Retinal Atrophy Induced by Intravitreous Colchicine

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Colchicine is known to inhibit axoplasmic transport in ganglion cells. Previous studies have shown considerable, but largely reversible, retinal changes after low dosages of intravitreal colchicine in experimental animals. In the present study, the effects of 1.0 to 100 μg of intravitreal colchicine in monkeys were studied by ophthalmoscopy, light microscopy, and electron microscopy. Optic atrophy was ophthalmoscopically evident within 4 weeks after a single dose of 10 μg or more. Morphologic changes included progressive swelling of retinal neurons, accumulation of a fibrillogranular material, displacement of organelles, and loss of microtubules. Plasmalemmal rupture was observed in ganglion cells and in photoreceptors after as little as 1 μg of colchicine. Relative sparing of the cone cells was noted. The retina of monkeys appears to be more sensitive to intravitreal colchicine than that of certain lower animals. Invest Ophthalmol Vis Sci 24:301–311, 1983

Colchicine, a mitosis-arresting phenanthrene derivative extracted from various species of Colchicum (especially C. autumnale), is a drug commonly used in the prophylaxis and treatment of gout. The precise pharmacologic activity of colchicine in gout is uncertain, but it is known to cause depolymerization of microtubules by binding to tubulin, the protein subunit of microtubules. Colchicine is known to be neurotoxic, and peripheral neuropathy associated with its use has been described clinically. However, according to Grant,1 only one case of ocular involvement associated with systemic toxicity has been reported. This was a case of fatal poisoning accompanied by keratitis, hypopyon, pupillary membrane, cataract, and disturbance of extraocular movements.

The interruption of axoplasmic transport by colchicine was first demonstrated by Dahlström2 and by Kreutzberg.3 Since then, the action of colchicine upon neural tissue has been evaluated under various experimental conditions.4–20 Norström and co-workers21 have described severe ultrastructural alterations in the neurons of the rat hypothalamo-neurohypophyseal tract after subarachnoid injection of colchicine. Their findings included: enlargement of the nerve cell body and nucleolus, deep folding of the nucleolemma, perinuclear accumulation of organelles, mildly increased numbers of neurofilaments, and slightly decreased numbers of neurotubules.

Similar ultrastructural changes have been described in the retina after intravitreal injection of colchicine in the rabbit,22 cat,23 and rat.24 However, a toxic effect of colchicine upon the primate retina has not been reported previously. For that reason, our objective was to investigate, by light microscopy and electron microscopy, the early and long-term effects of colchicine on the retina and optic nerve of rhesus monkeys.

Materials and Methods

Rhesus monkeys (Macaca mulatta) of both sexes, weighing 2.2 to 2.6 kg, were used. Commercially available colchicine (Sigma Chemical, St. Louis, MO) was diluted with phosphate buffer (pH 7.4) to obtain 1.0, 10.0, and 100 μg in 0.05-ml aliquots.

The monkeys were anesthetized using a dosage of 0.3 ml of phencyclidine HCl (Sernylan) at a strength of 20 mg/ml, and 0.5 ml of atropine sulfate (0.5 mg/ml) given intramuscularly. The intraocular pressure of the test and controls eyes was first lowered by anterior chamber paracentesis with a fine knife-needle. The test solution of colchicine was then injected slowly via the pars plana into the central area of the vitreous under direct visualization. The same amount of phosphate-buffered saline (pH 7.4) was similarly injected into control eyes. All animals were examined ophthalmoscopically at various intervals, and fundus photographs were obtained of specific abnormalities encountered.

Eyes were obtained at the following intervals (after colchicine dosage as indicated): 1 μg: 1, 2, and 4 hrs; 10 μg: 1, 2, 4, 8, 24, and 48 hrs, 19 days, and 6 0146-0404/83/0300/301/$1.35 © Association for Research in Vision and Ophthalmology

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months; 100 μg: 1, 2, and 4 hrs, 19 days, and 6 months.

At the selected time the eyes were quickly enucleated, incised in the pars plana region superiorly, and were placed in a 2.5% solution of glutaraldehyde in phosphate buffer (pH 7.2) for 4 to 8 hrs. After washing in the buffer, the eyes were opened coronally, and 1 × 3 mm portions of the macular retina, choroid, and sclera were excised. Small blocks of the optic nerve were similarly obtained. These specimens were fixed secondarily with 2% osmium tetroxide in phosphate buffer for 2 hrs. The tissues were dehydrated in a graded series of water-ethanol mixtures and were embedded in epoxy (Araldite) resin. Semithin sections were prepared and stained with toluidine blue and paraphenylenediamine (PPDA) for orientation. Ultrathin sections were obtained with an ultramicrotome (Porter-Blum MT-2), were stained with uranyl acetate, and were examined with an electron microscope (JEM 100-B).

Eyes used for light microscopic examination were fixed in 10% formalin for 48 hr, washed overnight, and opened horizontally. The central portion of the eye—containing the pupil, optic nerve, and macula—was dehydrated and embedded in paraffin in a manner that included these areas in the same section. The tissue sections were monitored during the sectioning to insure the inclusion of the foveal area. Cross-sections of the optic nerve were also obtained. Stains used included hematoxylin-eosin, periodic acid-Schiff, Verhoeff-van Gieson, and Luxol fast blue.

**Results**

**Ophthalmoscopic Observations**

All eyes were ophthalmoscopically normal when the experiment was begun, and the control eyes remained normal throughout the period of observation.

Three animals received 1 μg of colchicine. No ocular abnormality was detected at 1 hr, but at 2 and 4 hrs there was questionable macular edema present.

Eight monkeys each received 10 μg of colchicine. At 1 and 2 hrs there was questionable macular edema in both eyes, which became more apparent at 4 hrs but still remained slight at 8 hrs. By 24 hrs, moderate edema was present with loss of the foveal reflex in both eyes. At 48 hrs the media were slightly hazy in both eyes, with increased edema and elevation of the macula. In two animals, which were followed-up for 19 days and 6 months, respectively, before killing, the macular edema disappeared by 1 to 2 weeks, and optic atrophy with retinal vessel narrowing ensued.

Four monkeys each received 100 μg of colchicine. At 1 hr a schisis-like lesion was evident, and also macular edema and exudates (snowflake-like opacities) along the superotemporal vessels. The animal that was killed at 2 hrsshowed only questionable macular edema in both eyes, whereas the monkey killed at 4 hrs showed more prominent macular edema in both eyes. Before enucleation at 19 days, one eye had extensive posterior synechiae, moderately cloudy media, retinal exudates, and optic atrophy. Yet another eye had little remaining macular edema at 19 days, definite optic atrophy and retinal vessel narrowing at one month, and a posterior subcapsular cataract at 5 months. The animal was killed at 6 months.

**Light Microscopy**

Monkey eyes that had received 1 μg of colchicine were examined by light microscopy at 1, 2, and 4 hrs after colchicine, and showed only slight edema of the outer plexiform layer.

Monkey eyes injected with 10 μg of colchicine were examined at 1, 2, 4, 8, 24, and 48 hrs, 19 days, and 6 months. Progressive retinal edema, most prominent in the outer plexiform layer, and elongation of the outer segments of the photoreceptor cells were noted up to 48 hrs. There was a slight fullness of the prelaminar portion of the optic nerve at 48 hrs, and a conspicuous inflammatory infiltrate was present in the fibrovascular pial septa of the retrolaminar portion of the nerve. At 19 days, the retinal edema was more widespread, and diffuse glial hypercellularity was evident in the optic nerve. Sections obtained at 6 months (Fig. 1) showed a slight but definite reduction in the depth of the nerve fiber layer and in the number of ganglion cell nuclei. The normally distinct alternating columns of astrocytes and nerve fiber bundles in the nerve head were difficult to discern (Fig. 2). There was moderate reduction in the size of the nerve fiber bundles of the optic nerve, slight thickening of the fibroplastic septa, and partial demyelination.

Those eyes receiving 100 μg of colchicine were examined at 1, 2, and 4 hrs, 19 days, and 6 months. Chronologically early sections were similar to those obtained after 10 μg of colchicine. Microscopic examination after 19 days and 6 months respectively disclosed extensive atrophy of the nerve fiber and ganglion cell layers of the retina, conspicuous thinning of the outer nuclear layer in the macula, and reduction in the number of outer segments. The optic nerve was atrophic, with loss of the alternating columns of astrocytes and nerve fiber bundles, glial hypercellularity, and diffuse partial demyelination.
Electron Microscopic Observations

Rhesus monkey eyes were examined at 1, 2, and 4 hrs after 1 μg of colchicine; at 1, 2, 4, 8, 24, and 48 hrs, 19 days, and 6 months after 10 μg of colchicine; and at 19 days and 6 months after 100 μg of colchicine.

Usually, retinal changes were evident at 1 hr with either the 1- or 10-μg dosage. These changes intensified with time, and they were only slightly greater with 10-μg dose than with the 1-μg dose. Extensive destruction followed the 100-μg dosages. For simplicity, the changes will be described for dosages of 1 to 10 μg, except as otherwise indicated.

Retinal Pigment Epithelium (RPE)

Numerous markedly swollen mitochondria were present in the basal portion of the cells at 1 hr and persisted until at least 8 hrs. The retinal pigment ep-
ithelium (RPE) was not examined by electron microscopy later than 8 hrs.

**Outer Segments**

Moderate swelling of some of the outer segments was detectable at 1 hr. The segments were distended by a fibrillogranular (FG) material, which separated the cell membrane from the lamellar discs in some areas. This distention was increased at 2 hrs, when moderate distortion and disruption of the lamellar discs were apparent (Fig. 3). At 48 hrs there was marked focal disarray of the lamellar discs of many outer segments, and by 19 days only fragments of the outer segments remained.

**Inner Segments**

Overall, the inner segments of rod photoreceptors appeared to be much more affected by colchicine than
Fig. 3. Outer segments at two hours after 10 micrograms of intravitreous colchicine. There is swelling with separation of cell membrane from lamellar discs by a fibrillogranular material (asterisks, bracket and inset) and distortion and disruption of some lamellar discs (x3,300; inset, x8,300).

were the cone inner segments (Fig. 4). At 1 hr, rod ellipsoids exhibited diffuse mitochondrial swelling, distention by a finely granular material, and prominent loss of neurotubules, whereas cone ellipsoids displayed only focal, segmental mitochondrial swelling. Likewise, a finely granular material distended many of the rod myoids at 1 hr, with concomitant prominent but partial loss of neurotubules and displacement of organelles, but adjacent cone myoids exhibited numerous neurotubules and inappreciable swelling. Many membrane-bound vacuoles and polyribosomes were observed in the myoids of both rods and cones. These changes slowly intensified with time, leading to cellular disruption, and by 6 months only a few abbreviated inner segments were recognizable.

Outer Nuclear Layer (ONL)

There was marked intumescence of the perinuclear cytoplasm of the rod cells, with accumulation of FG material and polyribosomes and much loss of neurotubules at as early as 1 hr. The perikaryon of the cone cells appeared unaffected until later, except for vacuolization similar to that seen in the myoids. Occasional dense nucleoli were found in the nuclei of both rods and cones. The cell distention increased with time (Fig. 5), and by 19 days after a single 100-μg dosage, it was obvious that many of the photoreceptor cells had ruptured, leaving behind large cystic spaces containing FG material and cellular debris. Müller’s cell processes in this layer contained abundant neurotubules throughout the period of study and remained quite intact.

Outer Plexiform Layer (OPL)

There was conspicuous but highly variable swelling of receptor fibers throughout the OPL at 1 hr. In favorable sections, saccular and fusiform dilatations of otherwise intact fibers were observed (Fig. 6). Within the dilated portion, there was partial to total disappearance of neurotubules and an increase of FG material. Scattered swollen mitochondria were noted. By 48 hrs, virtually all fibers were distended with FG substance, and at 19 days, many of the fibers had burst; no microtubules could be identified, and the entire OPL appeared deranged. Surprisingly, Müller’s cell processes and their microtubules were remarkably well preserved.

Inner Nuclear Layer (INL)

At 1 hr there was much perinuclear swelling, with deposited FG material, numerous polyribosomes, and scattered turgent mitochondria in all cells except Müller’s cells, which appeared nearly normal (Fig. 7). In a few cells, the acute swelling had caused rupture of the plasmalemma within 2 hrs. By 19 days, the cytoplasmic swelling was greatly diminished, and although many vacuoles and distended mitochondria
remained, most of the cells appeared viable. The Müller cell nuclei contained a more homogeneously distributed chromatin than before, but their cytoplasm was not altered noticeably.

**Inner Plexiform Layer (IPL)**

Bipolar cell axons and ganglion cell and amacrine cell processes were decidedly tumescent within 1 hr after intravitreal colchicine. They contained FG material, scattered polyribosomes, and swollen mitochondria (Fig. 8). Very few neurotubules could be found except in Müller cell processes, where they abounded. There was only a mild increase in the intensity of these changes at 48 hrs. However, by 19 days there was definite loss of neuronal processes, further distention of many of the remaining processes with FG substance, prominence of the rather well-preserved Müller cell processes, and a general appearance of disarray.

**Fig. 4.** Inner segments at 2 hrs after 10 μg of intravitreal colchicine. Rod inner segments (R) are quite distended with fibrillagranular material, whereas cone inner segments (C) are less affected. Rod ellipsoids contain swollen mitochondria (arrowhead) and few remaining neurotubules (arrows, bracket, and upper inset). Vacuoles (asterisks) are present in both cone and rod myoids. Similar but less numerous vacuoles are present in the myoid of both rods and cones (asterisks, lower inset) at two hours after one microgram of colchicine, and few neurotubules (arrows) remain in rod myoids (top, ×3,300; upper inset, ×9,000; lower inset, ×12,000).
Fig. 5. Outer nuclear layer near external limiting membrane (arrowheads) at 2 hrs after 1 μg of intravitreous colchicine. Much swelling of the perinuclear cytoplasm of rod cells (R) is evident, whereas cone cells (C) show only scattered vacuoles. Higher-power view (inset) shows preservation of cone fiber neurotubules (asterisk), whereas only a few neurotubules (circle) remain in the rod perikaryon (x3,800; inset, x12,400).

Ganglion Cell Layer (GCL)

The effect of colchicine upon the individual ganglion cell was highly variable. At 1 hr, some of the cells seemed unaltered except for distention of mitochondria, and yet others showed a dense collection of FG material and polyribosomes in one portion of the cytoplasm, ballooning that end of the cell and displacing normal organelles (Fig. 9). The swelling became more diffuse and extreme with time, and by 19 days most of the cells had ruptured and disappeared, leaving large cystic spaces surrounded by Mueller cell processes. The few ganglion cells remaining at 6 months were distended by an osmophilic, vacuolated cytoplasm.

Fig. 6. Outer-plexiform layer at 2 hrs after one microgram of intravitreous colchicine. There is marked focal dilatation of photoreceptor fibers, with deposition of fibrillogranular material and partial loss of neurotubules in the distended portions. An abrupt transition (arrows) between relatively intact fibers and swollen degenerated areas is evident (x3,600).
Nerve Fiber Layer (NFL)

Many of the ganglion cell axons (which normally contain abundant neurotubules) were markedly turgid at 1 hr, with near total loss of neurotubules, prominent swelling of mitochondria, and amassment of FG substance (Fig. 9). The intensity of these effects was unaltered at 48 hrs, and by 19 days only a few moderately distended axons containing membranous debris and a few mitochondria were noted. No intact neurotubules could be identified in the vestigial axons, and even the surviving processes of Müller's cells had fewer microtubules than in earlier sections.

Optic Nerve (ON)

At 1 hr, mild mitochondrial swelling and a slight loss of neurotubules in a few of the myelinated axons...
Fig. 9. Ganglion-cell layer and nerve-fiber layer at one hour after 10 micrograms of intravitreous colchicine. The ganglion cells are variably affected. There is focal accumulation of fibrillogranular material (FG) and much turgescence of mitochondria (arrows). Ganglion-cell axons (bracket and inset) are also variably distended by fibrillogranular substance, and there is total loss of neurotubules. Müller's cell processes (asterisk) are unaffected (×3,300; inset, ×11,700).

Discussion

This study demonstrated that colchicine, when injected intravitreally in low dosages, causes profound and often irreversible damage to the primate retina and optic nerve. As little as 10 μg of the drug produced ophthalmoscopically evident atrophy of the optic nerve head, which was confirmed by light microscopy. A 100-μg dosage resulted in widespread destruction of ganglion cells and concomitant optic atrophy, which was virtually complete. Many of the

were the only noteworthy findings. By 2 hrs, mild and variable axonal turgescence, with accumulation of a fibrillar material and partial loss of neurotubules was noted (Fig. 10). Within 48 hrs there was near total loss of neurotubules from the myelinated axons, mild and variable shrinkage of the axoplasm, and partial collapse of the surrounding myelin sheaths. Optic atrophy—characterized by degenerated axons, frayed and irregularly collapsed myelin sheaths, and prominent, relatively well-preserved glial cells—ensued by 19 days (Fig. 10).
Ultrastructural alterations, although variable in intensity, were similar in the various neurons of the retina. These changes consisted of: swelling of the perikaryon and cell processes, distention of mitochondria, variable loss of cytoplasmic organelles, and prominent loss of microtubules. In the swollen cells, there was a conspicuous accumulation of a fibrillo-granular material, often containing numerous short fragments of rough endoplasmic reticulum. With time, the distention progressed to the point of plasma membrane rupture in many cells, especially in the ganglion cells and rod photoreceptor cells, which were the most severely affected. RPE cells and Müller cells, however, remained remarkably unaltered; tumid mitochondria were the only signs of damage to these supporting elements. It is noteworthy that the cone photoreceptors displayed a relative resistance to the action of colchicine.

Hansson and Sjöstrand have described an increased density and granularity of the nucleus, enlargement of the nucleolus, and the appearance of...
bundles of 70 Å filaments in the nucleoplasm of retinal cells in the rat after intravitreal injection of colchicine. Such changes were not a prominent feature of our present study. Although occasional dense nuclei were observed in various of the cell types, the nuclei of the retinal neurons of the monkey were not consistently altered in any evident manner by colchicine in the dosages used.

Colchicine is believed to cause the perinuclear accumulation of cytoplasmic constituents by inhibiting axonal transport to the periphery while the synthesis of proteins and organelles continues unimpeded. This inhibition of axonal flow is thought to be the result of the drug's effect on microtubules. Curiously, however, other authors22,24 have reported little or no loss of microtubules in the ganglion cells after intravitreal injection of colchicine. In striking contrast, our study revealed early and near total disappearance of the neurotubules from the intraretinal ganglion cell axons. Considerable loss of microtubules was also noted in many other retinal cells. These findings are consistent with the hypothesis that colchicine's toxic effect on neurons is mediated by the depolymerization of neurotubules. Other ultrastructural changes, however, such as mitochondrial swelling and the disruption of photoreceptor outer segments, are not explained easily on the basis of neurotubular dysfunction alone.

The retina of the rhesus monkey appears to be decidedly more sensitive to intravitreal colchicine than that of lower animals that have been studied. Karlsson and co-workers22 reported that intravitreal colchicine in a dose of 1 μg did not cause any substantial ultrastructural changes in the retinal ganglion cells, other retinal cells, or neuroglial cells of the rabbit. Vaccarezza et al23 observed a return of visual function and a complete ultrastructural recovery of the photoreceptor cells of cats within 30 days after intravitreal injection of 20 μg of colchicine. They described no retinal edema or loss of ganglion cells at that dosage.

In view of the extreme sensitivity of the primate retina and optic nerve to as little as 1 μg of intravitreal colchicine, we suggest that the possibility of a toxic retinopathy be considered in patients receiving colchicine or other agents that interfere with axoplasmic flow.

Key words: axoplasmic flow, axoplasmic transport, colchicine, gout, intravitreal injection, microtubules, neurotubules, optic atrophy, retinal atrophy, retinal toxicity

References

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