FULL PAPER *Anatomy*

Pulmonary Hypoplasia Induced by Liquid Paraffin Injection into Fetal Thoracic Cavity with Special Reference to Renal Development in Rats

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ABSTRACT. The present study was designed to clarify lung-kidney interrelation in fetal rats. On fetal day 20, liquid paraffin (LP) was injected into fetal thoracic cavity to produce pulmonary hypoplasia. No significant difference in body and renal weights were noted between the LP injected and control fetuses. The weight of lung, however, was significantly lower in the LP injected fetuses than in the control ones. Histological examinations on the lung and kidney of the LP injected fetuses revealed that the lung was hypoplastic characterized by rich interstitium and reduced air spaces. In the kidney, mature types of glomeruli and profiles of proximal tubules near them were increased in number. Furthermore, strong expression of EGF immunoreactivity was noted in the apical cytoplasm of epithelium of the proximal tubules in the LP injected fetuses. These findings indicate that lung-kidney interrelation exists in fetal rats during late gestational days, and suggest that interruption of the lung development induces accelerated growth of the kidney in fetal rats.—KEY WORDS: fetal rat, injection, kidney, liquid paraffin, lung.

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Pathogenesis of pulmonary hypoplasia (PH) has been extensively investigated. The primary factor involved in the PH pathogenesis is intrathoracic compression of the lung by the herniated abdominal viscera through defective diaphragm [8]. To investigate the PH pathogenesis, congenital diaphragmatic hernia (CDH) model is created by giving nitrofen (the herbicide, 2,4-dichlorophenyl-p-nitrophenyl ether) to pregnant rats [16, 23, 33] and mice [11, 34] or by surgical treatment in fetal lambs [6, 9, 13]. In fetal rats with CDH, which is created by maternal administration of nitrofen, no correlation suggesting a feedback mechanism of growth regulation between the kidney and lung is noted [1]. In fetal rabbits, bilateral nephrectomy results in small-for-gestational age status during birth without affecting the development of organic systems [17]. In newborn lambs with CDH, which is surgically created, significantly smaller lungs and larger kidneys are recognized when compared with those of non-treated newborns [15]. In fetal sheep, the kidney plays an important role in early lung growth [24]. In human newborns with CDH, significant renal enlargement is seen [8]. In human infants with renal agenesis, reduction of total lung volume is noted [14]. These findings indicate that correlation between the organs during developmental period is still controversial.

To investigate the correlation between the kidney and lung in rodents, fetal and neonatal CDH model has been produced by administrating nitrofen to pregnant animals. The nitrofen, however, has teratogenic effects and produces several developmental defects such as functional deficits of the reproductive system, cleft palate, heart anomalies, hydronephrosis, renal agenesis and exencephaly/encephalocele as well as diaphragmatic hernia [11, 16, 34].

In the present study, to exclude such teratogenic and/or hormonal effects as nitrofen has, liquid paraffin was used to produce PH. Liquid paraffin (mineral oil) is used for a variety of purposes in medical practice. When mineral oil is instilled in the bladder and ureter in animals, no significant histopathologic changes are noted in the ureteral or bladder urothelium and the renal parenchyma [21]. The present study was designed to clarify the lung-kidney interrelation in fetal rats by injecting liquid paraffin (LP) into fetal thoracic cavity to create PH.

MATERIALS AND METHODS

Rats of the Wistar strain (JCL:Wistar) purchased from Japan Clea (Osaka, Japan) were used. The animals were kept in light- and temperature-controlled room, and allowed free access to a commercial diet (NMF, Oriental Yeast Co., Tokyo, Japan) and water. They were mated overnight and examined the next morning for the presence of spermatozoa in the vaginal smear. The day when spermatozoa were found was regarded as day 1 of gestation. On day 20 of gestation the pregnant rats underwent midventral laparotomy to expose the gravid uterine horn under ether anesthesia. After the exposure of a portion of the horn, 0.05–0.1 m*l* of LP (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was injected into the right thoracic cavity of fetuses through the uterine wall using a syringe with a 27 gauge needle. The gravid horn was returned to the abdominal cavity and the incision was closed. On day 22 of gestation the fetuses were obtained under ether anesthesia. Fetuses were sacrificed by decapitation. The body, lung and kidney were weighed with an electronic reading balance (Shimadzu, LIBROR EB-280, Kyoto, Japan). Non-treated littermates were used as control.

The right kidney and cranial lobe of right lung were fixed

in methanol-Carnoy solution for 24 hr, dehydrated through a graded series of alcohols and embedded in Tissue Prep (Fisher Scientific Company, N.J., U.S.A.). Transverse serial sections of the tissues were made at 4 μm and stained with hematoxylin and eosin.

Counts of glomeruli were made on the largest transverse section of each kidney. The counts were made twice on the same section and the average of the values obtained from the section was regarded as the representative value for animal. Glomeruli in fetal rat kidney are classified into 5 types according to their developmental stages [22]. Types I, II, and III are considered as immature type of glomeruli. Types IV and V were classified as mature type of glomeruli, since they were clearly involved in filtration. First, we counted all types of the glomeruli seen on the section. Next, the ratio of the mature type of glomeruli (types IV and V) to all types of glomeruli was calculated and expressed as a percentage. Data were analyzed with Student's *t*-test. A P value less than 0.05 was considered statistically significant.

Further, to evaluate the effect of the hypoplastic lung on the kidney, immunohistochemical localization of epidermal growth factor (EGF) in the kidney was examined. After deparaffinization with xylene, the kidney sections were transferred to distilled water through a series of degraded alcohols and rinsed in phosphate buffered saline. The sections were incubated with rabbit anti-rat EGF antibody (IGG Corporation, Nashville, TN, U.S.A., 1:1,200) at 4°C overnight. Then, the sections were incubated with biotinized goat anti-rabbit IgG antibody (1:200) and avidin-biotin-peroxidase complex (1:200) for 30 min, respectively. Last, the sections were incubated with diaminobenzidine for 5 min.

RESULTS

Table 1 shows the changes of body, lung and renal weights, and percentage of mature types of glomeruli in the LP injected and control fetuses. There was no significant difference in the body and renal weights between the two groups. The lung weight of the LP injected fetuses was significantly lower than that of the control ones. Percentage of the mature types of glomeruli was larger in the LP injected fetuses than in the control ones.

Histologically, the lung of LP injected fetuses showed thicker septa and less enlarged air spaces compared with those of the control ones (Fig. 1). Immature types of glomeruli appeared in the superficial layer of the kidney and the mature types of the glomeruli, in the deeper layer of the kidney (Fig. 2). Larger number of mature types of glomeruli were noted in the deeper layer of kidney in the LP injected fetuses when compared with those in the control group (Fig. 2-B). Further, proximal tubules were increased in the LP injected fetuses (Fig. 2-B). Expression of EGF was noted in the apical cytoplasm of epithelium of the proximal tubules near the mature types of glomeruli in both groups (Fig. 3). However, the EGF immunoreactivity was stronger in the LP injected fetuses than in the control ones (Fig. 3-B).

DISCUSSION

To investigate pathogenesis of PH, experimental model of CDH has been produced by nitrofen in rodents. However, nitrofen has teratogenic effects and produces several types of anomalies simultaneously besides diaphragmatic hernia, and acts through modifications of the thyroid hormone status in both dam and fetus [4, 33]. These findings indicate that application of nitrofen is not a suitable method to demonstrate lung-kidney interrelation in fetal rats. Therefore, in the present study, to exclude such teratogenic and/or hormonal effects as nitrofen has, LP was used to create PH in fetal rats. The LP injection into fetal thoracic cavity interrupted the lung development and caused remarkable decrease in the lung weight. Histologically, the lung of LP injected fetuses was immature characterized by rich interstitium and smaller air spaces when compared with that of the control fetuses. Since the decrease in relative weight of the lung is considered as the index of pulmonary hypoplasia [5, 20], the LP injection into fetal thoracic cavity produced PH in the present study. These findings are in keeping with previous reports in rats and humans with CDH. Insufficient enlargement of the lung, reduction of air spaces and thickening of the septa were seen in fetal rats [3, 19, 32] and human fetuses and newborns [2, 28].

There was no significant difference in renal weight between the two groups. However, histological observations revealed that mature types of glomeruli and profiles of proximal tubules were increased in number, and augmented expression of EGF immunoreactivity was noticed in the apical cytoplasm of epithelium of the proximal tubules near

Table 1. Changes of body, lung and renal weights and percentage of mature type of glomeruli

Group	Number of animals	Body weight g	Lung weight mg	Renal weight (L+R) mg	Mature type of glomeruli %
		Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.
L P	7 (5)	3.54 ± 0.12	57.14 ± 1.79	26.42 ± 1.88	26.81 ± 1.60
p value		N.S.	< 0.001	N.S.	< 0.01
Control	8 (5)	3.66 ± 0.10	93.38 ± 1.88	26.63 ± 0.91	19.43 ± 1.29

The numbers in parentheses mean the number of litters. N.S. means not significant statistically. S.E.M. means standard error of mean. L means left kidney and R, right kidney. LP means liquid paraffin injected fetuses.

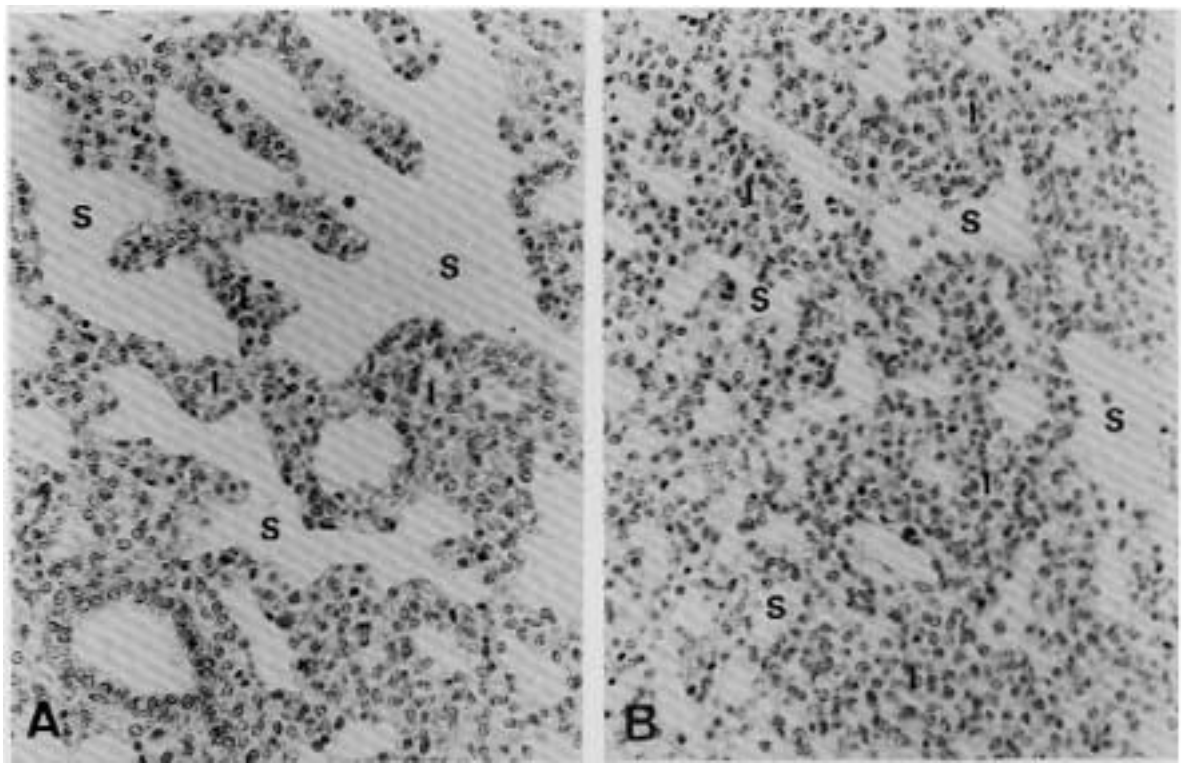

Fig. 1. Lung sections stained with hematoxylin and eosin. × 500. A: a control fetus. The air space is enlarged and the interstitium is thin. B: an LP injected fetus. The air space (S) is less enlarged and the interstitium (I) is rich when compared with those of the control fetus in Fig. 1-A.

the mature types of glomeruli in the LP injected fetuses when compared with those of the control ones. Raaberg *et al*. [27] reported that EGF first appears in the proximal convoluted tubules on fetal day 19 and increased on fetal day 20, expressing strong immunoreactivity of EGF on fetal day 20 as well as during the first 14 days after birth in rats. In addition, EGF and EGF receptor first appear in the proximal tubules near the mature types of glomeruli on fetal day 18 in rats [unpublished data]. In rat, mouse, and human kidney, EGF is produced as a part of a large membrane-anchored precursor molecule, which is found at the luminal site of the thick ascending limb of Henle and in the distal convoluted tubule [7]. Further, in adult rat kidney, EGF receptors are localized to basolateral membrane in the proximal tubule [12, 30]. Based upon these findings, the present results suggest that the EGF produced in fetal rat kidney might be involved in the renal development.

On the other hand, different accounts of the kidney's contribution to lung development have been reported. In fetal rats with CDH induced by nitrofen, the body and lung weights are significantly decreased and the kidney also appears smaller [33]. In fetal sheep, the kidney contributes to early lung growth [24]. In lambs, bilateral ureteral ligation has no effect on the lung development [26]. In human newborns, CDH always accompanies renal enlargement [8]. Also, in lambs, CDH created by surgical treatment accompanies significant renal enlargement [15]. In the present study, no enlargement of the kidney was noted in the LP injected fetuses. This might be dependent on the duration of compression in the lung or species difference in developmental pattern of the kidney. Nephrogenesis is not completed at birth in rats [10] and pigs [18], while the reverse is true in humans [25], sheep [29] and guinea pigs [31].

In conclusion, the present study demonstrates that the lung-kidney interrelation is present in fetal rats during late gestational days, and suggests that interruption of lung development induces accelerated growth of the kidney.

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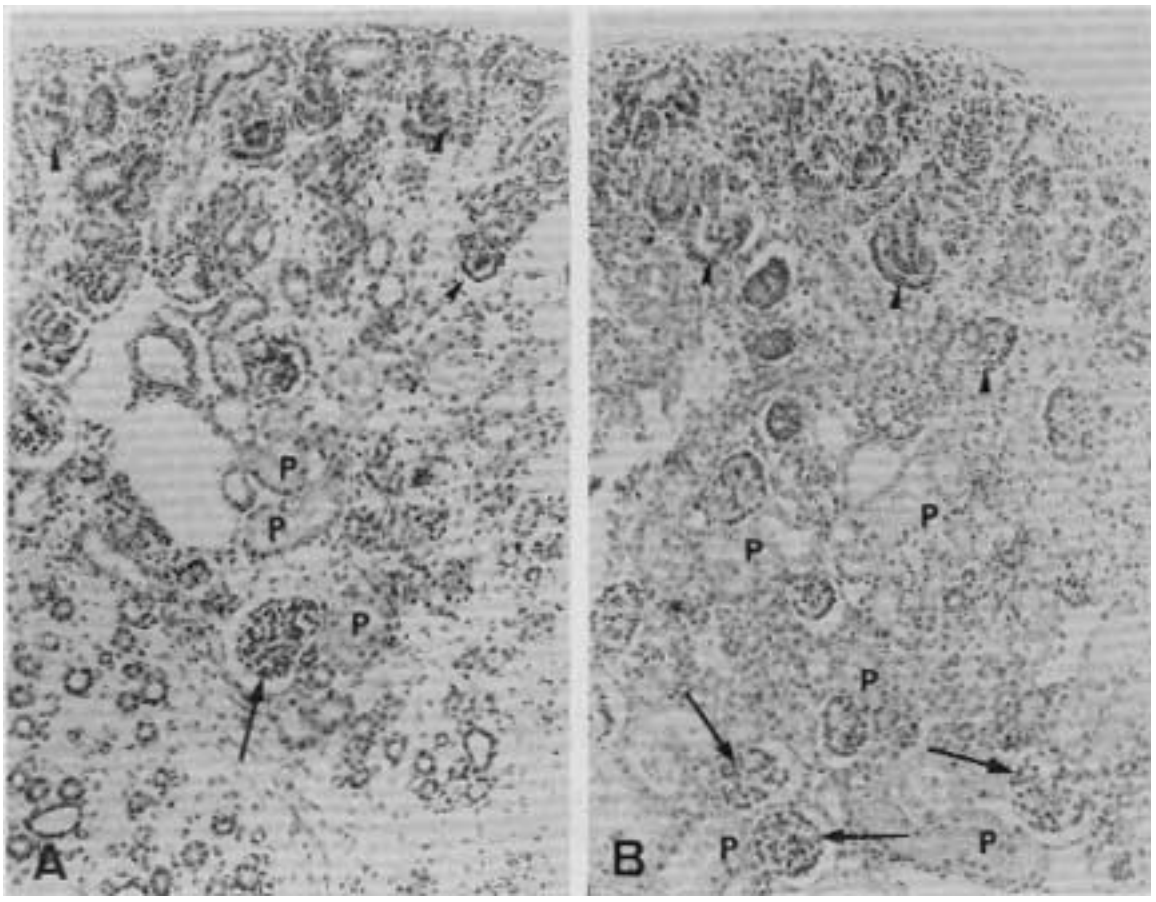

Fig. 2. Kidney sections stained with hematoxylin and eosin. × 250. A: a control fetus. Immature types of glomeruli (arrow heads) are seen in the superficial layer and mature types of glomeruli (arrow), in the deeper layer of the kidney. Profiles of the proximal tubules (P) are seen near the mature types of glomeruli. B: an LP injected fetus. Larger number of mature types of glomeruli (arrows) and of profiles of the proximal tubules (P) near the mature types of glomeruli are seen when compared with those of the control fetus in Fig. 2-A. Immature types of glomeruli (arrow heads) are also seen in the superficial layer of the kidney.

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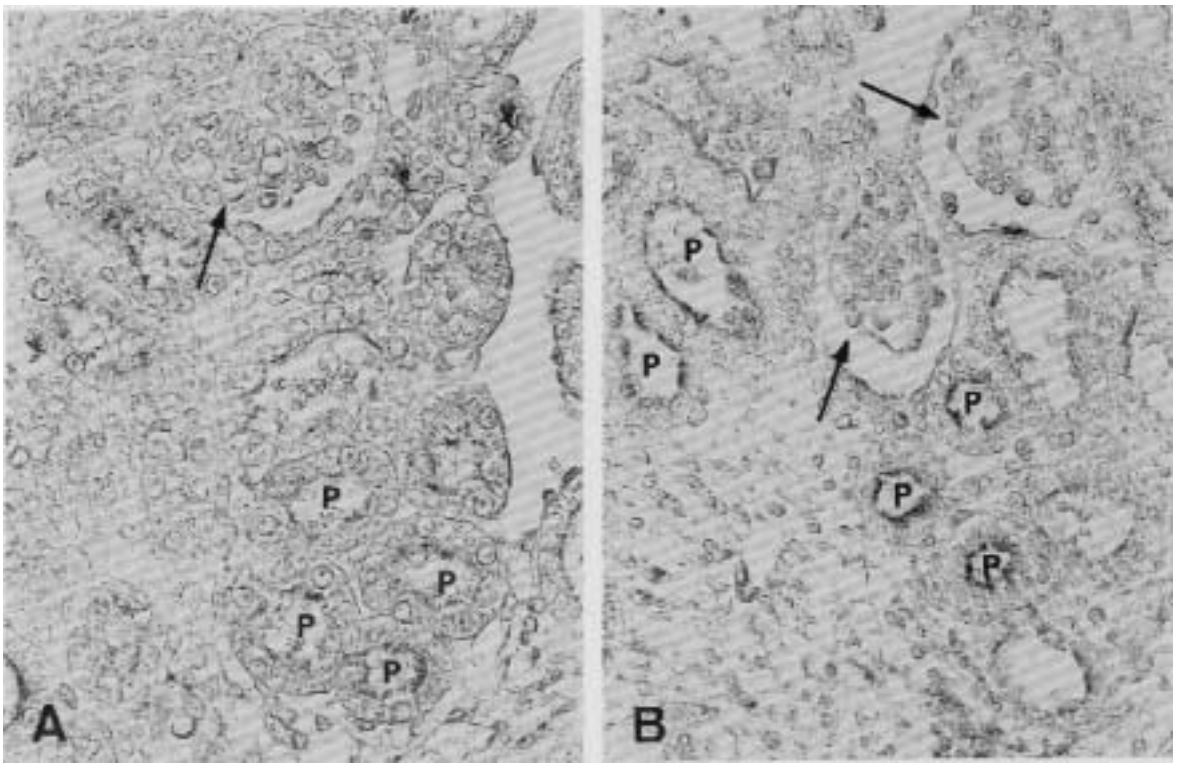

Fig. 3. Kidney sections stained with EGF antibody. × 400. A: a control fetus. EGF immunoreactivity is seen in the apical cytoplasm of epithelium of the proximal tubules (P) near the mature type of glomerulus (arrow). B: an LP injected fetus. Stronger expression of EGF immunoreactivity is seen in the apical cytoplasm of epithelium of the proximal tubules (P) near the mature types of glomeruli (arrows) when compared with the EGF expression of the control fetus in Fig. 3-A.

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