Ultrastructural Ocular Lesions of 6-Aminonicotinamide Toxicosis in Rabbits

J. A. Render, J. J. Turek, E. J. Hinsman, and W. W. Carlton

Department of Veterinary Microbiology, Pathology, and Public Health, School of Veterinary Medicine, Purdue University, West Lafayette, IN

Abstract. 6-Aminonicotinamide, given by intraperitoneal injection to male and female Dutch belted rabbits, produced swelling and vacuolation of ciliary and iridal epithelium plus vacuolation of the retinal pigment epithelial and outer plexiform layers of the retina. By transmission electron microscopy, inner and outer ciliary epithelial cells and inner iridal epithelial cells contained numerous coalescing, membrane-bound vacuoles of the cytocavitary network. These vacuoles were viewed as numerous interconnecting, intracytoplasmic cavities in scanning electron micrographs. Swelling of vacuolated epithelial cells and the presence of fibrin and proteinaceous fluid in the ciliary stroma resulted in thickening of the anterior ciliary processes with the formation of surface alterations detectable by scanning electron microscopy. In transmission electron micrographs the vacuoles in the retinal pigment epithelium were large, electron-lucent spaces and the vacuoles in the outer plexiform layer of the retina appeared to be intracytoplasmic spaces in axons of photoreceptor cells. Distention of cytocavitary structures has been reported in glial cells of animals given 6-aminonicotinamide and this change was apparently due to alterations in ion and water movement across cellular membranes that resulted in intracellular edema.

6-Aminonicotinamide, a potent antagonist of nicotinamide,11 has been used experimentally as a teratogen7 and a neurotoxin in rats.26 It has caused developmental anomalies in rabbit kits and was toxic to adult rabbits.10,22 Studies in rabbits were not numerous; ocular lesions have been reported however.22 By light microscopy, the principal alteration was vacuolation of the inner iridal, inner and outer ciliary and retinal pigment epithelial cells and of the outer plexiform layer of the retina.22

This study was undertaken to characterize vacuolar changes of 6-aminonicotinamide ocular toxicosis by scanning and transmission electron microscopy, including scanning electron microscopy of ultraplaned sections, a technique previously used to characterize glomerular lesions.12

Materials and Methods

Male and female Dutch belted rabbits which weighed between 1.35 and 2.0 kg were housed individually in metal cages with screened floors in a room with natural lighting and maintained at 22°C. The rabbits were given free access to commercial pelleted rabbit feed and fresh tap water containing sulfamethazine (12½% sodium sulfamethazine) at a dose of 1 ml/100 ml drinking water for treatment of coccidiosis. Each rabbit was acclimated for a minimum of three days prior to assignment to experimental groups.

Twenty-seven rabbits were assigned to treatment groups of 3, 4, 5, 6, 7, or 8 daily intraperitoneal injections of 6-aminonicotinamide at a dose of 3 mg/kg body weight. Surviving rabbits were killed 24 hours after the last injection. Three rabbits were untreated controls.

The 6-aminonicotinamide (Aldrich Chemical Company, Inc., Milwaukee, WI), 99+% pure, was dissolved in 100 ml of sterile water by heating a bottle that contained the compound in a water bath at a temperature of 60 to 70°C for approximately one to two hours. The solution was given intraperitoneally.

Prior to perfusion, rabbits were anesthetized with a mixture of ketamine and acepromazine. They then received sodium heparin intravenously at a dose of 500 USP units/rabbit. The rabbits were restrained in a V-shaped trough in dorsal recumbency with their heads pointing downward at an angle of approximately 45°. The thorax was opened, the left ventricle was cannulated, and the body was perfused with a chilled mixture of 1% glutaraldehyde and 4% formaldehyde. As gravity perfusion began, the right ventricle was incised to allow blood and fixative to drain from the heart. After perfusion for about five minutes, the globes were removed and stored in the same fixative at refrigerator temperature. One globe was processed for light microscopy and scanning electron microscopy of ultraplaned sections. The other globe was processed for transmission electron microscopy of the iris, ciliary body, and retina and scanning electron microscopy of the posterior surface of the iris and ciliary body.

Globes were dehydrated in a series of graded alcohols and then trimmed by making a sagittal cut adjacent and parallel to the optic nerve, perpendicular to the long posterior ciliary
arteries and through the pupil. Calottes of the globe were processed, embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin (HE).

For ultraplaned sections, paraffin blocks were trimmed to a block face of approximately ½ cm² with the iris and ciliary body in the center. Sections were cut at 1 to 2 μm with an ultramicrotome and mounted on glass coverslips. The ultraplaned sections were then rinsed twice for five minutes each in xylene and 100% ethanol and critical point dried with liquid carbon dioxide. Ultraplaned sections were mounted on aluminum stubs with colloidal graphite in isopropanol, sputter coated with approximately 20 nm gold, and examined with a scanning electron microscope.

For scanning electron microscopy of the posterior surface of the iris and ciliary body, rhomboidal sections, approximately 2 mm², were trimmed from perfused globes with a razor blade. Sections were washed in Millonig’s phosphate buffer, processed through a graded series of ethanol, critical point dried, and sputter coated with gold.

For transmission electron microscopy, approximately 1 mm³ samples of iris, ciliary body, and retina were washed in Millonig’s phosphate buffer (pH 7.3) and post fixed in 1.0% osmium tetroxide in Millonig’s phosphate buffer (pH 7.3). The tissues were dehydrated in a graded ethanol series, cleared with propylene oxide and embedded in Poly/Bed 812® (Polysciences, Inc., Warren Park, PA).

One μm sections were cut on an ultramicrotome, stained with methylene blue-azure II, and examined to select areas for thin sectioning. Thin sections were stained with uranyl acetate and lead citrate and examined using a JEM-100CX transmission electron microscope (JOEL Ltd., Tokyo, Japan).

The iris and ciliary body were divided into three areas for examination. Anatomical and histological terms used in the description of lesions were in accordance with Kozart. The three areas of the anterior uvea examined were: posterior ciliary processes; anterior ciliary processes, including the ciliary crest, consisted of marked cytoplasmic vacuolation and swelling of ciliary epithelial cells causing thickening of ciliary processes, especially in the anterior processes. These changes were evident in ultraplaned sections of the anterior uvea (fig. 2) and in scanning electron micrographs of the posterior surface of the anterior uvea. Cytoplasmic vacuolation and swelling of epithelial cells in posterior ciliary processes were mild. In scanning electron micrographs of the posterior ciliary processes, circumferential fissures were obliterated and circumferential ridges were flattened, but the thickness of the ciliary processes was slightly greater than that from control rabbits. Surface alterations were more severe in the anterior ciliary processes.

Light microscopic alterations of the epithelium of anterior ciliary processes, especially those of the ciliary crest, consisted of marked cytoplasmic vacuolation and swelling of ciliary epithelial cells causing thickening of ciliary processes, especially in the anterior processes. These changes were evident in ultraplaned sections of the anterior uvea (fig. 2) and in scanning electron micrographs of the posterior surface of the anterior uvea. Cytoplasmic vacuolation and swelling of epithelial cells in posterior ciliary processes were mild. In scanning electron micrographs of the posterior ciliary processes, circumferential fissures were obliterated and circumferential ridges were flattened, but the thickness of the ciliary processes was slightly greater than that from control rabbits. Surface alterations were more severe in the anterior ciliary processes.

Light microscopic alterations of the epithelium of anterior ciliary processes, especially those of the ciliary crest, consisted of marked cytoplasmic vacuolation and swelling of ciliary epithelial cells causing thickening of ciliary processes, especially in the anterior processes. These changes were evident in ultraplaned sections of the anterior uvea (fig. 2) and in scanning electron micrographs of the posterior surface of the anterior uvea. Cytoplasmic vacuolation and swelling of epithelial cells in posterior ciliary processes were mild. In scanning electron micrographs of the posterior ciliary processes, circumferential fissures were obliterated and circumferential ridges were flattened, but the thickness of the ciliary processes was slightly greater than that from control rabbits. Surface alterations were more severe in the anterior ciliary processes.

Light microscopic alterations of the epithelium of anterior ciliary processes, especially those of the ciliary crest, consisted of marked cytoplasmic vacuolation and swelling of ciliary epithelial cells causing thickening of ciliary processes, especially in the anterior processes. These changes were evident in ultraplaned sections of the anterior uvea (fig. 2) and in scanning electron micrographs of the posterior surface of the anterior uvea. Cytoplasmic vacuolation and swelling of epithelial cells in posterior ciliary processes were mild. In scanning electron micrographs of the posterior ciliary processes, circumferential fissures were obliterated and circumferential ridges were flattened, but the thickness of the ciliary processes was slightly greater than that from control rabbits. Surface alterations were more severe in the anterior ciliary processes.

Results

Most of the rabbits given 6-aminonicotinamide had matted soft feces on the perianal fur after four daily injections and some rabbits had diarrhea. Most were ataxic in the hindlimbs after five daily injections; ataxia quickly developed into paresis and/or paralysis and some rabbits remained in sternal or lateral recumbency until death.

Light microscopic findings consisted of swelling and vacuolation of ciliary epithelium (fig. 1), iridal epithelium, the retinal pigment epithelium and the outer plexiform layer of the retina. These light microscopic iridociliary alterations correlated well with the scanning electron microscopic findings. Cytoplasmic vacuolation and cellular swelling of ciliary epithelial cells caused thickening of ciliary processes, especially in the anterior processes. These changes were evident in ultraplaned sections of the anterior uvea (fig. 2) and in scanning electron micrographs of the posterior surface of the anterior uvea. Cytoplasmic vacuolation and swelling of epithelial cells in posterior ciliary processes were mild. In scanning electron micrographs of the posterior ciliary processes, circumferential fissures were obliterated and circumferential ridges were flattened, but the thickness of the ciliary processes was slightly greater than that from control rabbits. Surface alterations were more severe in the anterior ciliary processes.

Light microscopic alterations of the epithelium of anterior ciliary processes, especially those of the ciliary crest, consisted of marked cytoplasmic vacuolation and swelling of ciliary epithelial cells causing thickening of ciliary processes, especially in the anterior processes. These changes were evident in ultraplaned sections of the anterior uvea (fig. 2) and in scanning electron micrographs of the posterior surface of the anterior uvea. Cytoplasmic vacuolation and swelling of epithelial cells in posterior ciliary processes were mild. In scanning electron micrographs of the posterior ciliary processes, circumferential fissures were obliterated and circumferential ridges were flattened, but the thickness of the ciliary processes was slightly greater than that from control rabbits. Surface alterations were more severe in the anterior ciliary processes.

Light microscopic alterations of the epithelium of anterior ciliary processes, especially those of the ciliary crest, consisted of marked cytoplasmic vacuolation and swelling of ciliary epithelial cells causing thickening of ciliary processes, especially in the anterior processes. These changes were evident in ultraplaned sections of the anterior uvea (fig. 2) and in scanning electron micrographs of the posterior surface of the anterior uvea. Cytoplasmic vacuolation and swelling of epithelial cells in posterior ciliary processes were mild. In scanning electron micrographs of the posterior ciliary processes, circumferential fissures were obliterated and circumferential ridges were flattened, but the thickness of the ciliary processes was slightly greater than that from control rabbits. Surface alterations were more severe in the anterior ciliary processes.
6-Aminonicotinamide-induced Ultrastructural Ocular Lesions

Fig. 1: Iris (I) and anterior ciliary processes (CP) with vacuolated epithelium (arrow) from rabbit given 6-aminonicotinamide. HE.

Fig. 2: Thickening of anterior ciliary processes (CP) adjacent to contracted iris (I) from rabbit given 6-aminonicotinamide. Ultraplaned section.

Fig. 3: Posterior surface of anterior ciliary processes. a. Slender, cylindrical ciliary processes from control rabbit. b. Thickened and flattened anterior ciliary processes of rabbit given 6-aminonicotinamide.

ciliary processes. Cytoplasmic vacuolation observed by light microscopy was due to membrane-bound, electron-lucent, intracytoplasmic vacuolation of the inner iridal epithelium as seen in transmission electron micrographs, and these vacuoles were viewed as cavities in scanning electron micrographs of ultraplaned sections. By scanning electron microscopy of the posterior surface of the pupillary region of the iris of control rabbits, radial ridges and furrows were present and were parallel, straight and long. In treated rabbits, ridges varied in thickness and were irregular. The radial furrows were shallow.

By light microscopy, alterations in the retina included marked vacuolation of the retinal pigment epithelium, outer plexiform layer, and sometimes, of the inner and outer nuclear layers. Ultrastructurally, vacuoles in the
retinal pigment epithelium were membrane-bound, electron-lucent except for a minimal amount of membranous debris, varied in size, and they often formed large spaces (fig. 7). Large vacuoles sometimes communicated with the intercellular space external to terminal bars, and basilar infoldings were prominent in comparison to the retinal pigment epithelium of control rabbits. The vacuoles were readily differentiated from the non-membrane-bound, faintly electron-dense lipid droplets observed in the retinal pigment epithelium of control and treated rabbits.

In the outer plexiform layer, large electron-lucent membrane-bound vacuoles contained membranous debris arranged in loose whorls. Larger vacuoles compressed adjacent cells and some were too large to define their exact location. Smaller vacuoles appeared to be present in the axonal processes of photoreceptor cells (fig. 8), especially in the terminal synaptic enlargements.

Discussion

In this study, ocular and systemic clinical signs were similar to those previously reported, and the emphasis was placed on characterization of the ocular lesions of 6-aminonicotinamide toxicosis by scanning and transmission electron microscopy. Ocular findings attributed to administration of 6-aminonicotinamide were observed only in treated rabbits in comparison to control rabbits, therefore alterations were not due to inadequate perfusion. The principal alteration was coalescing intracytoplasmic vacuoles of various sizes in epithelial cells of both layers of the anterior ciliary processes and the inner layer of the iris. The distribution and size of vacuoles plus the vacuolation of photoreceptor cells and the spaces within the retinal pigment epithelium layer are unique features of 6-aminonicotinamide toxicosis.

Cytoplasmic vacuolation of the ciliary epithelium is not a change limited to 6-aminonicotinamide toxicosis. Intracytoplasmic vacuoles, often with dilated intercellular spaces, have been produced in the outer ciliary epithelium of rabbits by administration of Shigella sp endotoxin, by the intracameral injection of mineral oil, after freezing of the ciliary processes, and after colchicine administration. Both layers of the ciliary epithelium of rabbits were vacuolated after dosing with naphthalene. With naphthalene and 6-aminonicotinamide toxicosis in rabbits, vacuoles represent distentions of the cytocavitary network.

Vacuolation has been described previously as a change of 6-aminonicotinamide toxicosis. It has been described as a teratogenic change in cells of the lens and retina in fetal rats. In these tissues, cellular vacuolation was due to dilatation of perinuclear cisterns. Vacuolated astrocytes were observed in pigs and rats treated with 6-aminonicotinamide and vacuolated oligodendrocytes and neurons were described in 6-aminonicotinamide-treated rats. In these tissues, vacuoles were caused by distention of cisternae of endoplasmic reticulum and the perinuclear cisternae.

Dilatation of the endoplasmic reticulum is a reversi-
ble change associated with the movement of both water and sodium ions across the membrane into the cisternal lumen. The endoplasmic reticulum seems to act as a reservoir in which water accumulates in the injured cell. It has been proposed that vacuolation results from a disturbance of the ion pump in the cellular membrane of astrocytes and oligodendrocytes. This metabolic disturbance increases cellular uptake of water and sodium ions and the development of intracellular edema. Such disturbances of transport of fluid through biological membranes may be responsible for the ocular alterations in the anterior uveal cells.

Cystic changes within the outer plexiform layer of the retina due to a systemic toxicosis has not been recorded in experimental animals. In people, cystic spaces were found in the outer plexiform layer and inner nuclear layer of the retina following lens extraction. These spaces varied in size, contained proteina-
ceous fluid and their walls were comprised of either photoreceptor cell axons or Mueller cell processes. In the 6-aminonicotinamide-treated rabbits, the vacuoles appeared to be intracytoplasmic, although large spaces may have been between cells. The cause for the apparent vacuolation of photoreceptor cell axons was not established. Considering other ocular lesions, altered fluid transport would be a likely mechanism.

Distended spaces in the retinal pigment epithelium were produced by administration of 6-aminonicotinamide. This change differed from previously described distention of perinuclear cisternae in retinal pigment epithelial cells of fetuses from rats given 6-aminonicotinamide. The vacuoles appeared to be distentions of intercellular spaces instead of a coalescence of smaller intracytoplasmic vacuoles since the latter structures were not observed in retinal pigment epithelial cells. Intercellular clefts have been observed in rabbits anesthetized with a mixture of halothane and nitrous oxide. However, in this study, this alteration in the retinal pigment epithelium was observed only in treated rabbits and the severity appeared to be related to dose. The cause is unknown but may be due to an increased permeability to fluid by the choriocapillaris.

An increased vascular permeability to fluid may have been the reason for the greater susceptibility of the anterior ciliary processes to toxic changes. In addition to 6-aminonicotinamide administration, other conditions including systemic administration of bacterial endotoxin, thrombocytopenia, urethan or pentobarbital anesthesia, and topically applied prostaglandins produce severe alterations in the anterior ciliary processes. The reason for the greater susceptibility of this region to toxic change has not been elucidated. However, increased susceptibility of anterior ciliary processes to toxic compounds may be related to alterations in fluid transport and to morphological factors since fewer cellular junctions exist in anterior ciliary processes than in posterior ciliary processes.

Ciliary stromal edema and exudation of fibrin due to increased vascular permeability occurred in the anterior ciliary processes in addition to the intracytoplasmic vacuolation and swelling of epithelial cells. These changes contributed to the thickening of the ciliary processes of treated rabbits which was detectable by surface scanning electron microscopy. Such thickening has been produced by edema of the ciliary processes of rabbits suffering an acute immunogenic anterior uveitis. However, in scanning electron micrographs of ultraplaned sections and transmission electron micrographs, swelling of the vacuolated epithelial cells was the principal factor responsible for thickening of the ciliary processes.

Swelling of iridal epithelial cells was also found in
the treated rabbits and resulted in thickening and distortion of iridal radial ridges. Some of the ridge distortion was due to iridal contraction at the time of fixation since contraction of the iris has been associated with anesthesia at the time of perfusion.

In this study, scanning electron microscopy of ultra-planed sections has proved to be a useful adjunct technique in the study of ocular lesions in addition to its use in the study of glomerular lesions. The scanning electron micrographs provide a link between light microscopic and surface scanning electron microscopic findings and also give a 3-dimensional view to alterations viewed by transmission electron microscopy. By the use of these various techniques, the ultrastructural ocular lesions of 6-aminonicotinamide were more completely characterized.

Acknowledgements

This work, published as paper no. 10060 of the Agricultural Experiment Station, Purdue University, West Lafayette, IN, was funded in part by a grant from the Eli Lilly Company, Indianapolis, IN and in part by a grant from the Stauffer Chemical Company, Farmington, CT.

References

25 Sandor, S.; Checiu, M.; Fazekas-Todea, I.: Regeneration of retinal pigmentation epithelium damaged in rat foetuses


Request reprints from W.W. Carlton, Dept. Veterinary Microbiology, Pathology and Public Health, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907 (USA).