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Ocular Toxicity Caused by Paclitaxel in Neonatal Sprague-Dawley Rats

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Abstract. *Background: The toxic effects of paclitaxel (PTX) on neonatal eyes have not been evaluated. Materials and Methods: PTX was dissolved in solvent containing polyethoxylated castor oil and intraperitoneally administered to male and female Sprague-Dawley rats at a dose of 0, 2, 4 and 8 mg/kg at 0 day of age, 4 mg/kg at 14 days of age, or 8 mg/kg at 12-18 weeks of age. Eyes were histologically examined 1 and/or 7 days after PTX. Results: Male and female rats that received 4 mg/kg or more of PTX at 0 days of age developed cataracts and retinal dysplasias, while the rats that received other dosing regimens did not develop ocular lesions. Epithelial cells in the lens were apoptotic on day 1, and lens fibers were degenerative at day 7, indicating the development of cataracts. Scattered foci of apoptosis in the neuroblastic layer of the retina on day 1, and rosettes were seen on day 7, suggestive of retinal dysplasia. Conclusion: Neonatal rats that received a threshold dose of PTX (4 mg/kg) at a critical period (0 days of age) developed cataracts and retinal dysplasia; however, the 2 mg/kg dose at 0 days of age and the 4 or 8 mg/kg dose at 14 days of age or older caused no ocular damage. Thus, the determination of the dose and timing of PTX treatment administered during the early developmental stage requires great care to avoid ocular toxicity.*

Chemicals that are used systemically or topically for the treatment of medical disorders have the potential to induce adverse side-effects in the eye (1). Several chemicals are linked to the development of cataracts, which are lens opacities associated with visual impairment. Steroids are a risk factor for cataract development and children are more susceptible than adults to the development of steroid-associated cataracts (2). Antineoplastic agents have also been linked to an increased risk of cataract development. Young rats are more likely than older rats to develop cataracts after exposure to the anthracycline antibiotic doxorubicin (3, 4), the glycopeptide antibiotic bleomycin (5), and the alkylating agent busulfan (6). Retinal dysplasia is a developmental anomaly characterized histologically by an anomalous development of the retina with dysplastic rosettes composed of neuronal retinal cells (7). Retinal dysplasia can be experimentally induced by the exposure of newborn rats and mice to certain chemicals (8-13), such as the metal coordination complex cisplatin (14). Antineoplastic agents that target highly proliferative malignant cells also affect quickly dividing non-neoplastic cells that are found in young individuals. Paclitaxel (PTX), a member of the taxane family of drugs isolated from the plant *Taxus brevifolia*, is a microtubule stabilizer (anticontractile agent) and a mitotic inhibitor (antiproliferative agent) (15). Proliferative vitreoretinopathy is characterized by the proliferation of cells in the vitreoretinal surface and subsequent cellular contraction that results in retinal detachment. PTX suppresses cell proliferation and contraction in experimental proliferative vitreoretinopathy induced in animals (16). In humans, PTX is used to treat a range of cancer types. The intraperitoneal (*i.p.*) injection of PTX significantly inhibits the growth of human pediatric tumors transplanted into athymic mice (17), and subconjunctival injections of PTX significantly inhibit the intraocular growth of retinoblastoma in LH beta-Tag murine transgenic mice (18). In humans, PTX is used to treat children with pediatric tumors (19, 20) and pregnant women with cancer (21). Thus, the exposure of young patients to PTX, as well as their *in utero* PTX exposure, should be considered. Ocular toxicities, such as reduced vision, scintillating scotomas, and cystoid macular edema, have occurred in adults who received PTX infusion (22, 23). Fast-dividing cells that are more sensitive to PTX may exist within the ocular tissue during the developmental period of life. However, ocular toxicity caused by exposure to PTX in the

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Key Words: Paclitaxel, rat, newborn, lens, retina, cataract, retinal dysplasia, apoptosis.

Table I. *Body weight of rats that received paclitaxel at 0 days of age.*

PTX (mg/kg)	Body weight * (g)	
	Day 1	Day 7
0	7.45±0.21	20.21±0.66
2	7.69±0.45	17.11±0.47**
4	7.79±0.45	14.75±1.30**
8	7.10±0.34	11.21±1.22**

PTX, paclitaxel; *values are mean±SE. **$p<0.01$ compared with PTX-untreated rats.

neonatal period has not been precisely evaluated. Therefore, in the present study, PTX was intraperitoneally injected into neonatal Sprague-Dawley rats that were monitored for the development of ocular abnormalities.

Materials and Methods

Animals. Pregnant Sprague-Dawley rats were purchased from Charles River Japan (Atsugi, Japan). Animals were individually housed in plastic cages with paper bedding (Paper Clean, SLC, Hamamatsu, Japan) in a room maintained at 22±2˚C and 60±10% relative humidity with a 12-h light/dark cycle. Animals were maintained on a basal diet (CMF, Oriental Yeast, Chiba, Japan) and had free access to water. Mothers as well as their male and female offspring were used. All experimental animal protocols were approved by the Animal Experimentation Committee of Kansai Medical University.

Chemicals. PTX was obtained from Tokyo Chemical Industry (Tokyo, Japan) in a powder form and stored at 4˚C in the dark until use. Immediately prior to use, PTX (1 mg) was dissolved in a solvent containing 100 μl polyethoxylated castor oil (Cremophor EL, Sigma, St. Louis, MO, USA), 14 μl absolute ethanol and 886 μl physiological saline. The ethanol content was lower than that in the PTX solution in clinical use to reduce ethanol toxicity. Vehicle (solvent without PTX) was used as a control.

Experimental procedures. Male and female newborn rats received a single *i.p.* injection of 0, 2, 4, or 8 mg/kg PTX at day 0 (day of birth) or 4 mg/kg PTX at day 14. A single *i.p.* injection of 8 mg/kg PTX was given to the mother rats, which were 12-18 weeks old. The dose of PTX was determined from the LC_{50} values of adult animals. Animals were sacrificed by cervical dislocation at 1 or 7 days after the PTX treatment. All animals were weighed at the time of injection and when they were sacrificed.

Tissue processing. Eyes were fixed in methacarn overnight and selected specimens were fixed in 10% neutral buffered formalin. Fixed tissues were embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (HE). Sections including ora serrata and optic nerve were independently evaluated by all three authors.

TUNEL and PCNA staining. Formalin-fixed sections were used for cell death analysis. Cell death was observed by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP digoxigenin nick end-labeling (TUNEL) using an in situ apoptosis detection kit (Apop-Tag; Intergen, Purchase, NY, USA).

Methacarn-fixed sections were used for cell proliferation analysis. Cell proliferation was immunohistochemically evaluated by using antiproliferating cell nuclear antigen (PCNA) antibody (clone PC-10; Novocastra, Newcastle upon Tyne, UK). The reaction products of TUNEL and PCNA were visualized with 3,3'-diaminobenzidine as a chromogen.

Statistics. The body weight expressed as mean±SE was compared by using the Student's *t*-test. *P*-values less than 0.05 were considered statistically significant.

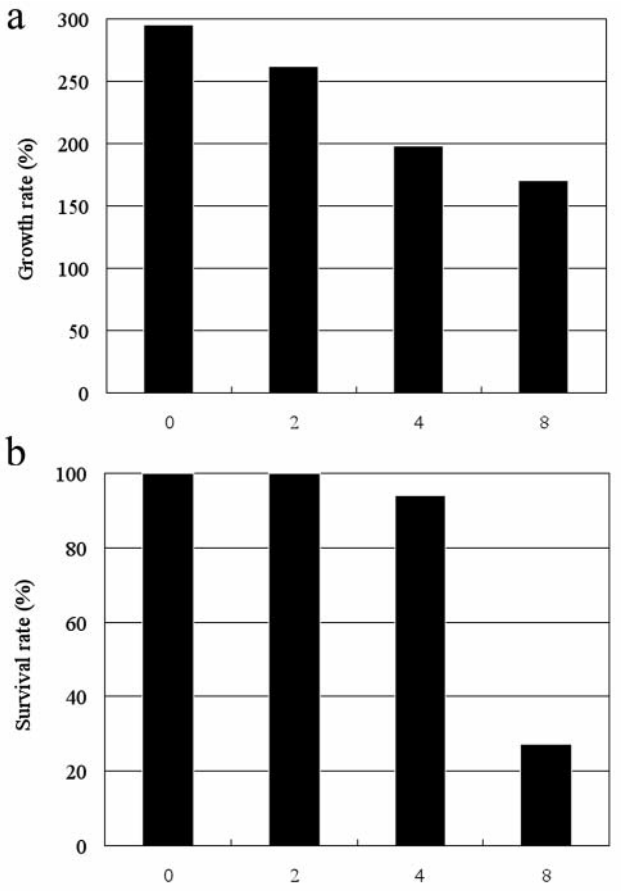

Figure 1. *Growth rate (a; mean body weight at day 7/ body weight at day 0) and survival rate (b) after different doses of paclitaxel (PTX) were given to 0-day-old male and female Sprague-Dawley rats. Rats were sacrificed at 7 days of age.*

Results

General remarks. PTX administered at different doses at different stages of development did not cause loss of body weight. However, acute toxicity occurred in day 0 PTX-treated rats. Their body weight on day 1 was similar to that of the other groups; but, it was significantly less at day 7 as

Table II. *Lens and retinal damage in rats after a single intraperitoneal injection of paclitaxel.*

Group	Age at treatment (days)	PTX (mg/kg)	No. of rats treated	No. of rats tested	Lens damage*		Retinal damage*	
					Day 1	Day 7	Day 1	Day 7
1	0	0	20	20	0/5	0/15	0/5	0/15
2	0	2	20	20	0/5	0/15	0/5	0/15
3	0	4	22	21	4/5	9/16	0/5	7/16
4	0	8	16	8	5/5	3/3	5/5	3/3
5	14	4	10	10	ND	0/10	ND	0/10
6	84-126	8	9	9	ND	0/9	ND	0/9

*No. of rats affected/no. of rats examined; ND, not determined.

compared with untreated rats (Table I). The gain of weight (growth rate) was inversely related to the PTX dose (Figure 1a), and the 7-day survival rate was low (27%) in the 8 mg/kg dose group (Figure 1b). When the 7-day surviving rats as well as rats on day 1 were autopsied, the most striking features were the dose-dependent suppression of extramedullary hematopoiesis in the spleen and liver (Figure 2a-d), indicating hematopoietic toxicity. This toxicity had already occurred 1 day after PTX treatment. No mortality occurred in the day 14 or older rats treated with 4 or 8 mg/kg PTX, respectively.

Ocular response. The lens and retina of day 0 neonatal rats were susceptible to PTX treatment. The 4 and 8 mg/kg dose of PTX induced lens and retinal damage, while the 2 mg/kg dose as well as the solvent only treatments, did not cause damage (Table II). However, no pathological ocular alterations occurred after the 4 or 8 mg/kg PTX treatment in day 14 or older rats, respectively. Therefore, we will focus on the lens and retinal damage caused by 4 mg/kg or more of PTX administered to neonatal rats at day 0.

In the lens on day 1, as compared with solvent-treated controls (Figure 3a) with TUNEL positivity restricted to the lens epithelial nuclei at the bow zone (Figure 3b, arrows), PTX caused abnormal lens epithelial cells beneath the capsule (Figure 3c) showing scattered TUNEL-positive cells (Figure 3d, arrowheads) in addition to cells at the bow zone (Figure 3d, arrows). On day 7, the lens fibers were degenerated, swollen, vacuolated and liquefied, and Morgagni-like water vacuoles appeared (Figure 3e), indicating the development of mature cataract. In solvent-treated rats, PCNA labeling was mainly seen in the germinative zone, sporadically beneath the lens capsule in the anterior portion, but not in the bow zone; the staining pattern of PTX-treated rat lenses did not dramatically differ from that of solvent-treated controls (data not shown).

The day 1 retina of control rats was composed of the neuroblastic layer (Figure 4a) with no TUNEL staining (Figure 4b). In contrast, the day 1 PTX-treated rat retina contained scattered foci composed of nuclear debris throughout the undifferentiated neuroblastic layer (Figure 4d). The nuclear debris contained TUNEL-positive cells (Figure 4e). At day 7, the control retina was separated into the inner and outer neuroblastic layer (Figure 4c), while the retinas of PTX-treated rats were marked by rosette formation on the outer neuroblastic layer, indicative of retinal dysplasia (Figure 4f). However, the inner neuroblastic layer and retinal pigment epithelial cells were free of involvement. Again, PTX treatment did not affect PCNA labeling. At day 1, most of the cells in the neuroblastic layer were positively stained, leaving the amacrine cells unstained. At day 7, the inner but not the outer neuroblastic layer was positively stained.

Discussion

A dose of 4 mg/kg or more of PTX injected into 0-day-old rats resulted in ocular toxicity; however, the same dose given to older rats did not cause ocular toxicity. The maximum tolerated dose in adult mice is 20-30 mg/kg (17, 24), and the median lethal dose (LD_{50}) in adult Sprague-Dawley rats is 8.3-8.8 mg/kg (24). In the present study, PTX treatment in 0-day-old rats dose-dependently lowered the body weight gain, and the 7-day survival rate decreased to 27% in 0-day-old rats given 8 mg/kg PTX. PTX causes embryotoxicity and increases the mortality rate of chick embryos (25). Extramedullary hematopoiesis was suppressed in parallel to the PTX dose in rats; this suppression was maximum in the day-1 liver and spleen of rats treated with 8 mg/kg PTX at 0 days of age. Bone marrow toxicity, which is the principal toxic effect of PTX (15), might be the cause of death in the present study. Zero-day-old rats treated with 4 mg/kg or more of PTX, but not older rats treated with the same dose, developed cataracts and retinal dysplasia. The 0-day-old rats treated with 2 mg/kg PTX, as well as the vehicle-treated controls, did not develop ocular toxicity. Because PTX is not sufficiently water soluble, it must be dissolved in a lipophilic solvent; dimethyl sulfoxide is frequently used as the solvent in animal experiments (18). Solvent toxicity is an important

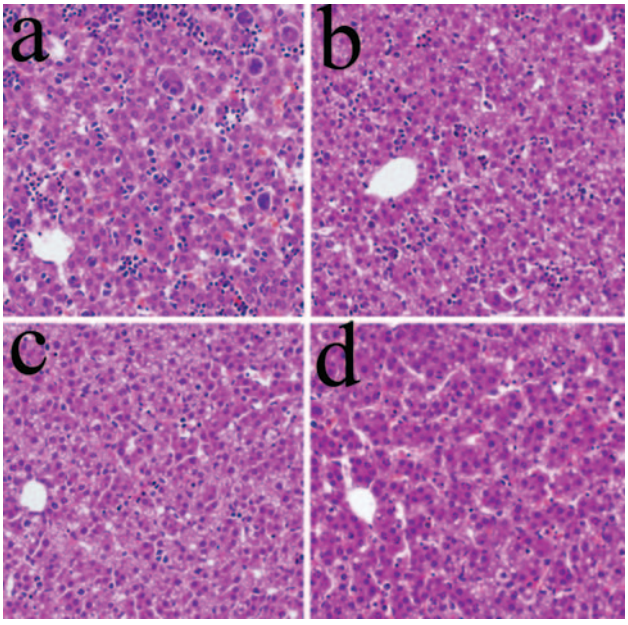

Figure 2. *Degree of extramedullary hematopoiesis of the liver after different doses of paclitaxel (PTX) were given to 0-day-old Sprague-Dawley rats that were sacrificed at day 1 of age. PTX was given at a dose of 0 mg/kg (a), 2 mg/kg (b), 4 mg/kg (c), and 8 mg/kg (d). Extramedullary hematopoiesis gradually decreased in parallel to the dose of PTX. (HE, ×100).*

confounder, and cataract formation has occurred in animals after dimethyl sulfoxide administration (18, 26). In the present study, the PTX-untreated vehicle controls did not develop ocular toxicity. Therefore, the delivery vehicle, polyoxyethylated castor oil (Cremophore EL), used in the present study did not cause any adverse ocular effects.

Chemically induced cataract formation in animals has been well documented (27). Cataracts can be rapidly induced in premature rats with the alkylating chemical *N*-methyl-*N*-nitrosourea (MNU) (28). When a single *i.p.* injection of 100 mg/kg MNU was given to 0-, 5-, 10-, and 15-day-old male and female Sprague-Dawley rats, gross lens opacity was recognized 7 days later in 0-day-old MNU-treated rats, 14 days later in 5- and 10-day-old MNU-treated rats, and 30 days later in 15-day-old MNU-treated rats; lens opacity was not evident in 20-day-old MNU-treated rats 30 days after the treatment. Thus, cataract formation was age-dependent, as the younger animals were more susceptible to the same dose of MNU. In agreement with the results of MNU studies, we found that the 0-day-old lens but not 14-day-old lens was susceptible to PTX-induced cataract formation. Microtubules exist in the crystalline lens epithelial cells and colchicine, an inhibitor of microtubule assembly, induces cataracts (29). Because PTX inhibits the disassembly of microtubules, PTX-treated microtubules are extraordinarily stable and dysfunctional. Normal microtubule dynamics are required for cell division and survival, so that PTX-induced cell death may be caused by disrupting the normal microtubule system (15).

Retinal dysplasia is induced when neonatal rats or mice receive more than 3 mg/kg of cisplatin; however, animals that are older than 7 days do not develop retinal dysplasia when given the same or higher dose of cisplatin (14). MNU administered on day 16 of gestation (30) or to newborn mice younger than 3 days of age (12) results in retinal dysplasia. The development of retinal dysplasia is related to the stage of retinal maturation, as dysplasia develops only in immature retina (31). Consistent with these results, we found that PTX caused retinal dysplasia only when applied to newborn rats. TUNEL-positive cell clusters were observed 1 day after PTX treatment, whereas the PCNA labeling was not altered. PTX selectively caused cell death in scattered neuroblast cells but did not influence the proliferative activity of growing retinal cells. Thus, the manifestations of rosettes are the result of selective cell death in the neuroblastic layer. When chick embryos are exposed to PTX, the resulting chicks have reduced eye pigmentation (22); the albino Sprague-Dawley rats used in the present study cannot be used to confirm this observation. However, the retinal pigment epithelial cells were not affected by PTX, as they remained as a single continuous layer above the Bruch's membrane.

Recently, phase 1 study on PTX in children with refractory leukemia and solid tumors have been performed (19, 20). In the present study, newborn rats developed lens and retinal toxicity. PTX is an effective treatment for pediatric cancer model (17, 18). The present results indicate that caution should be used when timing the administration of PTX to pediatric patients. The maturity of the rat retina at birth is approximately equivalent to that of the retina of a 4- to 5-month-old human fetus (32). The maternal age at birth is increasing; therefore, the chance of PTX use by older pregnant women, resulting *in utero* PTX exposure, may increase. The present results warn of the danger of PTX use during pregnancy. As the PTX dose necessary for ocular toxicity was below the human therapeutic dose (19), the ocular toxicity of PTX in the early developmental stage should be carefully considered.

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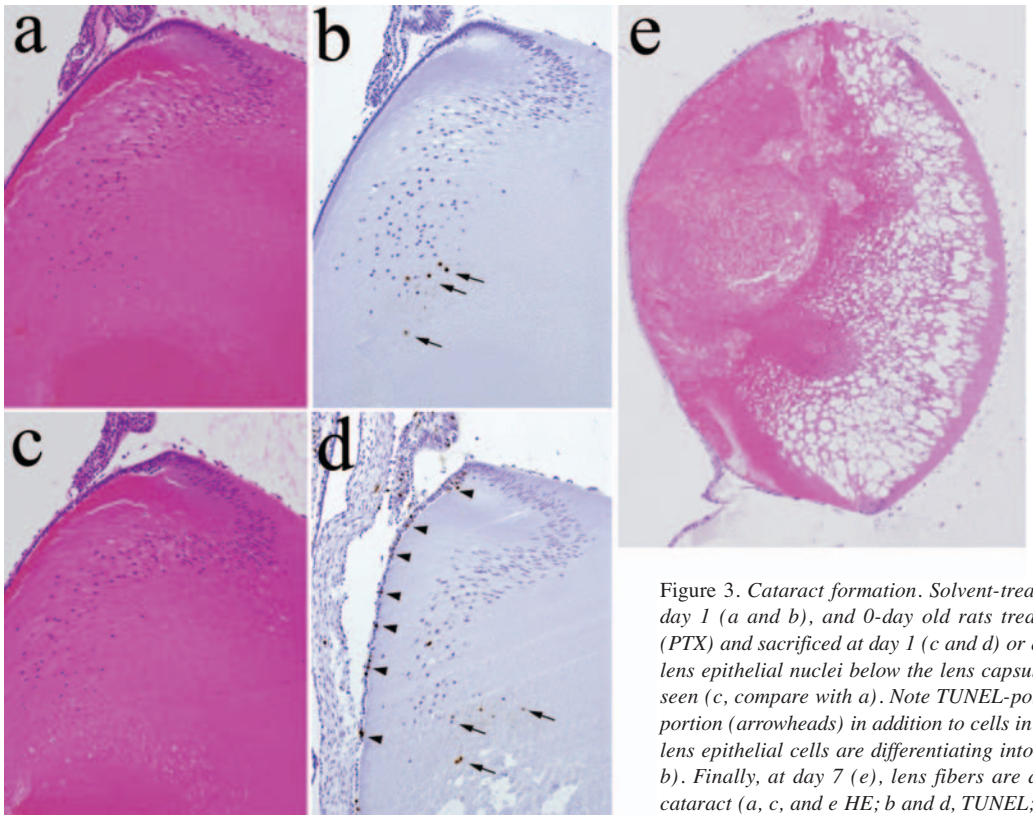

Figure 3. *Cataract formation. Solvent-treated control rats sacrificed at day 1 (a and b), and 0-day old rats treated with 8 mg/kg paclitaxel (PTX) and sacrificed at day 1 (c and d) or day 7 (e). At day 1, abnormal lens epithelial nuclei below the lens capsule at the anterior portion are seen (c, compare with a). Note TUNEL-positive staining at the anterior portion (arrowheads) in addition to cells in the bow area (arrows) where lens epithelial cells are differentiating into lens fibers (d, compare with b). Finally, at day 7 (e), lens fibers are degenerated, causing mature cataract (a, c, and e HE; b and d, TUNEL; a-d ×100, e ×60).*

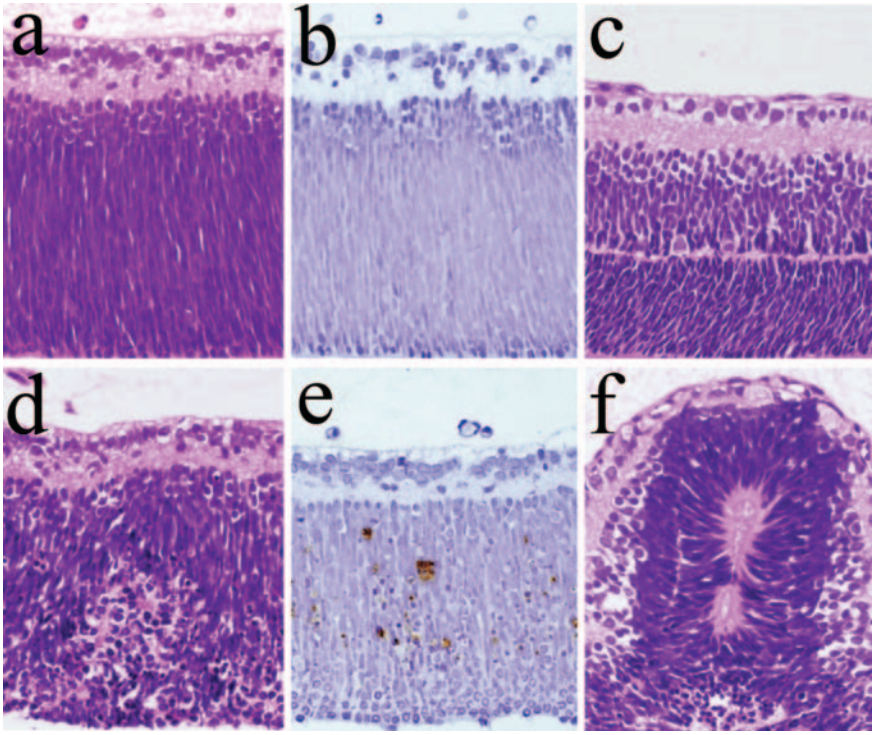

Figure 4. *Retinal dysplasia. Clusters of degenerative cells (d) showing TUNEL staining (e) are seen in the neuroblastic layer in 4 or 8 mg/kg paclitaxel (PTX)-treated rats at day 1, leading to rosette formation at day 7 (f), compared with normal retinal development in vehicle-treated retina (a and b, day 1; c, day 7). (a, c, d, and f, HE; b and e, TUNEL; ×150).*

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