CORRELATION OF TERATOGENICITY OF ASPIRIN TO THE STAGESPECIFIC DISTRIBUTION OF SALICYLIC ACID IN RATS

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Abstract—A study was made of the stage-specific teratogenicity in rat fetuses from dams given different dosages of aspirin throughout and also during 3 subdivided organogenetic periods of pregnancy (days 8–10, 11–13 and 15–17 of gestation). The stage-specific teratogenicity of the drug, from the aspect of the dam-fetus distribution pattern of salicylic acid during the respective periods of pregnancy was also determined. The stage when the malformations, typical of aspirin toxicity (cranioschisis, spondyloschisis, abdominal fissure, cleft palate) were most frequent was from day 8 to day 10 of gestation. Even in this period when the fetuses were most susceptible to this teratogen, however, the drug was not teratogenic unless it was ingested for 3 or more consecutive days. There were no significant alterations in the maternal plasma levels of salicylic acid during the respective periods. During the first 8–10 days of gestation, the fetal levels of salicylic acid remained significantly high (p<0.05) at any stage of measurement during the 10 hours after application of aspirin, compared with the maternal plasma levels. Furthermore, as pregnancy progressed, the amount of salicylic acid transferred to the fetuses tended to gradually decrease, despite of the fact that the maternal plasma levels of the agent remained fairly stable. On the other hand, the placental levels of salicylic acid tended to be increasingly elevated with the progression of pregnancy. Thus, salicylic acid is most readily transferred to the fetuses at high concentrations at days 8–10 of gestation. Also, from the finding that aspirin proved to be teratogenic when the fetuses were exposed to a given period (3 days or more), the teratogenic action of aspirin is attributed to a direct action of this compound.

The teratogenicity of aspirin and salicylic acid derivatives (1–4) has been reported, but there is little documentation of the fetal susceptibility to these drugs during gestation and of the factors relating to the susceptibility (5–7). Furthermore, the dose of aspirin which is teratogenic varies among the different reports (8–11). In an attempt to search for an animal model for malformations typical of aspirin (exencephaly, spondyloschisis, visceral ruptures and cleft palate) we gave aspirin in different doses to pregnant rats throughout the organogenetic stage (days 7–17 of gestation). The stage-specific teratogenicity of the drug during 3 different periods of the organogenetic stage was then studied (Experiment I). In Experiment II, the dam-fetus distribution pattern of salicylic
acid was studied as a factor relating to the aspirin susceptibility of the fetuses during the stages of gestation.

MATERIALS AND METHODS

Sprague-Dawley (S.D.) rats were used in all experiments. Ten to 12-week-old nulliparous females were placed with a male rat in the same cage overnight. Females with vaginal plugs detected on the following morning were considered to be in day 0 of pregnancy.

Experiment I: Aspirin was given in doses of 100, 250 and 300 mg/kg/day. The control animals were given the same volume of 0.5% CMC solution (vehicle). The dosing was performed in 4 periods: (1) days 7-17, (2) days 7-11, (3) days 10-15, and (4) days 13-17 of gestation; and in the respective periods, 300 mg/kg, daily p.o. by gastric tube. The rats were killed under ether anesthesia at day 21 of gestation; the abdominal cavities were opened, and examined for the number of implantation scars, number of viable fetuses, number of dead fetuses, and number of resorbed embryos. The viable fetuses were examined for the sex and abnormalities in external appearance, and then weighed individually. The fetuses with obvious malformations (exencephaly, spondyloschisis, abdominal fissure, etc.) were photographed.

Experiment II: Because aspirin at 300 mg/kg/day produced a marked weight loss in the dams and gave rise to high incidences of fetal resorption, 250 mg/kg/day was given in the experiment of the dam-fetus transfer of salicylic acid. The drug was given p.o. once daily in each of the following 3 different 3-day periods: (a) days 8-10 (early stage), (b) days 11-13 (middle stage), and (c) days 15-17 (late stage) of pregnancy. The dams (N=4-6) were decapitated 1, 4 or 10 hours after the final dosing in each period. The blood was collected and the fetuses removed. The fetuses together with the placentas were homogenized with 5 M NaH₂PO₄. All 8- to 10-day-old fetuses from the same dam were regarded as one sample. The 11- to 13-day-old fetuses from the same dam were separated into 2 to 3 groups and the 15- to 17-day-old ones also from the same dam were separated into 4 to 5 groups for homogenation. Because it was difficult to separate the 8- to 10-day-old placentas from the fetuses, the placentas and the fetuses were homogenized together; the 11- to 13-day-old and the 15- to 17-day-old placentas were divided into 2 to 3 parts for homogenation, respectively, as were the fetuses at the same ages. The aspirin and salicylic acid extracted from the bloods of the dams, fetuses, placentas and amniotic fluids were assayed by thin layer chromatography using a hydrogen flame ionizing detector (the Yatron scan model TH-10 TLC analyzer).

RESULTS

Experiment I

1. Effect on pregnant dams
   a. Groups dosed throughout the organogenetic stage: Figure 1 illustrates the weight gain curves of the dams, with the body weight at day 7 of gestation as 0. The animals at 100 mg/kg/day showed statistically significant weight gains on days 15-19 of gestation, compared with the control animals, while the animals given 250 mg/kg/day showed weight gains similar to those in the control group. In the dams given this dosage, the weight gain remained inhibited until the time of delivery. Figure 2 illustrates the food consumption of the dams. The rats on 300 mg/kg/day of aspirin showed no marked difference in food consumption from the control group, except for a slight decrease in the early stages of pregnancy. Gross examination revealed
piloerection and alopecia in the hindlimbs or the abdominal region in some of these rats.

b. Groups dosed in subdivided organogenetic periods: Figure 3 depicts the weight gain of dams given 300 mg/kg/day of aspirin in 3 subdivided periods of the organogenetic stage, i.e., days 7–17 of gestation. In each dosed group, statistically significant retardations (p<0.05) in weight gain were noted in the early stage of dosing. On withdrawal of the drug, the animals regained body weight, and at about day 19 of gestation, the weight was much the same as in the controls.
Fig. 3. Effects on body weight of aspirin given to dams p.o. 5–6 consecutive days during subdivided organogenetic periods of pregnancy.

Table 1. Effects of aspirin on fetuses following oral dosing to the dams during days 7–17 of gestation

| Dose (mg/kg/day) | Controla) | 100 | 250 | 300 |
|-----------------|----------|-----|-----|-----|
| No. of dams     | 6        | 3   | 8   | 6   |
| No. of implantations | 73      | 45  | 110 | 76  |
| Implantations/Dams | 12.2    | 15.0| 13.8| 12.7|
| No. of living fetuses (%) | 71   | 43  | 99  | 45  |
| (97.3)          | (95.6)   | (90.0) | (59.2)* |
| Living fetuses/Dams | 11.8    | 14.3| 12.4| 7.5 |
| Sex ratio       | 0.97     | 0.72| 1.41| 1.14|
| (Male/Female)   | (35/38)  | (18/25) | (58/41) | (24/21) |
| No. of resorptions (%) | 2       | 2   | 11  | 30  |
| (2.7)           | (4.4)    | (10.0) | (39.5)* |
| No. of macerations | 0      | 0   | 0   | 1   |
| No. of dead fetuses | 0      | 0   | 0   | 0   |
| Body weight of alive fetuses | $\pm 0.05$ | $\pm 0.08$ | $\pm 0.04$ | $\pm 0.08$ |

a) Control animals were given vehicle (0.5% CMC) solution. Significant difference from control: t-test, *p<0.01; $\chi^2$-test, $p<0.05$, $\chi^2_p<0.01$.

2. Effects of aspirin on the growth and abnormalities in external appearance of fetuses

a. Groups dosed throughout the organogenetic stage: Table 1 shows the ratios of the number of implantation scars to the number of viable fetuses in the dosed groups, in comparison with the ratio in the control group. The ratio was normal in the groups given 100 and 250 mg/kg/day. In the group on 300 mg/kg/day, however, the rate of viable fetuses was 59.2%, this being significantly low (p<0.01), compared with 97.3% in the control group. Also at 300 mg/
kg/day, the ratio of the number of resorbed embryos to the number of implantation scars differed significantly (p<0.01) from the ratio in the control group: there was a correlation between the dosage of the drug and the number of resorbed embryos (r=0.788). The viable fetuses in all dosed groups showed a tendency toward marked, dose-related retardation in weight gain (r=0.935).

b. Groups dosed in the subdivided organogenetic periods: Table 2 shows the abnormalities in the external appearance of viable fetuses. Abnormalities were nil in the 38 fetuses from the dams dosed on days 10–15 and in the 38 fetuses from the dams dosed on days 13–17 of gestation. However, abnormalities were found in the external appearance of 11 (23.4%) of 47 fetuses from the group dosed on days 7–11 of gestation, the being a significantly high incidence of malformations (p<0.01). The abnormalities included cranioschisis, spondylischisis, abdominal fissure, curvature of the tail and exophthalmos; and out of these abnormalities, the first 2 were the most frequent. Similar abnormalities were also noted in the group dosed (at 300 mg/kg/day) throughout the organogenetic stage (Photo 1).

Experiment II
Non-metabolized aspirin was rarely detected in the maternal blood and organs 30–60 minutes after ingestion by the dam.
When the maternal blood and fetal levels of salicylic acid in the group dosed in early pregnancy were compared, the fetal level of salicylic acid was significantly higher ($p<0.05$) at any stage after the dosing with aspirin (Fig. 4). In the group given aspirin half-way through the gestation period, the maternal blood level of salicylic acid decreased with passage of time from 1 hour after the application of aspirin onward, but the fetal level rather showed a tendency toward increase at 4–10 hour, and the levels continued to remain slightly higher than those in the maternal blood level (Fig. 5). In the group given aspirin during late gestation, the fetal level of salicylic acid was lower than that in the maternal blood level, at any stage after dosing (Fig. 6).

Figure 7 illustrates changes in the maternal blood level of salicylic acid with passage of time after the dosing with aspirin. The maternal blood level of salicylic acid in the group dosed during late pregnancy tended to be higher by about 20 µg/ml. However, no striking changes were noted either in the group dosed in early, middle or late pregnancy.

The fetal levels of salicylic acid in the groups dosed in the different stages of pregnancy were lower than those in the maternal blood level, at any stage after dosing.
pregnancy indicated that the amount of salicylic acid transferred into the fetuses showed a tendency toward a gradual decrease with the progression of pregnancy, despite the fact that the maternal blood level of salicylic acid remained all but unchanged (Fig. 8). In the early period of gestation, a large amount of salicylic acid was transferred to the fetuses, with a peak amount (186 μg per g of fetus) already attained at 1 hour; and the transferred amount decreased gradually over the following 10 hours (to 134 μg per g of fetus). In the middle and late periods of gestation, the transfer of salicylic acid was slow, with a peak amount (110–120 μg/g) seen at 4–10 hours. In late pregnancy, level of salicylic acid in the amniotic fluid were much the same as detected in the fetus. Levels in the placenta increased gradually with the progression of pregnancy and were remarkably high compared with levels in the maternal blood or fetus (Figs. 5 and 6).

DISCUSSION

The ratio of the number of viable fetuses, the number of resorbed embryos and the retardation in fetal weight gain were higher, depending on the test dose of aspirin and the the earlier in gestation the drug was given. There was a good correlation between these parameters and the dose. The incidence of abnormalities in the external appearance of viable fetuses was also dose-relatedly higher. When the findings in the group dosed at 300 mg/kg/day throughout the organogenetic stage and those in the group dosed at 300 mg/kg/day during days 7–11 of gestation were compared, the incidences of malformations in the 2 groups were much the same, despite of the fact that the latter group was given for a short period: days 7–11 of gestation, an early period of the organogenetic stage, proved to be the most critical period for the teratogenesis with aspirin.

There were evidences of causative factors for aspirin teratogenesis in man (12–16) and experimental animals (5–7, 17). However, decisive results were not yet obtained. From epidemiological research (12–16), regular aspirin ingestion during pregnancy (in the early stage or perinatal period) expanded higher incidence of anemia, pre- and post-natal bleeding, prolongation of delivery, hard labor, stillbirth and retardation in weight gain, but no statistically significant congenital malformations. These authors suggested that the troubles were probably due to the inhibitory excretion of pro-
staglandins and impaired coagulability of platelets.

From the effects of aspirin applied in different periods of pregnancy on fetal weight gain in Experiment I, i.e., the least retardation in fetal weight gain in the group dosed with aspirin in early pregnancy, or during days 7–11 of gestation, there was transient weight loss which did not necessarily correlate with teratogenesis.

The aforementioned 5-day period liable to teratogenesis, i.e., days 7–11 of gestation, was further examined for the period in which the fetuses were more susceptible to aspirin teratogenicity; such proved to be days 8–10 of gestation. The respective application periods were determined to be the 3-day period from day 11 to day 13 and that from day 15 to day 17 of gestation. Furthermore, the dosage of 250 mg/kg daily p.o. proved to be teratogenic.

Unpublished data from a previous study indicated that the incidence of malformations was extremely low when the rats were dosed with aspirin for 2 consecutive days in early pregnancy. These results differed from the data reported by Miyamoto and Nagahama (9). These authors demonstrated high incidences of skeletal abnormalities in rats given a single large dose (600 mg/kg, p.o.) of aspirin at the last stage (days 15–17) of gestation.

Beck et al. (18) reported that the manifestation of the teratogenic effect of trypan blue, even though the agent did not reach the fetal tissue, may have resulted from an impeded alimentation to the fetuses. Therefore, the high incidences of dead fetuses, fetal resorptions and abortions in middle and late pregnancy in our present study are presumably referable to the indirect effect of aspirin because of a malfunctioning placenta where salicylic acid accumulates at high concentrations.

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