CONSTRICTIVE EFFECTS OF PARAQUAT ON THE DUCTUS ARTERIOSUS IN FETAL RATS

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ABSTRACT—Rats on day 21 of pregnancy received a subcutaneous injection of paraquat (2, 7 or 25 mg/kg). The inner diameter of the ductus arteriosus (DA) of their fetuses was measured using the whole-body freezing method 1, 3, 6 and 24 hours after injection. The 7 mg/kg dose group showed a significant constriction of the DA 3 hours after paraquat treatment. The 25 mg/kg dose group showed a significant constriction 3 and 6 hours after treatment. The constrictive effect was dose-dependent, that is, the magnitude of constriction observed 3 hours after paraquat injection was greater in the 25 mg/kg dose group than in the 7 mg/kg dose group. The 2 mg/kg dose group showed no significant change in the inner diameter of the DA at any point of measurement (1, 3, 6 and 24 hours after injection), compared to the saline treated controls.

These results indicate that paraquat induces constriction of the fetal rat DA in a dose-dependent manner when administered in late stages of gestation, and that this effect of paraquat is not seen at a dose level of 2 mg/kg.

KEY WORDS : Paraquat, Ductus arteriosus, Fetal rat.

INTRODUCTION

The ductus arteriosus (DA) is patent during the fetal period and is responsible for most of the blood flowing from the pulmonary artery to the aorta, thus forming a part of the typical fetal circulation. The dilation of the ductus arteriosus is maintained by prostaglandins, while glucocorticoids cause it to constrict, this latter effect being due to their inhibition of prostaglandin biosynthesis.

Paraquat (1,1'-dimethyl-4,4'-bipyridylium) is a non-selective contact herbicide that is being used world-wide in approximately 130 countries. The toxicity is especially marked in the lung,

characterized by initial development of pulmonary edema and damage to the alveolar epithelium. The mechanism of pulmonary injury is believed to be related to the intracellular generation of free radicals of oxygen by the cyclic reduction and oxidation of paraquat leading to lipid peroxidation of cell membranes as a type of oxidant-induced injuries (Smith and Heath, 1976). On the other hand, since paraquat can cross the placenta to reach the fetus, it also may have fetal toxicity, although the degree of its toxicity may be low because of its low concentration in the fetuses compared with maternal tissues (Bus *et al.*, 1975). However, our previous study indicated that paraquat has a constrictive effect on the DA and that the onset of this effect occurs in the first half of day 19 of gestation in rats (Shirai *et al.*, 1994).

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The present work was designed to extend the studies on the dose dependency of the constrictive effect of paraquat on the DA in fetal rats by treatment with various dosages of paraquat using the whole-body freezing method.

MATERIALS AND METHODS

Female Wistar rats; CLEA Japan Inc., 12–15 weeks old at the time of mating, were used. They were maintained on a 12-hr light and 12-hr dark cycle with food (Labo-MR Breeder) and water both *ad libitum* at a room temperature of $22 \pm 3^\circ\text{C}$ and humidity of $55 \pm 10\%$. They were housed with males overnight and examined the next morning for the presence of sperm in the vaginal smear. The day on which sperm was found was designated as day 0 of gestation, and the pregnant rats were caged individually thereafter.

Maternal administration of paraquat dichloride was carried out 1, 3, 6 or 24 hr prior to cesarean section. Paraquat dichloride (ICI Japan Co., purity : 99.4 %) dissolved in physiological saline at a concentration of 1%, and was given subcutaneously to each pregnant rat at a dose of 2, 7 or 25 mg/kg. As controls, other rats were given the vehicle, physiological saline alone and killed 3 hr later. The time of caesarian section was 1 p.m. on day 21 of gestation. At autopsy of caesarean section, each male fetus was rapidly immersed in an acetone-dry ice mixture. Only male fetuses were used for observation to exclude the possible sex differences in the caliber of the DA and in the manner of its response to exogenous agents. The frozen fetuses were weighed individually, and 4 fetuses of similar weight were then selected from each litter and stored for several days at -20°C prior to observation.

At the time of observation, the caliber of the DA was determined by a method described previously (Arishima *et al.*, 1991). Briefly, the chest of the frozen fetus was shaved from the back toward the ventral side to expose the exact site where the DA was separated from the aorta. At this site, the DA was calibrated with an ocular micrometer set in a dissecting microscope.

Data were analyzed statistically by Student's *t* test, and values with a *P* value less than 0.01

were considered significantly different.

RESULTS AND DISCUSSION

Maternal treatment with paraquat at a dose of 7 or 25 mg/kg 3 hr after injection induced significant constriction of fetal DA. The DA of fetuses of rats given 25 mg/kg paraquat was smaller than that of rats given 7 mg/kg paraquat. There was also a significant constriction of the fetal DA even 6 hr after maternal treatment with 25 mg/kg paraquat. However, the caliber of the DA was comparable to the control value in all the groups treated with 2 mg/kg paraquat 1 to 24 hr after injection (Fig. 1).

The results of this study indicate that paraquat induces constriction of the DA of rat fetuses in late stages of gestation. Constriction of the DA was noted 3 hours after paraquat injection in the 7 mg/kg dose group and 3 and 6 hours after injection in the 25 mg/kg dose group. The inner diameter of the DA at 3 hours after injection was smaller in the 25 mg/kg dose group than in the 7 mg/kg dose group. These results suggest that the effect of paraquat in inducing constriction of the DA of rat fetuses is dose-dependent. The constrictive effect seemed to be increased and prolonged as the paraquat dosage increases. Because DA constriction was not seen at 6 hours in the 7 mg/kg dose group and 24 hours in the 25 mg/kg dose group, the paraquat-induced constriction of the DA seems to be reversible. That is, the DA, showing DA-induced constriction, can later dilate to its normal diameter. Similar reversible changes were also noted in our previous studies of the effects of indomethacin (Arishima *et al.*, 1991), thyroid hormone (Takizawa *et al.*, 1993) and alcohol (Arishima *et al.*, 1993). Therefore, it appears that the inner diameter of the DA in the fetuses returns to normal even when its constriction is induced by some factors.

The dilation of the DA is maintained by prostaglandins (PGs) (Clyman *et al.*, 1978; Coceani and Olley, 1973; Momma *et al.*, 1980; Starling and Elliott, 1974). On the other hand, glucocorticoids cause the DA to be constricted (Momma *et al.*, 1981; Waffarn *et al.*, 1979), the effect being due to their inhibition of PG biosynthesis (Hong and Levine, 1976; Kantrowitz *et al.*, 1975). The lung, a target for para-

Fig. 1. Changes in the caliber of the fetal DA 1, 3, 6 or 24 hr after maternal treatment with paraquat. A; pregnant rats given 2 mg/kg paraquat. B; pregnant rats given 7 mg/kg paraquat. C; pregnant rats given 25 mg/kg paraquat. Each vertical line indicates the standard error of the mean of data from 12 male fetuses obtained from 3 litters. * $P < 0.01$: significantly different from control.

quat toxicity, has been demonstrated to contain a large amount of PGs and to have a considerable capacity to synthesize and metabolize PGs (Christ and Van Drop, 1972; Hyman *et al.*, 1978). According to Giri *et al.* (Giri *et al.*, 1980), the plasma concentration of PGE_2 is increased in response to paraquat (45 mg/kg, i.p.) in rats. On the other hand, the same investigators reported decreased plasma PGE_2 levels in dogs and

decreased PGE_2 synthesis in guinea pig lung after treatment with paraquat (Chandler and Giri, 1981; Giri and Krishna, 1978). These reports suggest the presence of a species difference in the action of paraquat on PGs. However, the mechanism leading to the constriction of the fetal DA in the present study cannot be fully explained. If the patency of the DA is maintained only by prostaglandins, it likely follows that a medium dose of paraquat (7 or 25 mg/kg, s.c.) has an inhibitory action on PGs, resulting in the constriction of the fetal DA.

These results indicate that paraquat induces constriction of the DA of rat fetuses in late stages of gestation and that this effect of paraquat is not seen at a dose of 2 mg/kg.

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