Oxalate retinopathy: an experimental model of a flecked retina

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The clinical picture resembling fundus albipunctatus was seen to develop in rabbits following subcutaneous injection with dibutyl oxalate. On histologic examination, the flecks were found to be due to intracellular accumulation of calcium oxalate in the RPE cells. The clinical and histologic features of this animal model closely resemble the recently described entity of oxalate retinopathy in humans which was seen in the presence of high circulating oxalate levels. It is suggested that the presence of metabolic disorders or toxicity which are known to cause oxalate depositions should be sought in patients with fundus albipunctatus.

The term flecked retina syndrome was introduced into ophthalmology in 1965, to describe a group of diseases characterized ophthalmoscopically by the presence of numerous white or yellowish-white retinal flecks distributed throughout the fundus.1 The following entities were included in the flecked retina syndrome: fundus albipunctatus, fundus flavimaculatus, and drusen. Except for drusen, the pathogenesis of these entities remains obscure.

Recently, the histopathologic findings in a patient with apparent fundus albipunctatus were described.2 Crystalline deposits of calcium oxalate were observed in the retinal pigment epithelium (RPE) and appeared to account for the flecks that were seen ophthalmoscopically. It was found from the past history that this patient had undergone prolonged general anesthesia with methoxyflurane, an anesthetic agent which undergoes degradation to oxalate in the liver.3 To test the hypothesis that excess of oxalate may be a cause of flecked retina we have attempted to produce experimentally similar lesions in animals.

Materials and methods

Animals. Male Dutch rabbits weighing approximately 2 kilograms were maintained in individual metal cages and fed Purina rabbit pellets and tap water ad libitum.

Chemicals. The following chemicals were used: dibutyl oxalate (Eastman Organic Chemicals, Rochester, N. Y.), calcium oxalate monohydrate powder, 99.9 per cent pure, calcium chloride hexahydrate, sodium oxalate, 99.9 per cent pure (Fisher Scientific Company, Pittsburgh, Pa.).

Procedures. Dibutyl oxalate-treated rabbits. Three groups of five rabbits received daily subcutaneous injections of 1.0, 2.0, and 4.0 ml dibutyl oxalate, respectively, with an equal volume of 0.5 M calcium chloride solution administered subcutaneously at a distant site. A control
Fig. 1. Rabbit fundus, following four days treatment with daily 2.0 ml intramuscular dibutyl oxalate, in which white flecks are apparent.

A group of three rabbits received 2.0 ml normal saline and an equal amount of 0.5 M calcium chloride solution subcutaneously. Ophthalmoscopic observation with an indirect ophthalmoscope through dilated pupils was carried out on all animals prior to the first injection and on a daily basis thereafter. Animals were killed at 20 days or when signs of neurological impairment such as ataxia and stupor appeared. Postmortem examination was performed on all the animals. The eyes, kidneys, testes, heart, liver, and spleen were fixed in 10 per cent buffered formalin and processed for light microscopic examination. Six micron-thick paraffin sections were cut. Two slides were stained with hematoxylin and cosin, two slides with the Pizzalato stain for calcium oxalate, and two slides were left unstained and the "bubble test" for calcium oxalate was performed. In addition, the periodic acid-Schiff, Masson trichrome, and alcian-blue stains were performed.

Procedures for additional experiments. Additional procedures were carried out as follows: (1) Calcium oxalate (6.2 mg) suspended in 30 ml autologous blood was infused slowly into the left, common carotid artery by means of a Harvard pump in five rabbits. (2) Unilateral retrobulbar injections of 1.0 ml dibutyl oxalate were given to three rabbits. (3) Unilateral retrobulbar injections of 1.0 ml methoxyurethane were given to three rabbits. (4) Unilateral intravitreal injections of 0.5 ml 0.25M sodium oxalate solutions were given to three rabbits. (5) Unilateral intravitreal injections of 0.1 Cm calcium oxalate powder through the pars plana were given to three rabbits. (6) Unilateral instillation of 0.1 Cm calcium oxalate powder into the anterior chamber was done in three rabbits.

Animals were maintained for a maximum of 50 days after administration of the respective drugs. The nontreated eyes served as controls. The clinical and histological studies were carried out in a similar manner to those described above for the rabbits receiving systemic injections of dibutyl oxalate.

Results

Dibutyl oxalate-treated rabbits.

Clinical findings. The five animals which received 1.0 ml of dibutyl oxalate daily showed few toxic signs and were killed at 20 days. Two of the five animals receiving 2.0 ml of dibutyl oxalate survived four and five days, respectively; the remaining three animals in this dose range were killed at 20 days. The five animals receiving 4.0 ml died within three days, all terminally exhibiting signs of ataxia and stupor. Thus a total of ten treated rabbits survived three days or more. Seven of these rabbits exhibited a "flecked retina" appearance of the fundus prior to death, and the clinical and pathologic findings in these animals were more extensive in those that survived up to 20 days. The earliest fundoscopic changes were small, pin-point, depigmented areas of the retina apparently related to an RPE disturbance. These appeared on the third day following treatment. On the fourth day, discrete white flecks were observed throughout the posterior pole and in less concentration up to the equator in all seven animals (Fig. 1).

Eye pathology. On histopathological examination, the seven rabbits with flecked retinas demonstrated intracellular needle-like crystals within numerous cells of the RPE (Fig. 2). These crystals varied in number from one per cell to as many as ten or more. In some cells these coalesced to form a single large deposit (Fig. 2). The identity of the crystals as calcium oxalate was confirmed by their positive staining with the Pizzalato stain and their positive reaction to the "bubble test."

Examination of the RPE revealed cells which contained amorphous hyaline-like material (Fig. 3). Although present in the eyes of all experimental animals, these were
Fig. 2. Retinal pigment epithelium, left eye, same animal as Fig. 1. Calcium oxalate crystals are evident within the cells of RPE. The neurosensory retina is artificiatically detached. (Polarized light, alcian blue, ×875.)

Fig. 4. Lens, right eye, same animal. Calcium oxalate crystal is seen in the posterior subcapsular area. (Polarized light, hematoxylin and eosin, ×240.)

Fig. 3. Retinal pigment epithelium, right eye, same animal. Amorphous hyaline-like substances is noted within the RPE as an isolated finding (small arrow) and surrounded by crystals (large arrow). (Masson, ×875.)

Fig. 5. Posterior segment, fifty days following intravitreal sodium oxalate injection. A line of pigment-laden cells loaded with calcium oxalate crystals is seen in the region between atrophic neurosurgery retina and chorioid. No normal RPE is visible in this area. (Polarized light, hematoxylin and eosin, ×60.)

more frequent in the eyes of those that died four to five days after treatment. These deposits were surrounded by needle-like oxalate crystals. They appeared gray and did not stain by hematoxylin and eosin, periodic acid-Schiff, Masson trichrome, or alcian-blue stains.

The lenses of three animals with RPE oxalate deposition contained calcium oxalate crystals embedded in the posterior subcapsular area (Fig. 4). In addition, the lenses of all treated animals showed hydropic degeneration of the epithelium and posterior cortical vacuolization. The cornea, lens, ciliary body, choroid, neural retina, optic nerve, and sclera did not show crystalline deposits.

Autopsy. Extracellular calcium oxalate crystals were present in the kidney tubules of all oxalate-treated animals. In addition, seven animals that survived up to day 20 after injection exhibited myocardial oxalosis. In five animals of this group, calcium oxalate was seen in the seminiferous tubules of the testes. No oxalate crystal deposition was seen in any organ of the control animals.

Results from other experiments. RPE oxalosis of the type seen with systemically administered dibutyl oxalate could not be created by any other route or method of administration. Intracarotid injections of
oxalate produced immediate ischemic infarctions visible on ophthalmoscopy and emboli of calcium oxalate were found on histopathological examination in the uveal and retinal vasculature. Intravitreal injection of calcium oxalate caused necrosis and disorganization of the neural retina and RPE. In addition, a line of birefringent crystals which were within or adjacent to pigment-laden cells of apparent RPE origin was observed along Bruch's membrane (Fig. 5).

Discussion

Oxalic acid is an end-product of the metabolism of glyoxylic and ascorbic acids. Small quantities of calcium oxalate are normally present in the serum of healthy subjects. Elevated blood and urine levels of calcium oxalate and deposition of calcium oxalate crystals in various organs occur in several clinical conditions. The most significant of these entities are primary oxalosis types I and II, pyridoxine deficiency, thiamine deficiency, ethylene glycol poisoning, oxalic acid poisoning, excessive ingestion of foods with high oxalate content, in patients with ileal disease, or after ileal resection, subsequent to intravenous hyperalimentation with xylitol, or following methoxyflurane anesthesia. In the present experiment, hyperoxalemia presumably occurred due to breakdown of the dibutyl ester in the liver with the release of the oxalate ion.

Crystalline deposits of calcium oxalate have been noted in various organs in association with hyperoxalemia. These crystals have usually been described in the extracellular spaces, except for one report of intracellular calcium oxalate deposition within the renal tubular cells in association with primary oxalosis. The apparent presence of multiple calcium oxalate crystals within the cells of the RPE, as determined by light microscopy in the present study, and electron microscopy of a human case, seems to be characteristic of the RPE changes in oxalate retinopathy, and correlates well with the white flecks seen by ophthalmoscopy. Further studies are in progress to adapt methods for the determination of blood oxalate levels to these experiments, to determine the means by which calcium oxalate is transported in the blood, and to investigate the mechanism that leads to preferential localization within the RPE.

While ophthalmoscopic similarities exist among the varieties of flecked retina syndrome, varying opinions on causes and functional significance have been expressed concerning these diseases. Of the types of flecked retina syndrome originally described, the stationary form of fundus albipunctatus shows ophthalmoscopic appearance somewhat similar to oxalate retinopathy. No histopathologic specimens of fundus albipunctatus are available for comparison of the histopathology with that of oxalate retinopathy. The present experiments indicate that systemic oxalosis may give rise to ophthalmoscopic changes of flecked retina and the possibility of oxalosis should be considered when investigating affected patients.

REFERENCES