The effect of long-term administration of amopyroquin,* a 4-aminoquinoline compound, on the retina of pigmented and nonpigmented laboratory animals

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It has been reported that prolonged high dosage of the 4-aminoquinoline, chloroquine, is capable of producing destructive lesions of the retina in both humans and cats. On the basis of detailed studies, the concept has been derived that the avidity of melanin for chloroquine is primarily responsible for the pattern of retinal injury. In our laboratory, amopyroquin (Propoquin), another 4-aminoquinoline, was orally administered at several toxic levels to albino rats, beagle dogs, and rhesus monkeys for periods of up to one year. Degenerative atrophic lesions developed in the retinas of albino rats treated for 26 weeks and longer, and in beagle dog retinas after only 21 weeks, but failed to appear in monkey retinas after one year of drug administration. In general, the incidence of the lesion in dogs and rats was related to the duration of drug administration and the size of the dose. Furthermore, in some of the rats, the lesion became manifest after the drug was withdrawn from the diet. It is concluded that the presence or absence of melanin in the retina-choroid is not a primary factor in amopyroquin-induced retinal atrophy. Rather, retinal sensitivity to injury appears to be a species-related phenomenon. Examination of the chloroquine literature indicates that this conclusion is valid also for chloroquine.

Chloroquine, one of the 4-aminoquinoline compounds used originally as antimalarial drugs, has been found effective in the treatment of rheumatoid arthritis and discoid lupus erythematosus. Characteristically, the compound must be administered over a long period of time at relatively high doses, in order to achieve a successful therapeutic result. Among such patients treated vigorously with chloroquine, there developed what appeared to be a significant incidence of a dose-related retinopathy. Since the original report by Cambiaggi,1 more than 80 entries have appeared in international clinical and experimental literature attesting to this relationship. In addition to well-documented clinical features,2 histological changes in several patients3-5 who had taken prolonged or high dosages of chloroquine, and who later came to be autopsied as a consequence of either their collagen disease or of nonrelated causes were described. Sections of their eyes revealed that the layer of rods and cones was focally and diffusely atrophied. The ganglion cell layer was

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420
similarly affected. The lesion was duplicated in laboratory cats after chronic administration of chloroquine. This relationship between chloroquine administration and retinopathies in humans was recently challenged by Scherbel and associates, who found a similar or greater incidence of retinopathies in rheumatoid arthritic patients who were not treated with chloroquine than in those who were treated with the drug. However, as the authors pointed out, the two populations studied had important dissimilarities, and a strict classification of the encountered retinopathies was not offered.

Because of the known avidity of melanins and melanin-bearing ocular tissues for chloroquine, it was proposed that retinal damage was initiated by a high concentration of the drug in the pigmented portion of the eye. Indeed, retinal lesions in albino rats and rabbits failed to develop after 3 months and 11 months, respectively, of chloroquine administration.

The present study deals with the effect of long-term administration of amopyroquin, a 4-aminoquinoline, on the retina of both pigmented and nonpigmented laboratory animals. The results of the study establish that the presence or absence of natural pigment (melanin) in the retina-choroid is not the exclusive basis for the development of retinal atrophy, or of its failure to develop following prolonged drug administration.

Materials and methods

Albino rats (Holtzman source), beagle dogs, and rhesus monkeys were used in this study. Dosages of amopyroquin were selected on the basis of preliminary toxicity studies, so that varying degrees of general toxic reactions would be produced over the projected course of the experiment. In small groups of animals, interim death and drug withdrawal periods were utilized in order to define more clearly the temporal and dosage aspects of possible retinal toxicity.

Groups of 30 male and 30 female rats were fed the drug which was mixed with a standard ration at 0.03, 0.07, and 0.12 per cent in order to yield a daily intake of 10, 30, and 60 mg. per kilogram of amopyroquin for periods of up to 52 weeks. A control group of 30 male and 30 female rats was fed normal rations. Twelve beagle dogs were administered bulk amopyroquin daily (by capsule) in doses divided so as to achieve daily dosages of 10 mg. per kilogram, 40 mg. per kilogram, and 80 mg. per kilogram for periods of 12 to 52 weeks. Eight male and 4 female rhesus monkeys were dosed by gavage with the drug suspended in 10 per cent acacia in order to achieve daily dose levels of 5 mg. per kilogram, 25 mg. per kilogram, and 50 mg. per kilogram for periods of 24 to 52 weeks. These and other pertinent details of the experiment are summarized in Table I.

The rats were painlessly put to death with chloroform; the dogs and monkeys with an intravenous overdose of thiamylal sodium. Fixation was carried out by means of arterial perfusion with physiological saline, followed by 10 per cent buffered neutral formalin. Tissues from animals that died spontaneously during the course of the experiment were removed, and fixed by conventional immersion in 10 per cent formalin. The eyes were removed, quick-frozen, hemisectioned through the optic nerve, fixed, paraffin-embedded, sectioned, and stained with hematoxylin and eosin.

Results

Albino rats. Of the 30 females at the high dose level, 6 died within 26 weeks. At this time, 7 were painlessly put to death, and 17 were continued on standard ration for an additional 16 weeks. Six of the latter animals died, and were not suitable for histological study. At the mid- and low-dose levels, 4 males and 4 females were put to death from each group at 26 weeks, and 10 of each sex were given only standard rations for a reversibility period of 16 weeks. The remaining animals at these dose levels were continued on drug diet for 52 weeks. Histological examination of the eyes from animals treated for 26 weeks revealed normal retinas (Figs. 1 and 2). However, among animals surviving to the completion of the reversibility period (normal rations for 16 weeks), as well as among those dosed for the entire 52 week period,
Fig. 1. Retina of a normal control albino rat. (Original magnification x430.)

Fig. 2. Retina of albino rat treated with 30 mg. per kilogram amopyroquin for 1 year. There is loss of the inner plexiform and nuclear layers, as well as the layer of rods and cones. The remaining portions of the retina are edematous and distorted. (Original magnification x430.)

**Table I.** Pertinent details relative to administration of amopyroquin to rats, dogs, and monkeys

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight</th>
<th>Dose range mg/Kg.</th>
<th>No. animals/dose</th>
<th>Total No. animals</th>
<th>Length of dosing (days)</th>
<th>Length of observation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holtzman rats</td>
<td>177-227 Gm. (initial)</td>
<td>10, 30, 60</td>
<td>30</td>
<td>240</td>
<td>182-366</td>
<td>306-366</td>
</tr>
<tr>
<td>δ and Φ</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Beagle dogs</td>
<td>5.9-11.6 Kg.</td>
<td>5 (twice daily)</td>
<td>4</td>
<td>12</td>
<td>3-369</td>
<td>3-369</td>
</tr>
<tr>
<td>δ and Φ</td>
<td>20 (twice daily)</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>40 (twice daily)</td>
<td></td>
<td></td>
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<tr>
<td>Rhesus monkeys</td>
<td>2.90-3.82 Kg.</td>
<td>5, 25, 50</td>
<td>4</td>
<td>12</td>
<td>192-361</td>
<td>195-364</td>
</tr>
</tbody>
</table>

A characteristic retinal lesion was seen almost exclusively among females. Typically, the lesion consisted of retinal degeneration, ranging in its mildest form from edema of the inner and outer nuclear and plexiform layers, to progressive thinning of the outer nuclear layer, and distortion of the rods and cones layer. The more severe manifestations consisted of destruction of the rods and cones layer, the outer nuclear layer, and the outer plexiform layer of the retina. One or both eyes were involved in about equal numbers. The lesions were distributed focally or diffusely in the retina, but generally were observed earliest anteriorly near the ciliary body. One treated male had severe vacuolation of the retina. Except for the lack of pigment, the lesions seen here generally conform to those reported to be induced by the administration of chloroquine to humans and to cats.

The lesion was distributed almost exclusively among females; being present in only 2 male rats that were administered the low dose for 52 weeks. Considering the total number of female rats at each dose level that survived either the drug reversal period or 52-week drug regimen, the incidence of the lesion was 9/11 for the high-dose survivors, 8/25 for the mid-dose survivors, and 6/25 for the low-dose survivors.

A single control male rat had a small
Amopyroquin effects on retina

Fig. 3. Retina of a normal beagle dog. (Original magnification ×100.)

Fig. 4. Retina of a beagle dog that received 40 mg. per kilogram amopyroquin for 1 year. There is accumulation of pigment (arrow) in the pigment layer of the retina, and loss of rods and cones in the overlying area. (Original magnification ×100.)

Fig. 5. Retina of a beagle dog that received 10 mg. per kilogram amopyroquin for 1 year. The lesion is similar to that of Fig. 4, but more severe; with atrophy of the nuclear layers, in addition to loss of rods and cones. (Original magnification ×100.)

focus of retinal scarring and adhesion to the underlying choroid. Otherwise, the retinas of all control rats were normal.

Beagle dogs. One dog that received the drug at the 10 mg. per kilogram level had considerable amounts of a reddish-brown pigment in the choroid-retinal junction at 52 weeks. The overlying rods and cones and nuclear layers were atrophic (Figs. 3 to 5). In the 3 other dogs at this dose level, no retinal lesions were noted. At the 40 mg. per kilogram level, 3 out of 4 dogs had accumulations of a reddish-brown pigment in the pigment cell layer of the retina; 2 of these had focal atrophy and degeneration of the overlying retina. The fourth dog had marked atrophy and degeneration of the retina without the accumulation of pigment. Because of the development of severe toxicity, none of the dogs at the 80 mg. per kilogram level completed the scheduled dosing period. One dog died after 3 days of dosing at 80 mg. per kilogram, without developing retinal changes. The remaining 3 dogs at this dose level were in poor condition and put to death after 84, 86, and 146 days. The first 2 dogs had small accumulations of the reddish-brown pigment at the choroid retinal junction, but no changes in the retina proper. The third animal was put to death after 146 days of dosing, and had small amounts of pigment in the retina, with focal areas of atrophy or degeneration of the rod and cone layer. The lesion was similar to that reported to occur in humans\(^3\), \(^4\), \(^5\) and in cats\(^6\) following the administration of large amounts of chloroquine.

Rhesus monkeys. Destructive retinal lesions and/or pigment deposition were not seen in the eyes of monkeys given amopyroquin, regardless of the level or the duration of dosage.
Discussion

It is clear from the foregoing data that retinal sensitivity to injury by amopyroquin is related to species differences, rather than to the presence or absence of melanin in the eye. This may be reflected in earlier reports of failure to induce frank retinal atrophy in pigmented rats and rabbits following chronic chloroquine administration,10, 11, 12 and of success in producing the lesion in cats.9 The finding of focal and general retinal atrophy in the albino rat is particularly intriguing. The concept has evolved that at least the first step in the development of retinal atrophy by chloroquine involves the fixation of the compound in the pigmented portions of the retina and choroid. This concept was based on the findings that: (1) chloroquin accumulated preferentially in the pigmented portions of the eye during chronic administration of the drug; (2) disturbances of pigment distribution in the retina and choroid developed in advance of/or concomitantly with the development of the lesion; and (3) that in albino animals fed the drug over an extended period, there were neither excessive concentrations of drug in the eye, nor retinal lesions.13 The results found with albino rats in this study suggest the possibility that the 3 month studies on chloroquine in albino rats elsewhere may not have been continued long enough to produce the lesion at the dosage employed.

Although, in our study, the first incidence of retinal atrophy in rats was noted in animals put to death 10 months after the initiation of dosing, several of these affected animals had received no drug after the sixth month. Furthermore, there were no retinal lesions in randomly selected companion animals that were put to death at the time administration of the drug was discontinued. Therefore, while 26 weeks of exposure to the compound was adequate to trigger the lesion in some animals, the overt histological change appeared to require an additional period of time to become manifest. This phenomenon parallels descriptions of the clinical course of chloroquine retinopathy, in which the lesions progressed despite drug withdrawal.5, 13 This feature was ascribed to the known long half-life of the drug in the pigmented portions of the eye,5, 11 an explanation that cannot be invoked here for albino rats.

Of the 2 pigmented species used in this study, the dog proved to be very sensitive to retinal damage from chronic administration of amopyroquin, while the monkey developed no apparent retinal abnormality after one year of dosing. This failure to develop retinal lesions in the monkey was not due to lack of affinity of the retina-choroid for the drug, since chemical analysis of the dog and monkey retinas for the presence of amopyroquin14 revealed that eyes of both species accumulated comparably large amounts of the compound, and that its half-life was in excess of 68 days.

REFERENCES