

Effect of Aldose Reductase Inhibitors on Naphthalene Cataract Formation in the Rat

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Naphthalene feeding can result in cataract formation in rats and rabbits due to specific metabolites of naphthalene. The concomitant administration of the aldose reductase inhibitor A11576 to naphthalene-fed rats was proven to prevent cataract formation. To determine whether this effect was directly linked to the ability of A11576 to inhibit enzyme aldose reductase, a variety of structurally diverse aldose reductase inhibitors, including the carboxylic acids tolrestat, Ponalrestat, and FK366, and the spirohydantoins, sorbinil and A11576, were investigated for their ability to inhibit naphthalene-induced cataracts. Brown Norway rats, administered naphthalene by gavage, were fed normal rat chow containing these aldose reductase inhibitors at levels known to inhibit sugar cataract formation. The lens changes in these rats were monitored over a 90-day period by portable slit-lamp microscopy and histologic study. A11576 showed a dose-dependent reduction in naphthalene-induced cataract formation, with no naphthalene-associated deposits seen in toluidine blue-stained lens sections. Sorbinil also reduced lens changes, whereas tolrestat, Ponalrestat, and FK366 had no effect. These results suggest that inhibition of naphthalene-induced cataract formation by structurally diverse aldose reductase inhibitors was not linked to the inhibition of aldose reductase. Invest Ophthalmol Vis Sci 32:1630-1637, 1991

The first report of naphthalene-induced cataracts in rabbits was in 1886.¹ Since then, naphthalene-induced cataractogenesis has been extensively investigated in rabbits and rats.²⁻⁴ Generally, these cataracts develop more easily in pigmented strains of animals, presumably due to the presence of the melanin-synthesizing enzyme phenol oxidase (tyrosinase; E.C.1.10.3.1). Lens changes during naphthalene-induced cataract formation were first described by Adams,⁵ and detailed pathologic changes in rabbits were reported by Pirie.⁶ The morphologic changes in naphthalene-induced cataracts of rats have been documented by Scheimpflug and slit-lamp photography.^{7,8}

In rats fed naphthalene for 6 wk, a dense, well-defined zonular opacity is formed.⁸⁻¹⁰ This opacity results from the formation of 1,2-dihydroxynaphthalene, a metabolite of naphthalene, in the eyes of both

rats and rabbits.¹¹ This compound auto-oxidizes to 1,2-naphthoquinone, an agent that reacts with many metabolically active components of the lens.¹¹ In the rat, tyrosinase from pigmented tissue plays an active metabolic role, whereas catechol reductase in the lens is of no consequence. The reverse is true for rabbits.⁴

Interest in naphthalene-induced cataracts has been renewed by the observations that naphthalene-induced cataracts in rats can be prevented by the aldose reductase inhibitor A11576.^{8,12} To determine whether this effect was linked to specific structural attributes of A11576 or whether it resulted from the ability to inhibit the enzyme aldose reductase, we studied a variety of structurally diverse aldose reductase inhibitors for their abilities to prevent naphthalene-induced cataract formation.

Materials and Methods

Chemicals

Naphthalene (99+%) was purchased commercially (Sigma Chemical Co., St. Louis, MO). Methacrylate JB4 was obtained from Polyscience (Warrington, PA). Paraformaldehyde was purchased from Fisher Scientific (Fair Lawn, NJ). The aldose reductase inhibitors used were tolrestat (N-[[5-[trifluoromethyl]-6-methoxy-1-naphthalenyl]thioxomethyl]-N-methylglycine; Wyeth-Ayerst, Princeton, NJ), Ponalrestat ([3-[4-bromo-2-fluorobenzyl]-4-oxo-3H-phthalazin-

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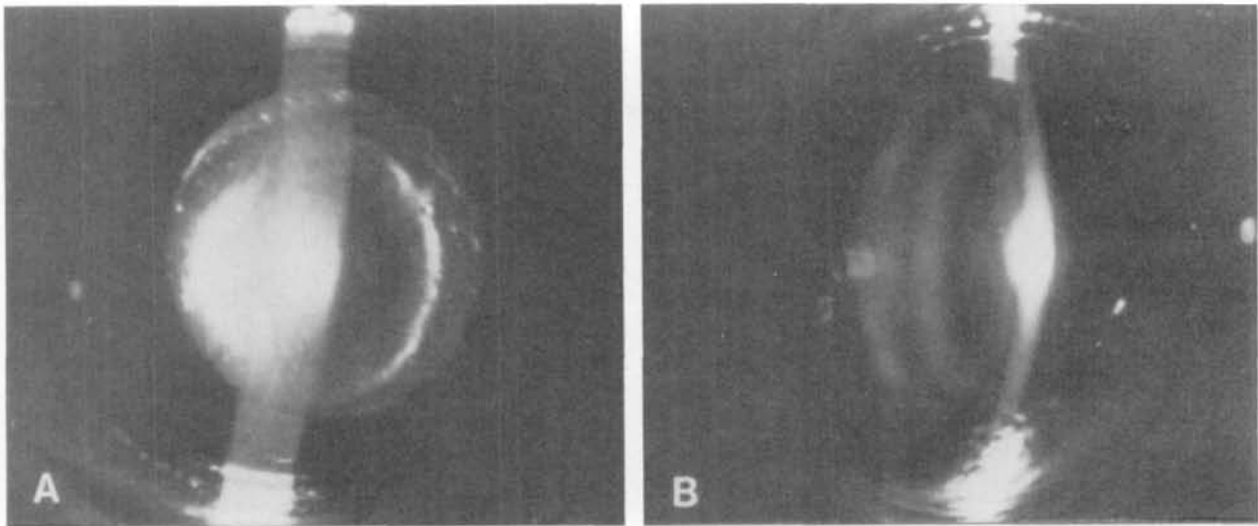


Fig. 1. Portable slit-lamp view of naphthalene-induced cataract development at 30 days. (A) Retroillumination. (B) Slit-lamp photography. Progressive presence of granular deposits were noted in the deep cortex near the nucleus of the cataractous lenses similar to zonular cataracts.

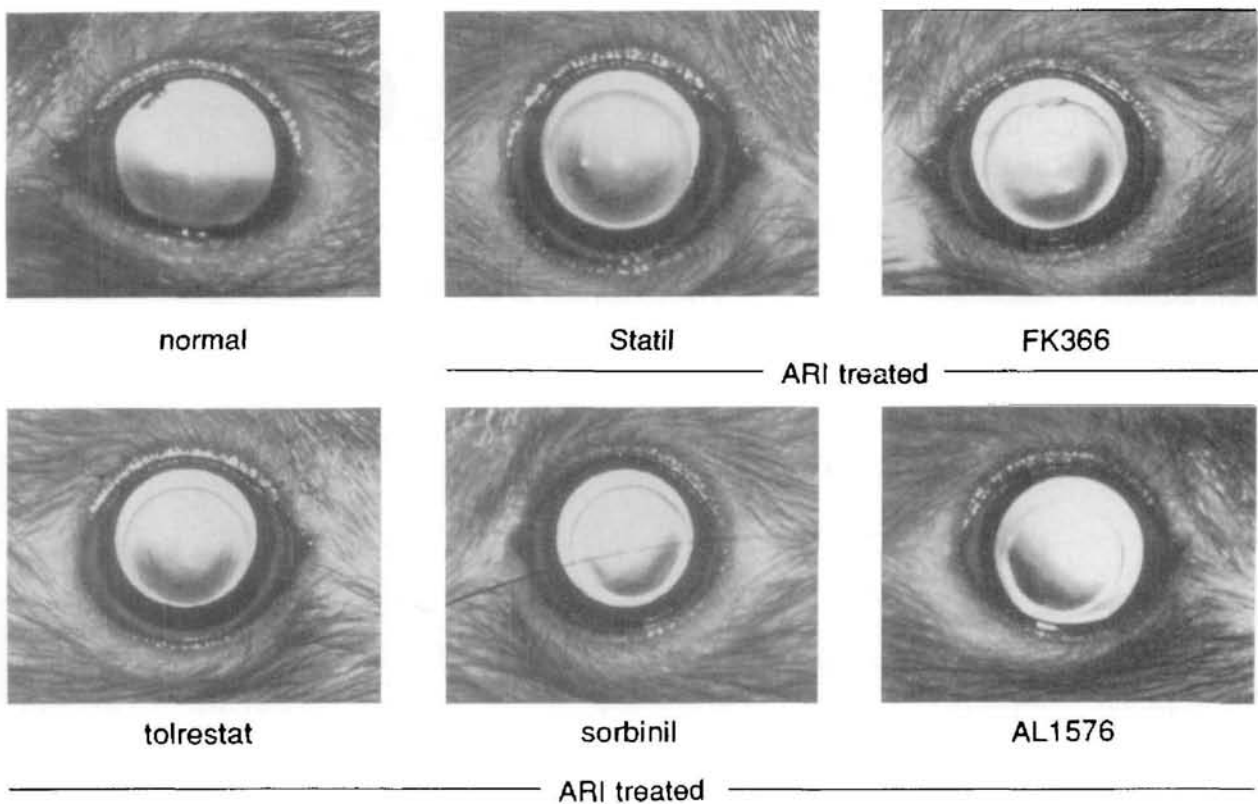


Fig. 2. Retroillumination photography showed granulation, such as rings. These deposits were reduced in naphthalene-fed rats concomitantly receiving aldose reductase inhibitors (A11576 and sorbinil)

1-yl]-acetic acid; ICI America, Wilmington, DE), FK366 ([3-[4-bromo-2-fluorobenzyl]-7-chloro-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-1-yl]-acetic acid; Fujisawa, Osaka, Japan), sorbinil ([S]-6-fluoro-spiro-

chroman-4,5'-imidazolidine-2',4'-dione; Pfizer Central Research, Groton, CT), and A11576 (2,7-difluoro-spiro-fluorene-9,5'-imidazolidine-2',4'-dione; Alcon Laboratories, Fort Worth, TX).

Naphthalene treated rat lens (3M)

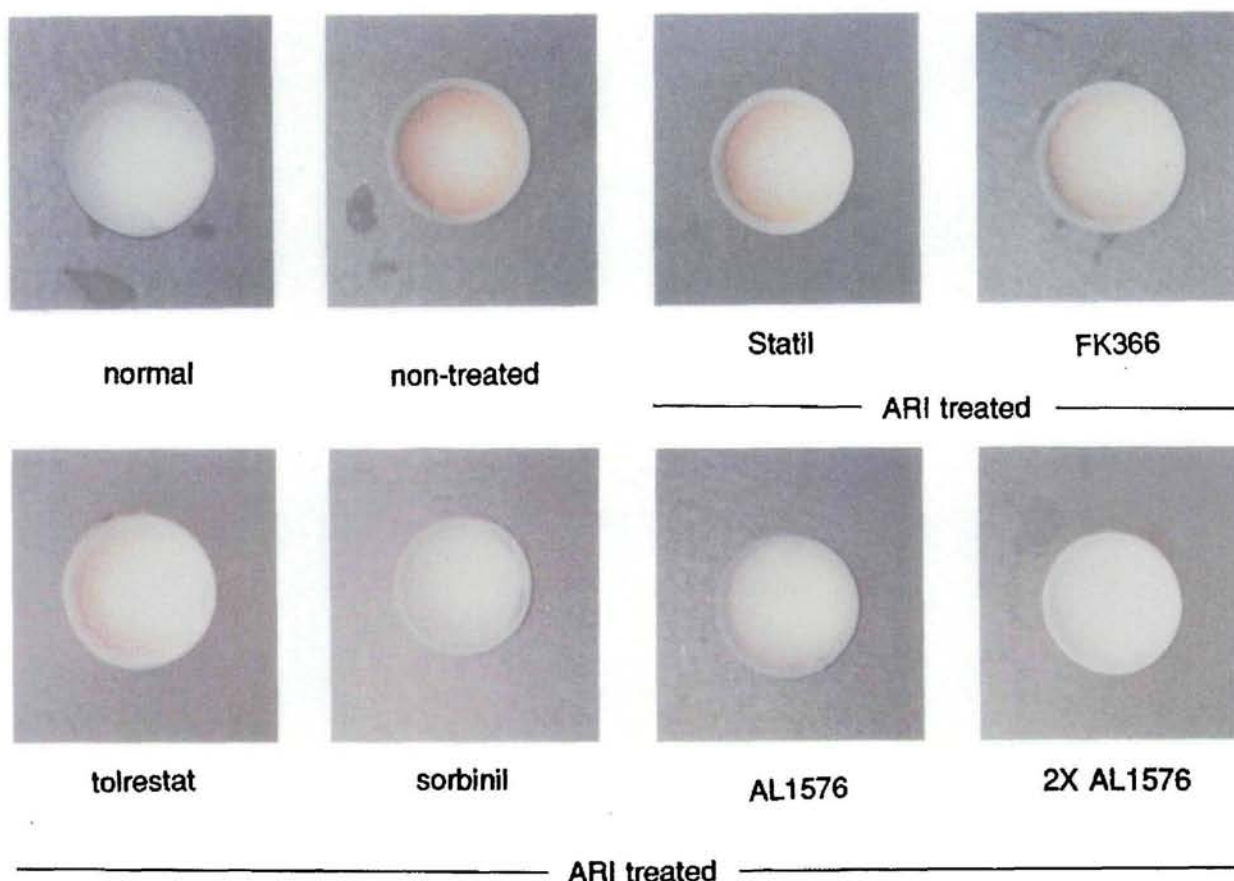


Fig. 3. Examination of the extracted whole lenses showed brown pigmentations, which were markedly reduced in the lenses of rats treated with hydantoin inhibitors (AL1576 and sorbinil).

Animals

Brown Norway rats, weighing 150–160 g, were randomly assigned to eight groups: one control group and seven groups receiving naphthalene with or without aldose reductase inhibitors. All animals were treated according to the ARVO Resolution on the Use of Animals in Research.

Naphthalene Feeding

A 10% solution (wt/vol) of naphthalene dissolved in corn oil (Mazola, Best Food, CPC Intl. Inc, Englewood Cliffs, NJ) was administered daily by gavage at a dose of 0.7 g/kg to all rats except for the controls, which received only corn oil.

Aldose Reductase Inhibitor Treatment

Six of the seven naphthalene-fed groups received normal Purina (Ralston-Purina, #5001) rat chow contain-

ing aldose reductase inhibitors at concentrations known to inhibit sugar cataract formation (0.04% sorbinil, 0.045% FK366, 0.008% and 0.016% AL1576, and 0.05% tolrestat and Ponalrestat), as described or communicated (Refs. 13–15; Y. Akagi and B. York, personal communication). The remaining animals in the naphthalene group and the control animals were given the same rat chow without inhibitors. These experimental diets were administered for approximately 100 days, and lens changes were monitored at 0, 30, 60, and 90 days by portable slit-lamp microscopy and histologic study.

Slit-Lamp Microscopy

Cataract formation in each group was examined by a portable slit-lamp microscope, and retroillumination photography was taken using a Kowa fundus camera (Kowa Co., Ltd., Tokyo, Japan) at monthly intervals. A 1% tropicamide solution was used as a mydriatic.

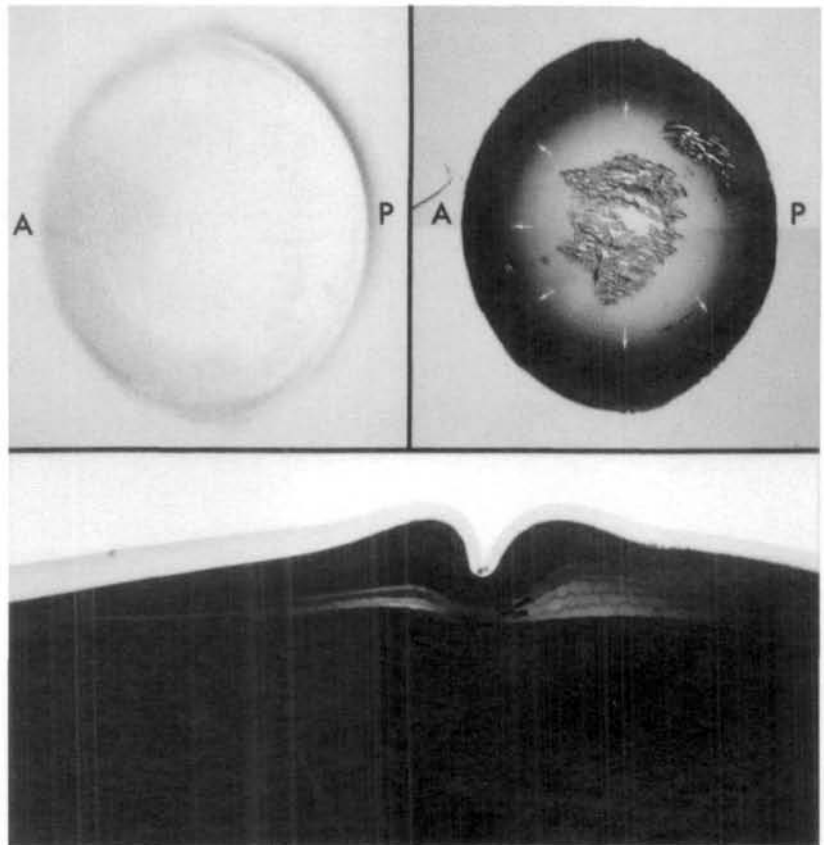


Fig. 4. Histologic examination of the naphthalene cataracts. (Top left) Sagittal dissection showed brown deposits in the deep cortex near the nucleus of the lenses. (Top right) Low-power view of the whole lens section stained with toluidine blue. (Bottom) View of the bow region under high-power magnification. A = anterior; P = posterior.

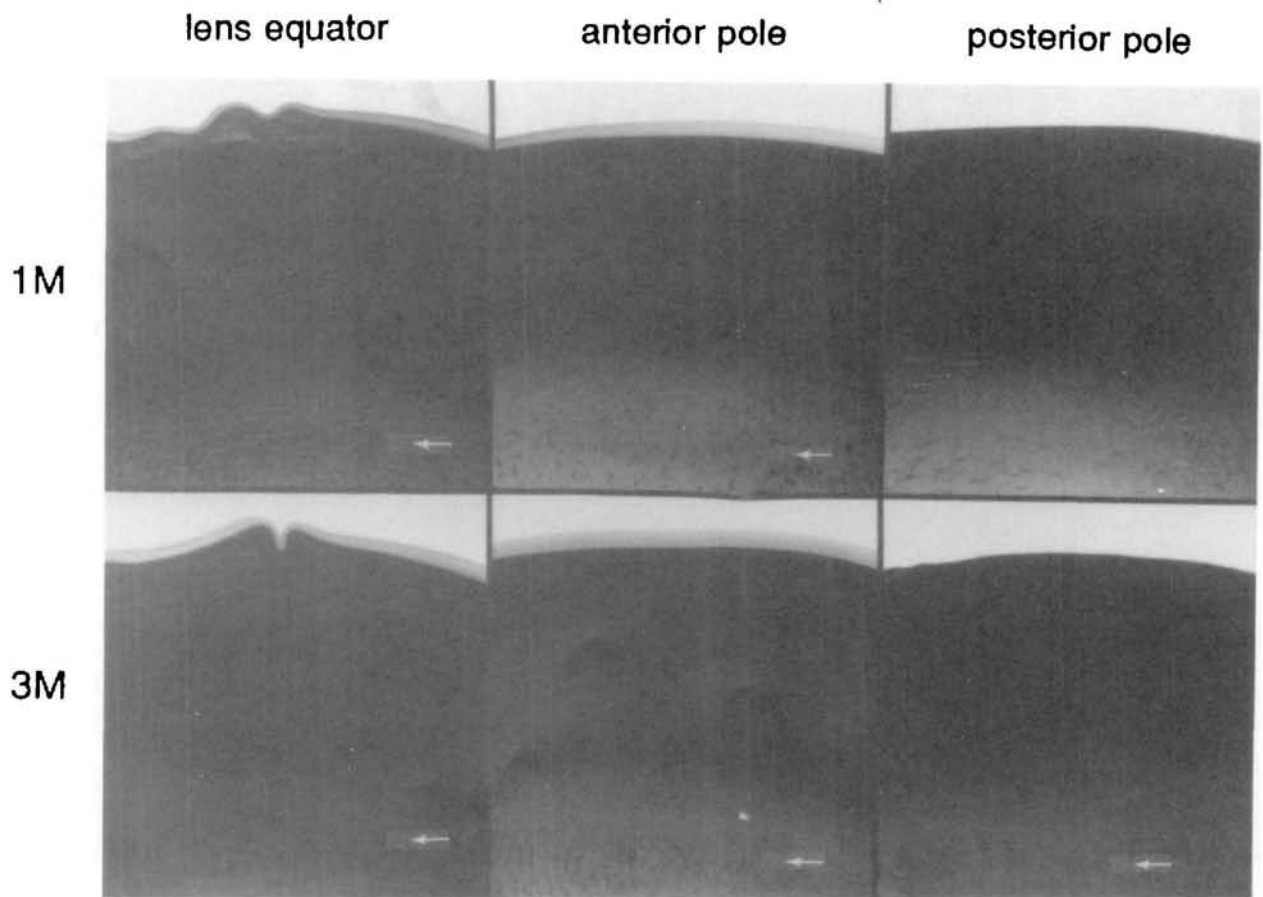


Fig. 5. Histologic view of the progression of pigmented ring formation (1 to 3 months) in rats treated with naphthalene.

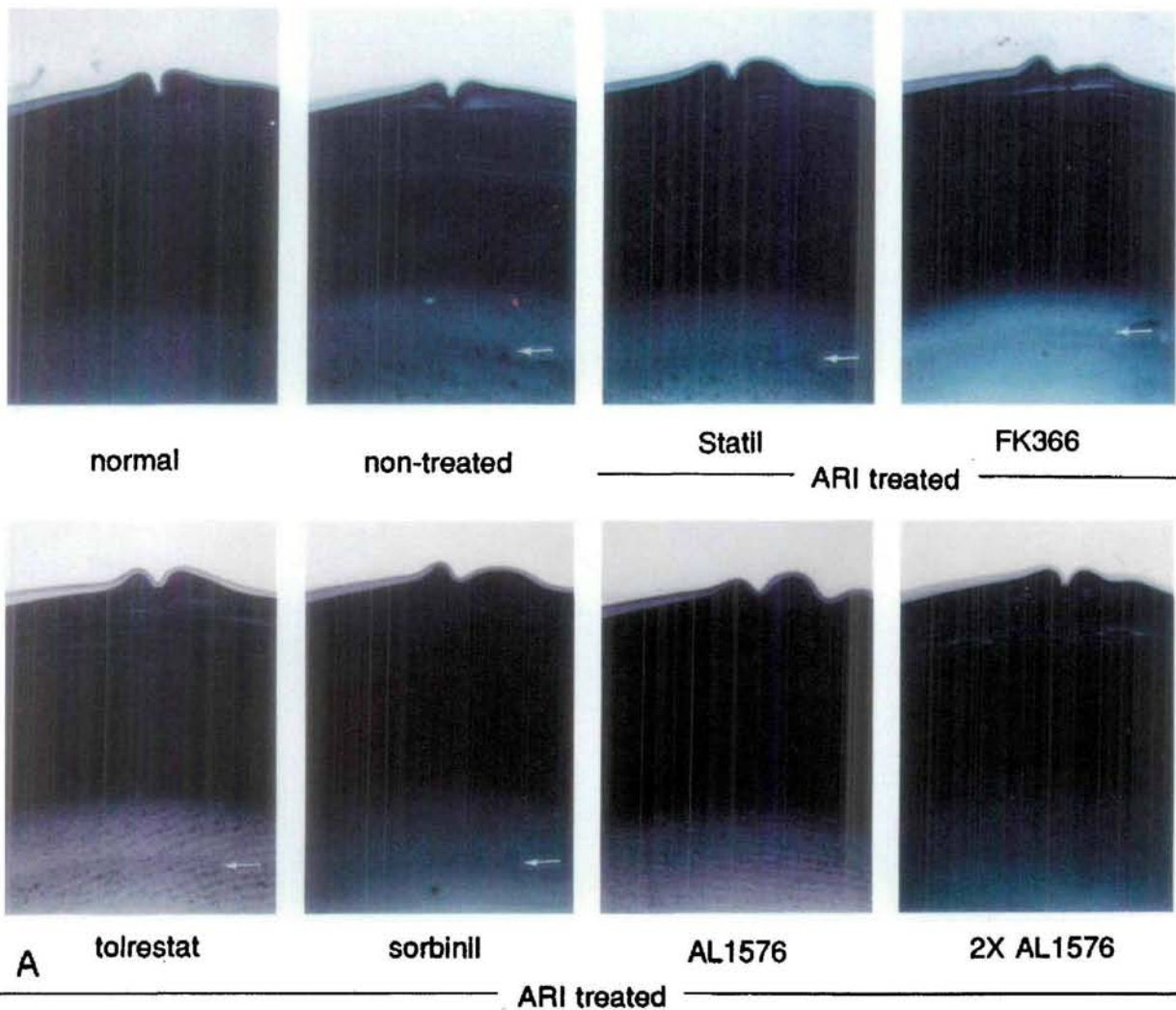


Fig. 6. Effects of aldose reductase inhibitors on naphthalene cataracts as verified by histologic techniques. (A) Lens equator. (B) Anterior pole. The development of pigmented ring was reduced in naphthalene-induced rats treated with AL1576 or sorbinil, but not with tolrestat, Ponalrestat, and FK366.

Whole-lens pictures were taken with the camera shortly after the lens was removed from the eyes.

Histologic Studies

With the animals under anesthesia by carbon dioxide inhalation, the eyes were enucleated. The lenses were removed from the eyes and fixed with 4% paraformaldehyde solution buffered at pH 7.4 in 0.1 M phosphate buffer for 48 hr. Dehydration was performed in a graded series of ethanol (50%, 75%, 85%, and $2 \times 95\%$). Tissues were embedded in methacrylate. Five-micrometer sections were cut and stained with toluidine blue.

Results

Brown Norway rats were administered corn oil, with or without naphthalene, by gavage daily. In addition, six groups of naphthalene-fed rats received rat

chow containing the aldose reductase inhibitors tolrestat, Ponalrestat, FK366, sorbinil, and AL1576. Portable slit-lamp examination indicated the progressive presence of granular deposits in the deep cortex near the nucleus in the lenses of rats receiving naphthalene. These appeared similar to zonular cataracts. By retroillumination photography, these granular deposits were seen as rings (Figs. 1,2). In naphthalene-fed rats concomitantly receiving aldose reductase inhibitors in their diets, these characteristic deposits were reduced with the aldose reductase inhibitors AL1576 and sorbinil (Fig. 2).

Four rats from each group were killed at 30-day intervals, and their eyes were enucleated for histologic studies. Examination of the isolated lenses showed that brown pigmentations were seen in cataractous lenses (Fig. 3). This pigmentation was reduced in the lenses of rats receiving the aldose reductase inhibitors AL1576 or sorbinil.

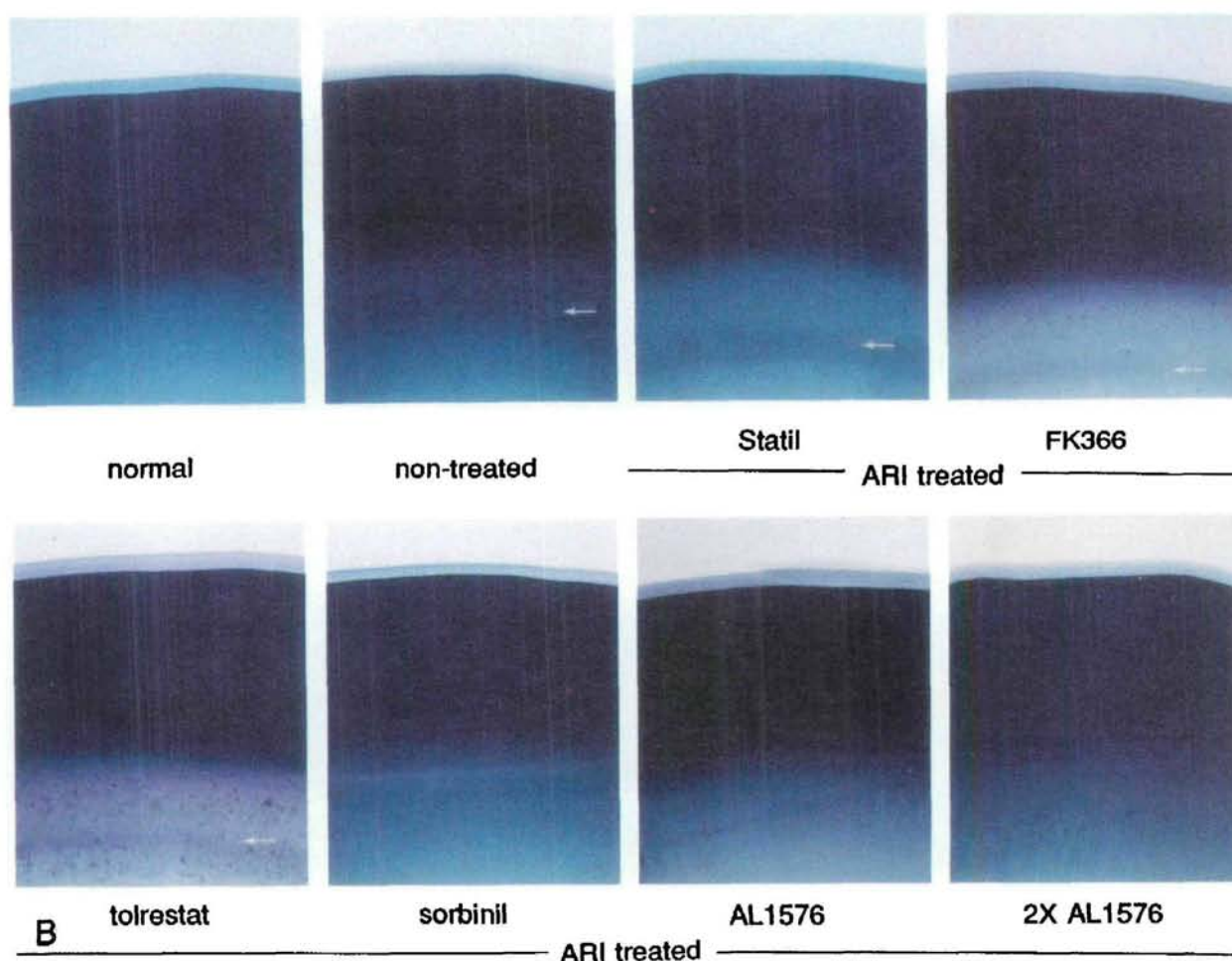


Fig. 6. (continued)

Histologic examination of these lenses showed the presence of colored deposits in the deep cortex near the nucleus of the cataractous lenses (Fig. 4). These deposits appeared as blue-stained rings (arrow) in the toluidine blue-stained whole lens sections (Fig. 4, upper right). The blue-stained ring was more prominent in the anterior pole and equator region. This pigmented ring formation progressed with the naphthalene feeding over the 3-month study period (Fig. 5). No histologic changes were seen in the bow region (Fig. 4, bottom).

The reduction in naphthalene-induced cataract formation by the aldose reductase inhibitors A11576 and sorbinil was histologically verified. As shown in Figures 6A and 6B, the development of the pigmented ring was reduced in naphthalene-fed rats treated with A11576 or sorbinil, but not with tolrestat, Ponalrestat, or FK366.

Discussion

The effect of aldose reductase inhibitors on naphthalene-induced cataract formation was studied in

rats. Brown Norway rats, rather than Black-Hooded rats^{9,10} were used because these animals were more docile and easier to handle. Naphthalene dosing differed from that used by Hockwin et al⁸ in that a lower dose (0.7 g vs 1 g/kg body weight) was administered on a daily basis rather than on alternating days. The development of naphthalene-induced cataracts in these animals was predictable and reproducible, as reported.⁸⁻¹⁰ Because aldose reductase inhibitors may be beneficial in ameliorating cataract formation, five structurally diverse aldose reductase inhibitors were used in this study to treat naphthalene-induced cataracts in rats. These compounds can be classified as either hydantoins or carboxylic acid (Fig. 7).

Our evaluation of naphthalene-induced cataract development by slit-lamp microscopy, retroillumination photography, and histologic studies indicated that brownish pigments, first seen in the deep cortex near the nucleus, form ringed opacities. The histologic studies indicated that the amount of these granular deposits is greatly reduced in naphthalene-treated rats concomitantly treated with the hydantoin-type aldose reductase inhibitors A11576 and sorbinil, but

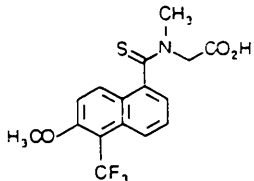
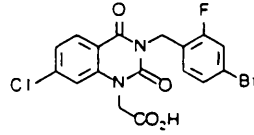
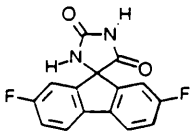
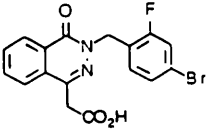
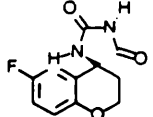
Inhibitor	Name	Type
	Alredase tolrestat. AY 22,273	Carboxylic Acid
	FK366 FR74366	Carboxylic Acid
	Imirestat HOE 843. AI 1576	Hydantoin
	Ponalrestat Statil. ICI-128,426.	Carboxylic Acid
	sorbinil CP 45,634	Hydantoin

Fig. 7. Structure of aldose reductase inhibitors.

not by the carboxylic acid-type aldose reductase inhibitors tolrestat, Ponalrestat, or FK366. These studies, which used two doses of AI1576, confirmed the reports of Hockwin et al⁸ and showed that sorbinil also inhibited naphthalene-induced cataract formation.

Van Heyningen and Pirie¹¹ inferred that metabolites of naphthalene induce this cataract formation. Xu et al¹² corroborated the earlier observations by showing that 1,2-naphthoquinone can induce cataract formation in cultured lenses; however, coadministration of AI1576 did not prevent cataract formation. The mechanism by which AI1576 prevents naphthalene cataract formation in the rat *in vivo*, but not *in vitro*, is not understood.

The role of aldose reductase in the development of cataracts in diabetic patients has been well established and reviewed.¹³ Moreover, a variety of structurally different compounds has been shown to slow down or prevent the onset of cataract formation (both *in vivo* and *in vitro*) through their ability to inhibit lens polyol formation.¹³ This inhibition of aldose reductase does not result from a free radical scavenger mechanism.¹⁴

The observation that naphthalene-induced cataracts can be ameliorated only by hydantoin-type aldose reductase inhibitors suggests that the preventive mechanism is not linked to the inhibition of the en-

zyme aldose reductase. Further studies are required to elucidate the biochemical mechanism(s) involved.

Key words: naphthalene-induced cataract, aldose reductase inhibitor, slit-lamp microscopy, histologic study, rats

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