis for the investigation of teratologic effect. Pranoprofen, pharmacologically similar to non-steroidal anti-inflammatory, analgesic, and antipyretic agent such as indomethacin is reported to be more effect than indomethacin. Indomethacin and other non-stroidal anti-inflammatory agents were reported as an inhibitor of prostaglandin (PG) synthesis, resulting an inhibition of ovulation, decreased uterine contraction, prolongation of parturition and a delayed initiation. Dosages were selected on the base of the maximum tolerance dose from the chronic toxicity tests of pranoprofen which has strong anti-inflammation effect. In this study, the 1.0, 2.5 and 5.0mg/kg/day pranoprofen was administered to pregnant rats body weight decrease in the 5mg/kg/day pranoprofen treated group and two deaths cases were observed. From postmortem examination of these dead rats, intestinal adhesions and ulcerations were observed.

These were quite similar symptom as other non-steroidal anti-inflammatory agents have (Wanka, 1964; Lövgren, 1964; Hucker, 1966; Rothermich, 1966; Menguy, 1967; Selye, 1969; Menasse Gdynia, 1974).

In the indomethacin group body weight decrease was remarkably shown ten outs of twenty dams died during experiment and four cases of the copulatory plug were not pregnated in this group.
The other six cases also showed severe body weight decrease.
Toxic symptom and deaths of dams such as these results were reported other animal experiments (Physician's, 1985). At the 5mg/.kg/day pranoprofen treated group, toxic symptom were apparently observed. There was high fetal mortality rate in the indomethacin treated group as 88.9% by maceratis embryo but in the pranoprofen treated group motality rate was 50% and it was caused by the absorption of implantation site.
It was reported that the increase of fetal absorption and the decrease of fetal body weights when administered indomethacin.
The fetal body weight of indomethacin treated group was a little bit lower than the control or pranoprofen treated groups but not significantly different. From external anomalies of fetuses, one case of mandibula and one case of hypoplasia of cranial bone were found in the highest pranoprofen treated group and one case with club–foot in the indomethacin treated group but internal organ anomalies of fetuses were not found. The skeletal anomalies were found one case in the 5mg/kg/day pranoprofen treated group and four cases in the indomethacin treated group. The skeletal variations were observed several cases only in the pranoprofen treated groups. Compared with Palmer's report(1972), occurrence of spontaneous fetal deformities were considered the incidence of spontaneous abnormalities and appearance. The incicdence of deficient ossification of sternebra was obtained 25.7% in indomethcin treated group and this result was significantly different compared that of 1.3% of control group. Also, the hypoplasia of parietal bone was found only in the indomethacin treated group. In the 2.5mg/kg/day pranoprofen treated group one case of the 13th rib absence shortening of the 12th rib and one case of the tibia absence were observed. In the 1.0mg/kg/day pranoprofen treated group, 10 cases of the hypoplasia of ossification centers in vertebral bone, the absence of 1 umber, caudal, tibia were observed. Two cases of hypoplasia of ossification centers in vertebral bone was observed in the 5.0mg/kg/day pranoprofen treated group.
It seems that incidence of anomalies and the anomalous types were not caused by the drug administration compared with control group and other reports.

Especially, eye ossification was observed 4 and 5 cases at the 1, 5mg/kg/day pranoprofen treated groups respectively and 5 cases at the indomethacin treated group. This case was not found at the studies on teratogenicity of pranoprofen in Japan. Although there were the reports on the occurrence of corneal deposit and retinal disturbance in clinical data, but there was no case reported from animal experiment. Furthermore, it is considered to study further on the teratologic effect of these kind of drugs. Following the guidelines for the method of specific toxicity test on drugs of NIH, pranoprofen was administered to pregnant rats during the organogenesis from day 7th to 17th of gestation.

The pranoprofen was given once daily at doses of 1, 2.5 and 5.0mg/kg/day and also 5.0mg/kg/day of indomethacin was administered as positive control. From this studies, we obtained these results as below.

1. In the pregnant rats, 1 and 2.5mg/kg/day pranoprofen treated groups and control groups were not showed significant difference in general symptoms, the average number of corpora lutea, implantation sites and the average weight of uterus throughout the gestation period. 5.0mg/kg/day of pranoprofen treated group was noted in the decreased body weight gain and 2 death cases. Indomethacin treated group, as a positive control was shown 10 death cases 4 cases of nonpregnant and abruptly decreased body weight gain.
2. Among the 20 day of gestation fetus, below 5.0mg/kg/day of pranoprofen treated groups

were not significant on the average number of live fetus, body weight and the death rate, but indomethacin treated group was showed 36.5% of high death rate.

3. From external organ observation, eye ossification cases were noted at 1, 5.0mg/kg/day pranoprofen treated groups and indomethacin treated group. From the above results, it seemed that these abnormalities were caused by spontaneous occurrence but not the drugs. In conclusion, pranoprofen was not teratogenic agent to the rat and maximum tolerence level was estimated as 2.5mg/kg/day.

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