

In Vivo Dilatation of the Ductus Arteriosus Induced by Furosemide in the Rat

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ABSTRACT: Furosemide increases prostaglandin production and may be associated with patent ductus arteriosus (PDA). We aimed to clarify the *in vivo* ductus-dilating effects of furosemide in neonatal rats. Near-term rat pups delivered by a cesarean section were housed at 33°C. After a rapid whole-body freezing, the DA diameter was measured using a microscope and a micrometer. Pregnant rats (gestational day 21) were s.c. injected with furosemide 4 h before delivery, and the neonatal DA was examined 0, 15, 30, 60, and 120 min after birth. Furosemide was also s.c. injected into 60-min-old rats and the DA diameter was examined 30, 60, and 120 min later. The control rats showed a rapid postnatal DA constriction (diameter: 0.80 and 0.08 mm at 0 and 60 min after birth, respectively). Prenatally administered furosemide delayed postnatal DA closure (0.36 mm at 60 min after birth). Furosemide injection in 60-min-old rats dilated the constricted DA at 60 min (0.25 versus 0.02 mm in the controls). Indomethacin inhibited furosemide-induced DA dilatation. Furosemide delays DA closure and dilates the constricted DA in neonatal rats. If furosemide has similar effects in human preterm neonates, caution may be warranted in its use in the treatment of infants with PDA. (*Pediatr Res* 67: 173–176, 2010)

Furosemide is used to potentiate the natural diuresis seen in the preterm neonate to attenuate the severity of RDS in premature infants in the immediate postnatal period (1). It is also used to treat preterm infants with symptoms of renal toxicity induced by indomethacin, which is used to ameliorate a symptomatic patent ductus arteriosus (PDA) while preparing for pharmacological closure by indomethacin (2,3). Further, furosemide stimulates the renal production of prostaglandin E₂, a potent ductal smooth-muscle dilator (4–8). A previous retrospective study has provided evidence that treatment with the diuretic furosemide may increase the incidence of preterm PDA (9). In addition, a randomized controlled trial wherein furosemide and chlorothiazide were compared has revealed that furosemide increases the incidence of PDA in premature infants with RDS, presumably through a prostaglandin-mediated mechanism (10). Furosemide treatment may help in preventing heart failure due to PDA or indomethacin-induced toxicity but may also affect the ductal response to indomethacin. In a recent systematic literature review, it was found that furosemide treatment does not significantly increase

the risk of failure of ductal closure; however, the sample size of the review was insufficient in the above-mentioned risk (11).

The effects of furosemide on DA remain to be elucidated. We hypothesized that furosemide may increase prostaglandin E (PGE) secretion and dilate the DA of neonates *in vivo*. There is a transplacental effect of furosemide (12). The objective of this study was to elucidate the effect of furosemide on the patency of the ductus arteriosus in neonatal rats.

METHODS

Furosemide (Lasix) was purchased from Sanofi-Aventis Co. (Tokyo, Japan). Water-soluble indomethacin that could be used for injection was purchased from Banyu Pharmaceutical Co. (Tokyo, Japan).

The treatment protocol conformed to the guidelines issued by the American Physiologic Society, and our Institutional Ethical Committee for Animal Experiments approved the experimental protocol. Virgin Wistar rats were mated overnight from 1700 to 0900 h; the day on which sperm were detected in vaginal smears was regarded as gestational d 0 (total pregnancy period, 21.5 d). The rats were housed in a room under controlled environmental conditions, acclimatized to a 12-h light/12-h dark cycle, and maintained on commercial solid food and tap water available *ad libitum*. Experiments were performed on newborn rats delivered on gestational d 21.

Maternal administration. For studying the effect of furosemide in delaying postnatal DA closure, near-term pregnant rats (gestational day 21) were s.c. injected with furosemide (1, 10, and 100 mg/kg). The pregnant rats were subjected to atlas dislocation, and the pups were delivered by a cesarean section 4 h later. The newborn rats were incubated in a room at 33°C. To examine the *in situ* morphology of the postnatal DA, we used a rapid whole-body freezing method as described previously (13,14). In brief, the newborn rats were frozen at 0, 15, 30, 60, or 120 min after birth in acetone that had been cooled to –80°C in dry ice. The frozen thorax was cut along the frontal plane by using a freezing microtome (Komatsu Solidate Co. Ltd., Tokyo, Japan), and the inner diameters of the ascending aorta, main pulmonary artery, and DA were measured by observation under a microscope (Nikon Binocular Stereoscopic Microscope; Nihon Kogaku Co., Tokyo, Japan), using a micrometer (Nikon Ocular Micrometer; Nihon Kogaku Co., Tokyo, Japan). The DA of the newborn rats was 800–1200 μm in length, tubular along the middle three quarters of its length, and horn shaped at the proximal and distal ends (15). We measured the DA at 100-μm intervals, in 8–12 planes. The short axis was measured in ellipsoid images, assuming that the DA was round *in situ* (15). The smallest diameter recorded was used as an indicator of constriction. The ductus diameter was measured at 0, 15, 30, 60, and 120 min after birth (6–12 newborn rats for each dose and time point). The control rats showed a rapid postnatal DA constriction (diameter: 0.80, 0.28, 0.12, 0.08, and 0.02 mm at 0, 15, 30, 60, and 120 min after birth, respectively).

Immediate neonatal administration. In addition, we examined the effect of furosemide in delaying postnatal DA closure by performing the following experiment.

Near-term pregnant rats (gestational d 21) were subjected to atlas dislocation, and the pups were delivered by a cesarean section. Newborn rats were s.c. injected with furosemide (1 and 10 mg/kg in 5 mL saline) within 3 min

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Abbreviations: PDA, patent ductus arteriosus; PGE, prostaglandin E

after birth. The newborn rats were incubated in a room at 33°C. Using a rapid whole-body freezing method, the newborn rats were frozen at 0, 15, 30, 60, or 120 min after birth in acetone that had been cooled to -80°C in dry ice. As well as the above-mentioned method, the DA diameter was measured at 15, 30, 60, and 120 min after birth (6–14 rats for each dose and each time point). The control rats showed a rapid postnatal DA constriction (diameter: 0.80, 0.28, 0.12, 0.08, and 0.02 mm at 0, 15, 30, 60, and 120 min after birth, respectively).

Administration to neonates after postnatal DA closure. Reopening of the DA was examined as follows. Near-term pregnant rats (gestational d 21) were subjected to atlas dislocation, and the pups were delivered by a cesarean section. The newborn rats were incubated in a room at 33°C. Newborn rats were s.c. injected with furosemide (1 mg/kg in 5 μ L saline), either alone or along with indomethacin (10 mg/kg) at 60 min after birth. Using a rapid whole-body freezing method, the newborn rats were frozen at 0, 30, 60, or 120 min after the s.c. injection with furosemide in acetone that had been cooled to -80°C in dry ice. As well as the above-mentioned method, the DA was examined at 30, 60, and 120 min after the injection (6–11 rats for each time point). The control rats showed a postnatal DA constriction (diameter: 0.02 and 0.0 mm at 120 and 180 min after birth, respectively).

Photographs. To observe constriction of the DA, the vessel was photographed in the frontal view with the help of a binocular stereoscopic microscope (Wild M400 Photomicroscope, Wild Heerbrugg Ltd., Heerbrugg, Switzerland) and color film (Reale; Fuji Film Co., Tokyo, Japan).

Statistics. The results are expressed as the mean \pm SEM. The statistical significance of the differences between the group means was determined using a modified two-way ANOVA and the Bonferroni's method (16). The difference was considered significant if the *p* value was <0.05.

RESULTS

Maternal administration. In the control neonates, a rapid DA constriction was noted after birth. DA closure was delayed in the rats that were prenatally exposed to furosemide. All three doses of transplacentally administered furosemide induced a similar and significant delay in postnatal DA closure, as shown in Figure 1. Moreover, the degree of delay induced by the two higher doses of furosemide did not significantly differ from that induced by the clinical dose of furosemide (1 mg/kg). The DA diameter, 15 min after birth, was 0.28 mm in control, and 0.68, 0.64, and 0.54 mm after administration of 100, 10, and 1 mg/kg of furosemide, respectively. The DA diameter, 30 min after birth, was 0.12 mm in control, and 0.30, 0.31, and 0.39 mm after administration of 100, 10, and 1 mg/kg of furosemide, respectively. The DA diameter, 60 min

after birth, was 0.08 mm in control, and 0.23, 0.27, and 0.23 mm after administration of 100, 10, and 1 mg/kg of furosemide, respectively. The DA diameter, 120 min after birth, was 0.02 mm in control, 0.01, and 0.05 mm after administration of 100 and 10 mg/kg of furosemide, respectively.

Immediate neonatal administration. Subcutaneous injection of furosemide (1 mg/kg) at birth significantly delayed DA closure, as shown in Figure 2. The higher dose (10 mg/kg) induced a significantly greater delay in DA closure than the lower dose (1 mg/kg; Fig. 3). The DA diameter, 15 min after birth, was 0.28 mm in control, 0.82 mm and 0.69 mm after administration of 10 mg/kg and 1 mg/kg of furosemide, respectively. The DA diameter, 30 min after birth, was 0.12 mm in control, and 0.48 mm and 0.36 mm after administration of 10 and 1 mg/kg of furosemide, respectively. The DA diameter, 60 min after birth, was 0.08 mm in control, and 0.28 mm and 0.18 mm after administration of 10 and 1 mg/kg of furosemide, respectively. The DA diameter, 120 min after birth, was 0.02 mm in control, and 0.14 mm and 0.02 mm after administration of 10 and 1 mg/kg of furosemide, respectively.

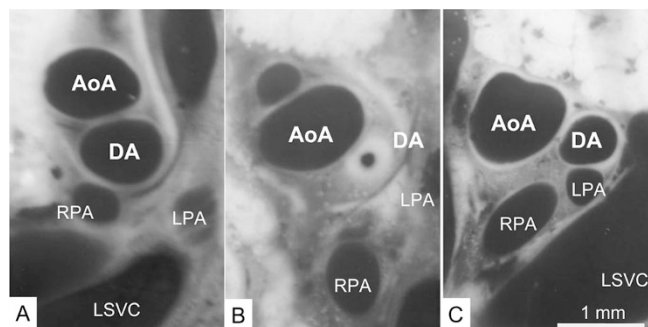


Figure 2. The neonatal thorax cut along the frontal plane, at the level of the DA. A, The dilated DA in a newborn rat (at 0 min after birth; control). B, The constricted, thick-walled DA in a 30-min-old rat (control). C, The semiconstricted DA in a 30-min-old newborn rat s.c. injected with furosemide (1 mg/kg) at birth. AoA, aortic arch; DA, ductus arteriosus; LPA, left pulmonary artery; L SVC, left superior vena cava; RPA, right pulmonary artery.

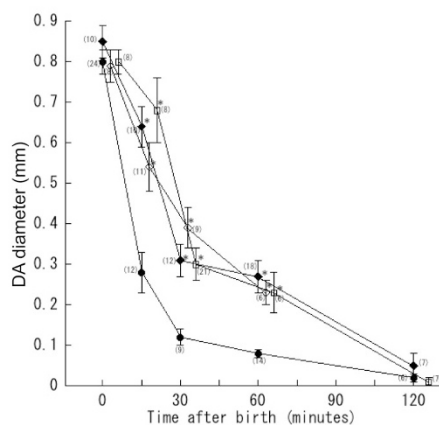


Figure 1. The time course of postnatal DA closure in the control rats (●) and in rats that were transplacentally exposed to furosemide (1 mg/kg (◇), 10 mg/kg (◆), or 100 mg/kg (□) injected s.c.) at 4 h before birth. The x axis shows the time after birth in minutes. The y axis shows the DA diameter. Each point was obtained from the study of 6–24 neonates. Each value expressed as mean \pm SEM. **p* < 0.05 vs the controls.

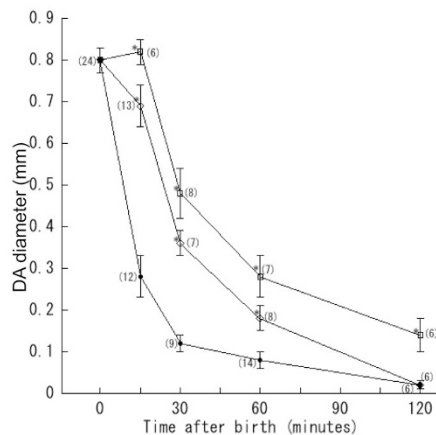


Figure 3. The time course of postnatal DA closure in the control rats (●) and in rats that were administered furosemide at a dose of 1 mg/kg (◇) or 10 mg/kg (□) at birth. The x axis shows the time after birth in minutes. The y axis shows the DA diameter. Each point was obtained from the study of 6–24 neonates. Each value expressed as the mean \pm SEM. **p* < 0.05 vs the controls.

Administration to neonates after successful DA closure.

Figure 4 shows that s.c. injection of furosemide at 60 min after birth induced DA reopening to a moderate degree. Peak dilatation was observed at 60 min after the injection (Fig. 5). The DA diameter, 30 min after injection (90 min after birth), was 0.06 mm in control, and 0.14 mm after administration of furosemide (1 mg/kg) alone and 0.05 mm after administration of furosemide (1 mg/kg) with indomethacin (1 mg/kg), respectively. The DA diameter, 60 min after injection (120 min after birth), was 0.02 mm in control, and 0.25 mm after administration of furosemide (1 mg/kg) alone and 0.10 mm after administration of furosemide (1 mg/kg) with indomethacin (1 mg/kg), respectively. The DA diameter, 120 min after injection (180 min after birth), was 0.00 mm in control, and 0.04 mm after administration of furosemide (1 mg/kg) alone and 0.10 mm after administration of furosemide (1 mg/kg) with indomethacin (1 mg/kg), respectively. The furosemide-induced DA dilatation was considerably, but not completely, inhibited with the simultaneous administration of indomethacin (Fig. 5).

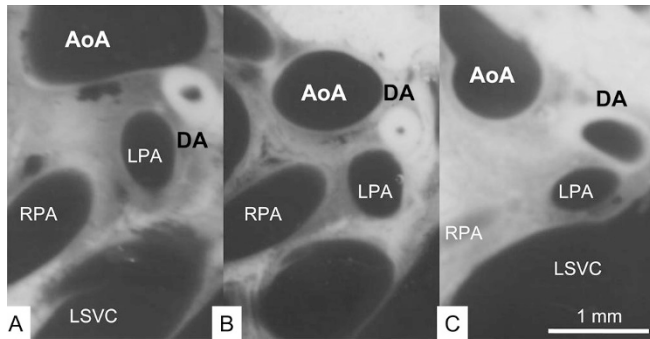


Figure 4. The neonatal thorax cut along the frontal plane, at the level of the ductus arteriosus (DA). A, The constricted DA in a 60-min-old rat (control). B, The constricted, thick-walled DA in a 120-min-old rat (control). C, The dilated DA in a 120-min-old rat that was s.c. injected with furosemide (1 mg/kg) 60 min after birth. AoA, aortic arch; DA, ductus arteriosus; LPA, left pulmonary artery; LSVC, left superior vena cava; RPA, right pulmonary artery.

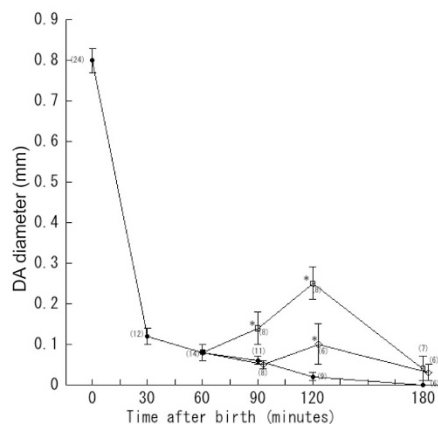


Figure 5. The time course of postnatal DA closure in the control rats (●) and in rats that were administered 1 mg/kg furosemide alone (□) or in combination with 10 mg/kg indomethacin (◇) at 60 min after birth. The x axis shows the time after birth in minutes. The y axis shows the DA diameter. Each point was obtained from the study of 6–24 neonates. Each value expressed as the mean \pm SEM. * $p < 0.05$ vs the controls.

DISCUSSION

This is the first *in vivo* study to experimentally demonstrate that postnatal DA closure is delayed after furosemide exposure in rats.

A previous randomized controlled trial wherein furosemide and chlorothiazide were compared has revealed that furosemide increases the incidence of PDA in preterm infants with RDS (9), presumably through a prostaglandin-mediated mechanism (10). Green *et al.* (9,10,17,18) suggested that furosemide treatment might adversely affect the patency rate of the immature DA. Apart from these studies, there has been limited research on the effects of furosemide on preterm individuals with PDA (11). In a recent systematic literature review, it was found that furosemide treatment does not significantly increase the risk of failure of DA closure; however, the sample size of the review was insufficient to rule out even a 31% increase in the risk (11). We hypothesized that if furosemide stimulates the renal synthesis of PGE in preterm infants, it should be able to delay postnatal DA closure and inhibit the constrictive effect of indomethacin in infants. In this study, we found that postnatal furosemide treatment delays ductal closure and dilates the constricted DA in neonatal rats in a dose-dependent manner. However, the ductus-dilating effect of furosemide, even when administered at a high dose (10 mg/kg) is merely modest. Various clinical and experimental studies have demonstrated that furosemide alters systemic vascular resistance in a manner that is independent of its diuretic action. Most previous studies have implicated prostaglandins synthesized in the kidney (5,8,19,20) or DA wall (21) as mediators of this effect of furosemide. Friedman *et al.* studied the urinary excretion of PGE in seven sick, low birth weight infants. They found that the excretion rate increased (from 0.4 to 1.3 ng/mg Cr) in all the patients after furosemide treatment but decreased in two patients after indomethacin treatment (6). These results indicated that furosemide enhances the urinary excretion of PGE by mechanisms that may reflect increased prostaglandin synthesis, decreased prostaglandin metabolism in the kidneys, or both (6). Neonatal blood PGE concentrations were not studied in our study. In our study, the administration of furosemide at 60 min after birth dilated the constricted DA of the neonates and indomethacin attenuated the effects of furosemide because it still differs from the controls (Fig. 5). We speculate that prostaglandins synthesized in the kidney, in response to furosemide treatment, may mediate the effects of furosemide on the DA.

In our study, on pregnant rats and their neonates, we found that when administered at the usual clinical dose (0.5–1.0 mg/kg) for the mother and the newborn infant, furosemide has a significant effect in delaying postnatal DA closure and reopening the constricted DA. In fetuses with indomethacin-induced DA constriction, PDE-3 and PDE-5 inhibitors dilate the DA more sensitively in preterm rats than in near-term rats (22,23). Clyman (24) has demonstrated that compared with the DA in near-term lambs, the DA in immature lambs is considerably more sensitive to the dilating effects of PGE. Therefore, we speculate that a) the DA in preterm animals is more sensitive to furosemide than that in full-term animals

and (b) in clinical settings too, furosemide exposure may result in delayed postnatal DA closure and attenuate the constrictive effect of indomethacin in premature infants. We recommend that furosemide be used with caution in the treatment of preterm infants with symptomatic PDA and heart or renal failure. However, the other beneficial effects of furosemide, for example, its diuretic effects, may balance its harmful effects in patients with PDA. In our study, the duration in ductus-dilating effect of furosemide is at least short in the newborn rats. Therefore, clinical observations on the effect of furosemide on the DA are warranted.

In conclusion, furosemide attenuates postnatal DA constriction in neonatal rats. If the ductus arteriosus is affected in a similar manner in human preterm neonates and this is an assumption, caution may be warranted in the use of furosemide in the treatment of preterm infants with PDA.

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