

# The effects of PSK, a biological response modifier, on congenital ocular abnormalities induced by X-ray irradiation

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**Summary.** The antiteratogenic effects of PSK, a biological response modifier, were examined using histological and developmental analysis. The whole bodies of pregnant mice were irradiated with X-rays and injected with PSK within ten minutes after irradiation on day 7 of gestation (E7). The foetuses on E18 were examined and a high incidence of malformations were observed in X-ray irradiated embryos. Microphthalmia was the most frequent malformation. PSK administration suppressed the X-ray irradiation-induced ocular anomalies in not only the frequency, as deduced by external observation, but also in histopathological changes in the retina, lens, and cornea. In particular, the incidence of lens aplasia was significantly decreased by PSK administration. Developmental analysis using E10 and E13 embryos revealed that the decrease in the incidence of histopathological changes was first observed within 72 hours after PSK administration. In addition, X-ray irradiation-induced early foetal death (E10-13) was also suppressed by PSK administration. The possible mechanisms of the antiteratogenic effects of PSK are discussed.

**Key words:** PSK, Microphthalmia, Antiteratogen, Mice

## Introduction

X-ray irradiation has been used as one of the therapies against cancer, and is known as one of the most famous teratogenic treatments. The process of ocular teratogenesis by X-ray irradiation has been well documented (Russell, 1950; Majima, 1961). PSK, a protein-bound polysaccharide extracted from *Basidiomycete*, has been used as a biological response modifier (BRM) in cancer therapies (Kondo et al., 1981; Hattori et al., 1982; Shimura et al., 1983; Tsukagoshi et

al., 1984), and has been suggested to restore the host defense mechanism from damage caused by anticancer drugs or irradiation (Tsukagoshi, 1975; Mikuria et al., 1985). Recently, the suppressive effect of PSK on the digital defects induced by 5-azacytidine (5-AC), an inhibitor of DNA methylation, was reported (Kurishita, 1990). In our previous study, we demonstrated that incidences of some external anomalies induced by X-ray irradiation or an alkylating agent, chlorambucil, were decreased by PSK administration (Naora et al., 1994). The incidence of X-ray irradiation-induced microphthalmia was most prominently decreased by PSK administration. In the present study, to further analyze the suppressive effect of PSK on X-ray irradiation-induced ocular abnormalities, a detailed histological analysis and developmental studies were performed.

## Materials and methods

Animals used in this study were Jcl: ICR mice (CLEA Japan Inc., Tokyo, body weights: 22-26 g, 7-9 weeks of age). An estrous female was placed overnight in the same cage as a potent male, and those showing a vaginal plug on the next morning were taken as day 0 of gestation (EO). Pregnant animals were divided into 13 groups (A-M, Table 1). On E7, they were settled in a plastic box and a whole body X-irradiation was carried out. ML-15 MDX (Mitsubishi Co, Japan) was used as the X-irradiation generator. Within ten minutes after irradiation, PSK (Krestin<sup>®</sup>, Kureha Chemical Ind. Co. Ltd, Tokyo, Japan) dissolved in physiological saline (0.1 ml) was injected intraperitoneally. The doses of PSK and X-ray irradiation are shown in Table 1. Physiological saline was used as control.

The females were sacrificed by cervical dislocation and embryos were obtained on the scheduled day described in Table 1. Subsequently, the body weight (BW), crown-rump lengths (CRL) of embryos in groups A-I were measured, and external anomalies were inspected under a dissecting microscope. All embryos were fixed by immersion in Bouin's solution or 10%

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Table 1. PSK-treated and control groups.

GROUP	SACRIFICED DAY (E)	X-RAY (Gy)	PSK (mg/kg)	No. DAMS	No. TOTAL FOETUS	No. LIVING FOETUS	No. MALFORMED FOETUS	No. MICROPH-THALMIA	No. SHORT TAIL	No. CLEFT PALATE	No. CRANIAL ANOMALY <sup>a</sup>	No. MICROITIA
A	18	0	0	2	26	26	0	0	0	0	0	0
B	18	2	0	8	107	61	43	39	15	8	17	0
C	18	2	100	7	98	63	38	30	11	10	8	0
D	18	2	200	8	109	78	36	31	8	7	15	0
E	18	2	400	8	111	73	38	37	10	8	11	0
F	18	3	0	8	108	16	16	16	8	7	15	0
G	18	3	200	8	105	16	16	16	9	5	13	2
H	18	4	0	4	53	0						
I	18	4	200	4	53	0						
J	13	2	0	8	108	63	35					
K	13	2	200	8	111	82	30					
L	10	2	0	8	109	78						
M	10	2	200	8	109	82						

\*:  $p < 0.05$ ; \*\*:  $p < 0.01$ , (Chi-square test). <sup>a</sup>: cranial anomalies include oxycephaly and exencephaly.

formalin neutral buffer solution (pH 7.4), embedded in paraffin, and serial sections (5  $\mu$ m) were made from the whole body on E10 and E13, and from the head on E18. Sections were stained with haematoxylin and eosin and examined histopathologically. Statistical analysis was done by chi-square test throughout the study.

## Results

The effect of PSK on X-ray-induced external anomalies.

The results about external malformations are summarized in Table 1. Observed malformations were microphthalmia, short tail, cleft palate, cranial anomaly,

and microtia (Table 1). By X-ray irradiation (2 Gy: group B, 3 Gy: group F) live foetuses decreased in number and incidence of malformations dramatically increased when compared with control (group A). In the 2 Gy-irradiated groups (groups C-E), the ratio of live to total foetuses at E18 was significantly increased by 200 mg/kg PSK treatment (group D) ( $p < 0.05$ ). And the frequencies of externally malformed foetuses, including microphthalmia and short tail at E18, were significantly decreased by 200 mg/kg ( $p < 0.01$ ) and 400 mg/kg ( $p < 0.05$ ) PSK administration, and that of microphthalmia by 100 mg/kg ( $p < 0.05$ ). In our previous study, in which similar protocol was used with a different X-ray generator, similar results were obtained (Naora et al.,

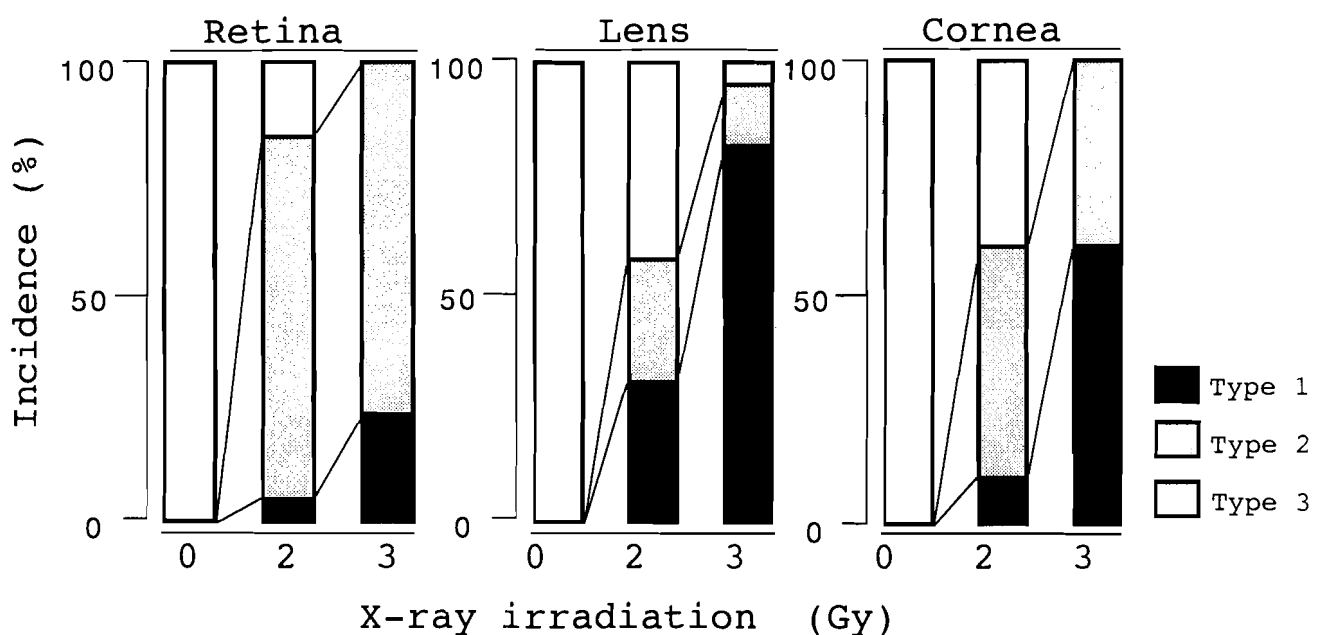


Fig. 1. Dose responses between X-ray irradiation doses and histological damage to the retina (left), lens (centre) and cornea (right) respectively. The incidence of damaged tissue (type 1 and type 2) increases and that of normal tissue (type 3) decreases in each tissue in a dose-dependent manner.

1994).

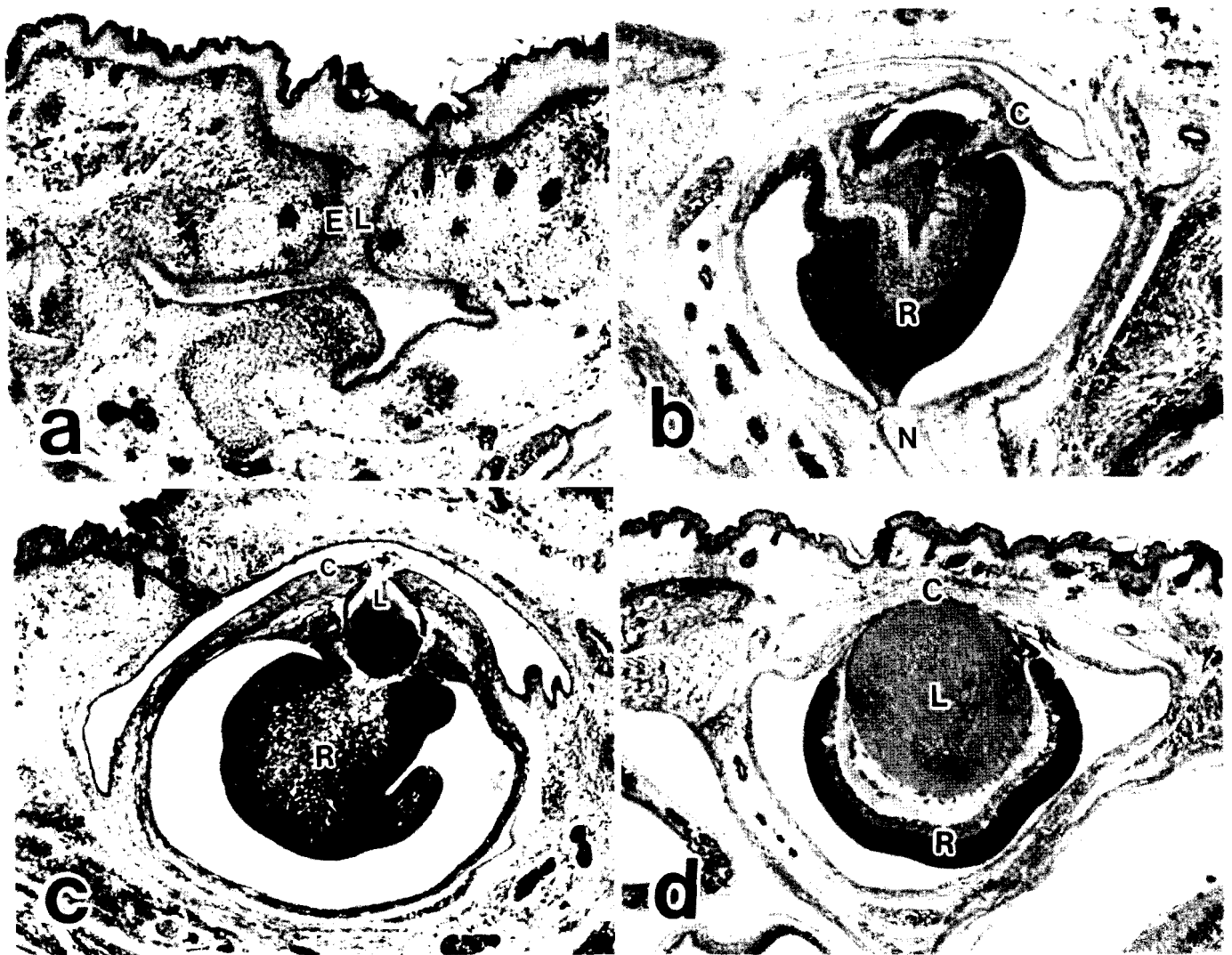
In the 3Gy-irradiated groups (groups F and G), the incidence of live foetuses and malformed foetuses at E18 was not affected with PSK treatment. In the cases of 4 Gy-irradiated groups (groups H and I), all foetuses were dead on E18 in both 200 mg/kg PSK treated group and control.

The body weight and CRL of the foetuses were not affected by any PSK administration in either 2 or 3 Gy-X-ray irradiated group (data not shown).

#### *Histological evaluation of microphthalmia*

For the evaluation of the degree of ocular anomaly

induced by X-ray, we classified the affected eyes into three groups based on the histopathological observations of the retinas (R), lenses (L), and corneas (C) (Table 2). Type 1 (1) was either aplasia of lens and cornea or rudiment of the retina. Type 2 (2) was classified as being other ocular abnormalities, and Type 3 (3) was classified as normal (Table 2). For example, pathological changes of type 1 in the retina were designated R-1. Three panels in Fig. 1 show the dose responses between the X-ray irradiation doses and the histological damage to the retina, lens, and cornea, respectively. The incidences of R-3, L-3, and C-3 were decreased, and those of R-1, 2, L-1, 2, and C-1, 2 were increased in a dose-dependent manner. Figs. 2-4 show representative examples



**Fig. 2.** Cross-sections of irradiation-induced abnormal and normal eyes on E18. **a.** An eyelid fissure (EL) is observed. However, the lens, cornea and typical retina tissue are not present. This is classified into R-1, L-1, C-1. **b.** An irregularly-shaped retina (R), which connects with the optic nerve (N) is observed. The cornea (C) is small and the corneal stroma is thickened. This eye is classified into R-2, L-1, C-2. **c.** A small lens (L), which is invaginated into the cornea (C) is observed. The retina (R) is irregularly shaped and poorly developed. This is classified into R-2, L-2, C-2. **d.** Here, the anterior chamber, the space between the cornea (C) and lens (L), is not formed. However, the lens, cornea, and retina are well developed and classified into R-3, L-3, C-3. x 50

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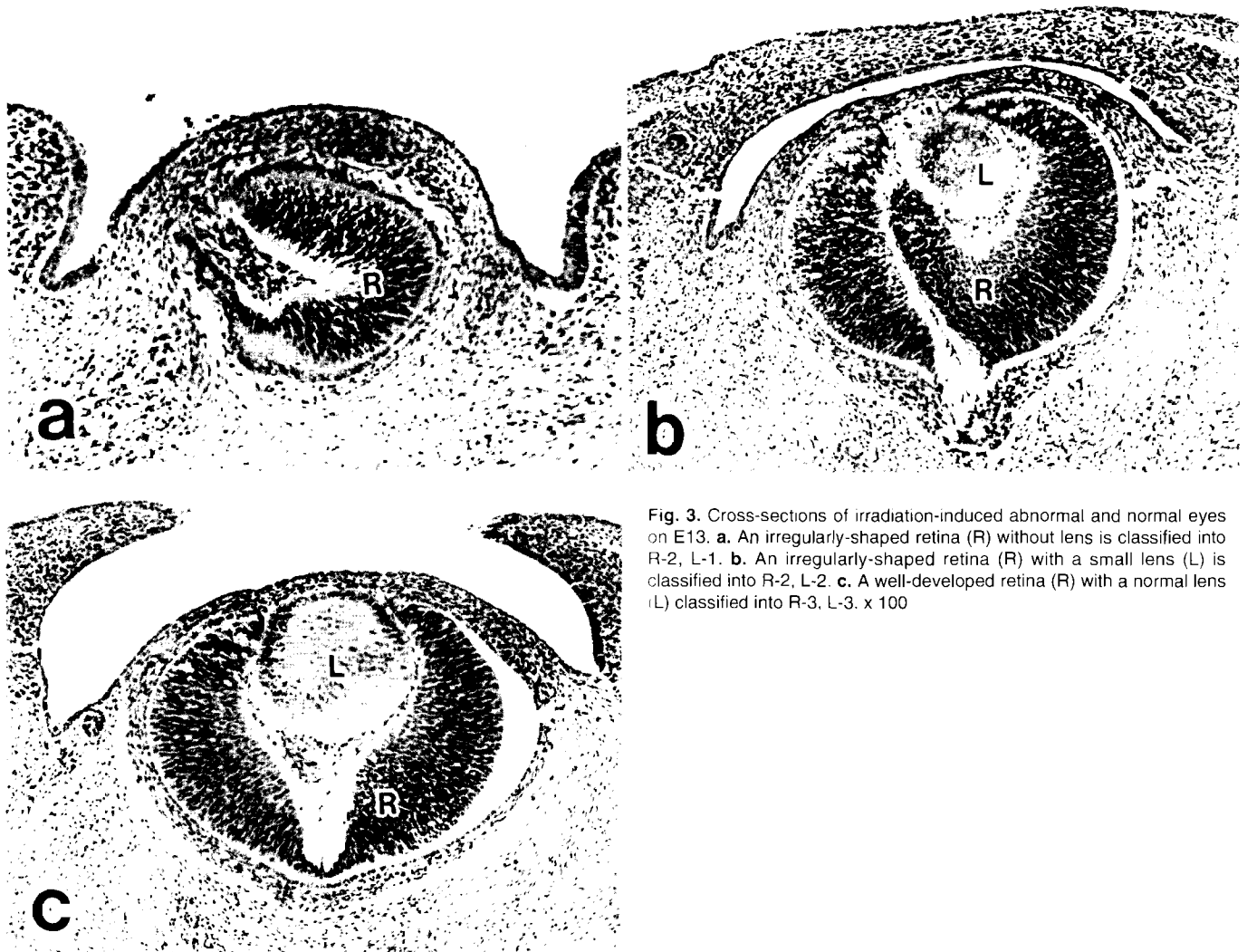
**Table 2.** Classification of histological findings.

	RETINA (R)	LENS (L)	CORNEA (C)
Type 1 (1)	Rudiments of optic cup (E10, 13, 18)	Lack of lens (E10, 13, 18)	Lack of cornea (E18)
Type 2 (2)	Eversion of inner wall (E10, 13, 18) Thickened retinal layer (E10, 13, 18) Failure to surround lens (E13, 18) Cylindrical appearance of outer layers (E18)	Small lens (E10, 13, 18) Cavities in lens (E10, 13, 18) Irregular staining of lens fibers (E10, 13, 18) Cylindrical form of lens epithelium (E18)	Irregular stroma (E18) Epithelial defect (E18) Lens invagination in cornea (E18)
Type 3 (3)	Normal retina (E10, 13, 18)	Normal lens (E10, 13, 18)	Normal cornea (E18)

classified by our criteria on E18, 13, and 10, respectively.

In the 2 Gy-irradiated groups (groups B-E), the effects of PSK treatment on the retina, lens and cornea on E18 are shown in Fig. 5. By 200 and 400 mg/kg PSK administration, the incidence of R-3 was significantly increased, and that of R-2 was significantly decreased. Although PSK administration at 100 mg/kg also significantly increased the incidence of R-3, that of R-2

was not affected. The incidence of R-1 was not affected by PSK administration. The incidence of L-1 was decreased by 200 and 400 mg/kg PSK administration, and the incidence of L-3 was increased by 200 mg/kg PSK administration. The incidence of C-2 was decreased and that of C-3 was increased by 200 mg/kg PSK treatment. No significant differences were obtained between the 100 and 400 mg/kg PSK-treated groups and control. As observed in the effect of PSK on X-ray-



**Fig. 3.** Cross-sections of irradiation-induced abnormal and normal eyes on E13. **a.** An irregularly-shaped retina (R) without lens is classified into R-2, L-1. **b.** An irregularly-shaped retina (R) with a small lens (L) is classified into R-2, L-2. **c.** A well-developed retina (R) with a normal lens (L) classified into R-3, L-3. x 100

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induced external anomalies (Table 1), 200 mg/kg PSK was the most effective in suppressing the 2 Gy X-ray irradiation-induced histological ocular abnormalities.

In the 3 Gy-irradiated groups (groups F-G), the results of the histological analysis of the retina, lens and cornea on E18 are shown in Fig. 6. The incidence of L-1 was decreased and that of L-2 was increased significantly by 200 mg/kg PSK treatment. The histological damage to the retina and cornea were not affected by PSK treatment.

#### Developmental study of the effect of PSK

To obtain further insight into the mechanism of PSK-induced antiteratogenic effects, fetuses treated as described above on E7 were examined during earlier days of gestation (E10 and E13) (Table 1: groups J-M). As the development of the optic cup is finished on E10, it was difficult to examine the ocular abnormalities before E10 using these criteria. By

external observations, the frequency of living foetuses on E10 and E13, which were treated with 2 Gy-irradiation with or without 200 mg/kg PSK administration, are shown in Table 1. On E13, incidence of living foetuses was significantly increased ( $p < 0.05$ ) in the PSK-treated group. Interestingly, these ratios of living to total foetuses in both PSK-treated and untreated groups on E13 were remarkably similar to those on E18 (Table 1, compare groups J to B, K to D, see Discussion). On E10, there was no significant difference between the 200 mg/kg PSK treated group and control.

A summary of histological observations of the eye on E10 and E13 is shown in Fig. 7. On E13, incidences of R-2 and L-1 were significantly decreased and those of R-3 and L-3 were increased by PSK administration. On E10, the incidence of L-1 was statistically decreased by PSK administration. Since differentiation of the cornea begins on E14-15, we could not examine the effects of PSK on the cornea on E10 and E13.

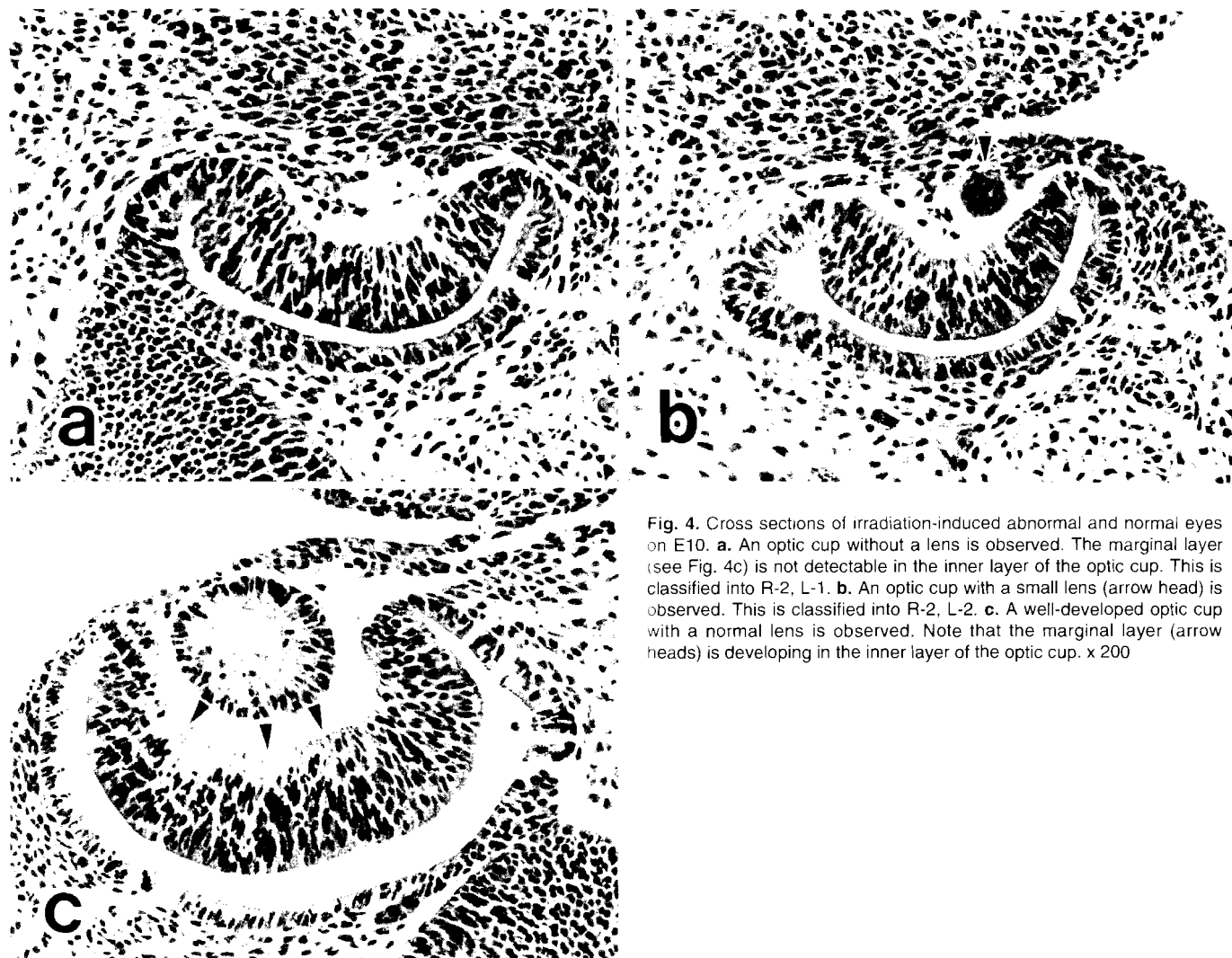


Fig. 4. Cross sections of irradiation-induced abnormal and normal eyes on E10. a. An optic cup without a lens is observed. The marginal layer (see Fig. 4c) is not detectable in the inner layer of the optic cup. This is classified into R-2, L-1. b. An optic cup with a small lens (arrow head) is observed. This is classified into R-2, L-2. c. A well-developed optic cup with a normal lens is observed. Note that the marginal layer (arrow heads) is developing in the inner layer of the optic cup. x 200

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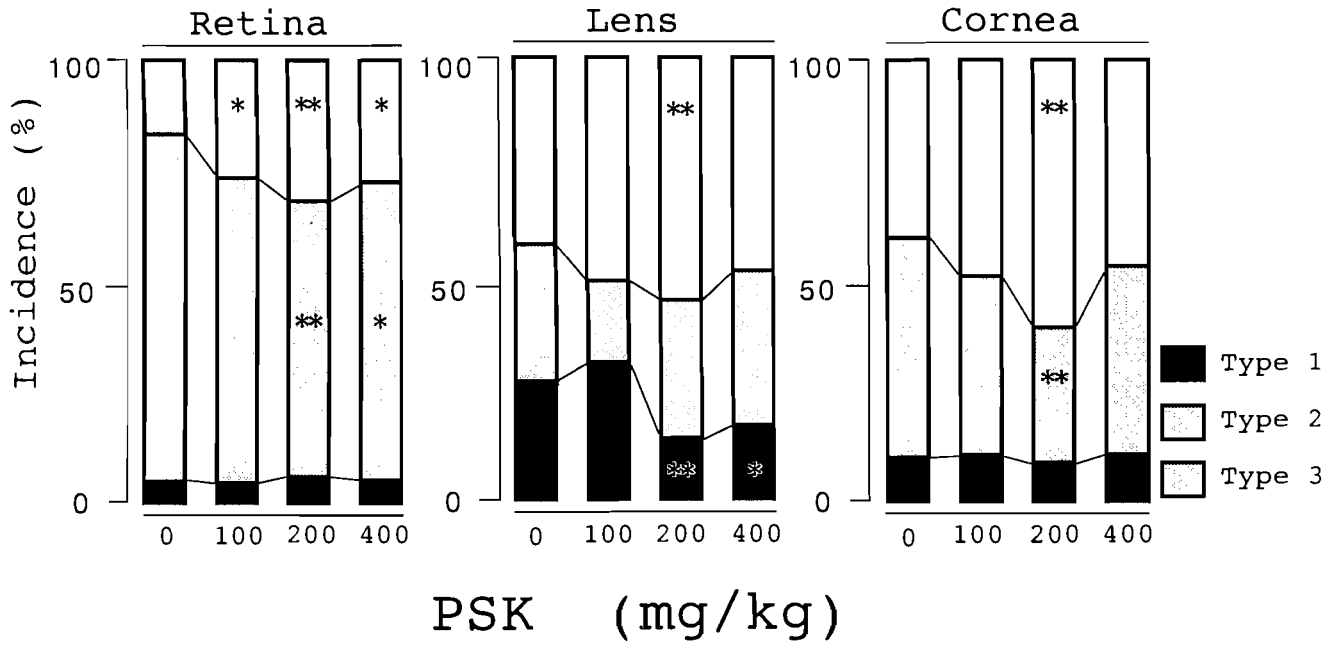


Fig. 5. The effect of PSK administration on the histological changes in the eye on E18 in 2 Gy-irradiated groups (Groups B-E). \*: p<0.05; \*\*: p<0.01.

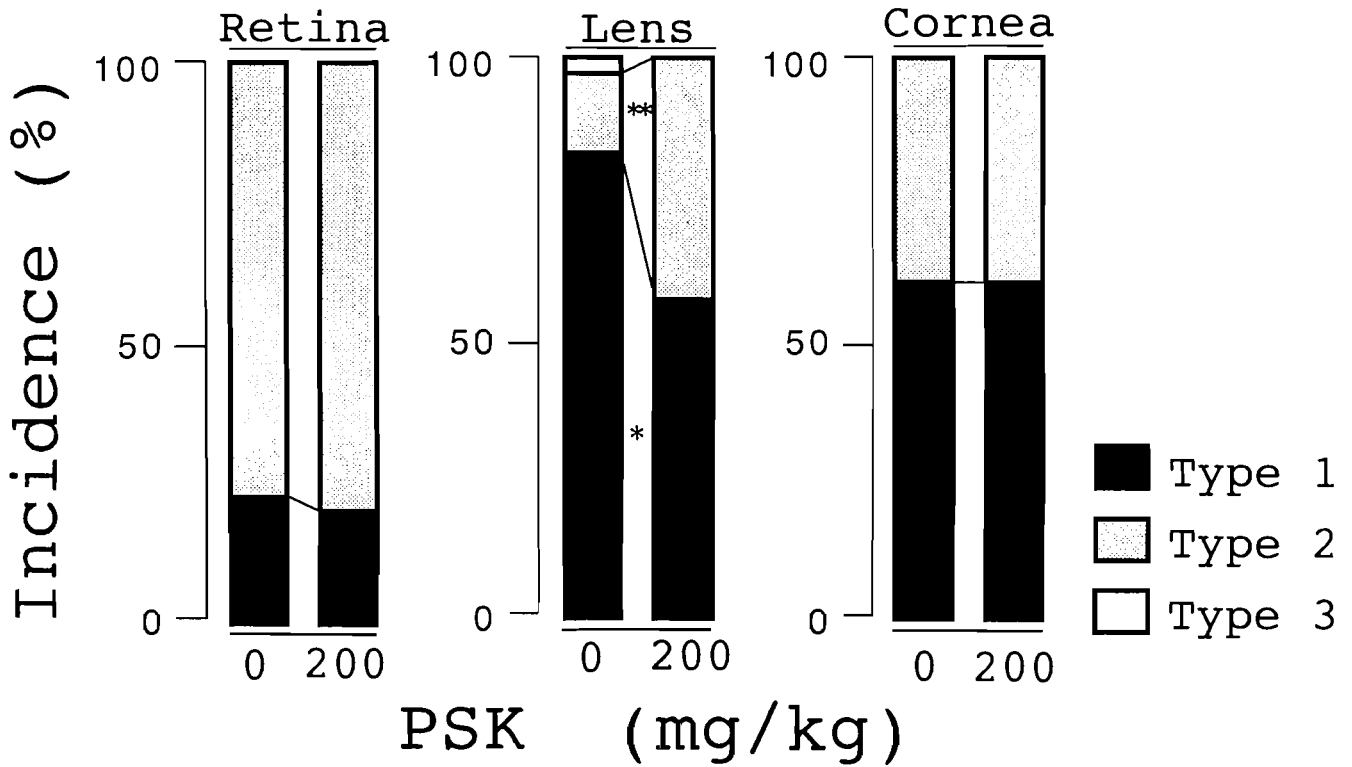


Fig. 6. The effect of PSK administration on the histological changes in the eye on E18 in 3 Gy-irradiated groups (Groups F-G). \*: p<0.05; \*\*: p<0.01.

## Discussion

In the present study, 200 mg/kg PSK administration was most effective for decreasing the incidence of X-ray-induced malformed fetuses on E18. In addition, this dose was also the most effective for decreasing X-ray-induced foetal death on E18. Therefore, we regard 200 mg/kg of PSK as the most effective dose against X-ray irradiation.

By external observation, the incidences of living foetuses were not affected by PSK administration on E10. On E13, the incidence of living foetuses was increased by PSK administration, and the incidences of living foetuses with or without PSK administration were almost the same between E13 and E18. These results suggest that the living foetuses on E13 survived until E18 with or without PSK administration. Therefore, PSK seems to suppress X-ray-induced early foetal death (E10-13), and it is unlikely that the elimination of severely damaged foetuses in later gestation (E13-18) is the mechanism of the antiteratogenic effect of PSK. According to Kurishita (1990), PSK decreased 5-AC-induced digital defects, when injected between 24 hours before to one hour after 5-AC treatment when injected 30 hours before or 3 hours after 5-AC treatment, PSK had no effect. In the present study, we injected PSK within ten minutes of X-ray irradiation and observed a decrease in the incidence of X-ray-induced ocular abnormalities. This report and our study suggest that the

antiteratogenic effect of PSK acts in the early period after X-ray irradiation.

Histopathologically, PSK administration suppressed the X-ray-induced damage in the lens most dramatically. The incidence of L-1 (aphakia) was significantly decreased by PSK administration in 2 and 3 Gy-irradiated groups on E18, but not those of R-1 (rudimental retina) and C-1 (corneal agenesis). In the present study, the incidence of X-ray-induced lens aplasia was already decreased by PSK administration on E10 (72 hours after X-ray irradiation). According to Rugh and Wolff (1955), after X-ray irradiation, mitosis was arrested and acute cell death was observed within several hours. Twenty-four hours after X-ray irradiation, the scavenging of dead cells occurred. After this, a repairing response finished within 72 hours of irradiation (Rugh and Wolff, 1955). After the repairing process, remaining severe X-ray-induced damage of the lens placode may be one of the causes of lens aplasia. Therefore, it was suggested that PSK may enhance protection against X-ray-induced damage and/or the early repairing process of foetuses, especially in the lens placode. Some pharmacological effects of PSK which support this possibility have been reported. PSK administration enhances the phagocytotic activity in mice (Mayer and Drews, 1980). Torigoe (1980) showed the antimutagenic effect of PSK at chromosome level. PSK has also been shown to possess a free radical scavenging effect (Yoshikawa, 1980), which acts as

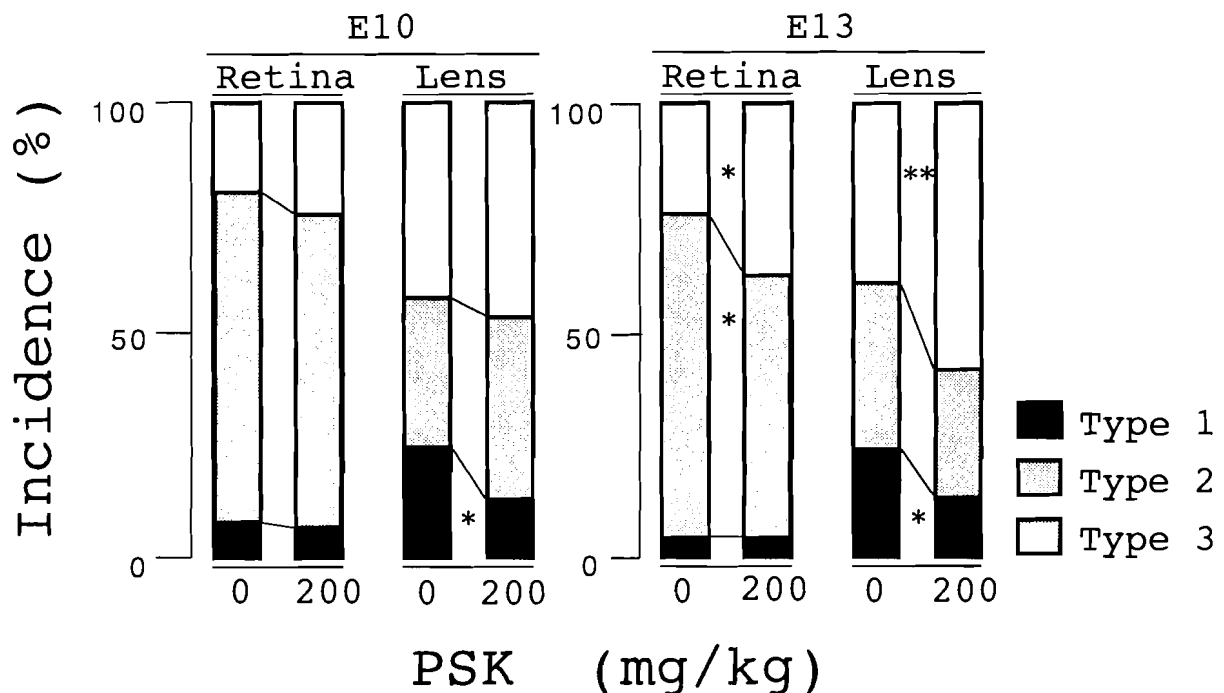


Fig. 7. The effect of PSK administration on the histological changes in the eye on E10 and 13 (Groups J-M). \*:  $p < 0.05$ ; \*\*:  $p < 0.01$

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protection against radiation (Jones et al., 1990).

On the other hand, without severe damage of the lens placode, complete failure in the invagination of the lens placode could also be the cause of lens aplasia (Saha et al., 1989). Without close contact between the optic vesicle and the surface ectoderm, the invagination of the lens placode cannot be induced (Webster et al., 1984). However, retinal damage was observed at the same incidence with and without PSK administration on E10, when the incidence of X-ray-induced lens aplasia was already reduced by PSK administration. PSK thus appears to effect primarily the lens placode rather than the retina, decreasing the incidence of lens aplasia.

We have demonstrated that PSK suppressed X-ray-induced abnormal ocular development not only externally but also histologically. Especially, we have observed a suppressive effect of PSK on X-ray-induced abnormal lens formation, which was already observed on E10. The cytological basis of the antiteratogenic effect of PSK remains to be investigated including studies on lens development in the earlier organogenetic period before E10.

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