7. H. Araki, K. Kawashima, and H. Aihara, Jpn. J. Pharmacol., 35, No. 1, 67 (1984).
8. F. Borsini, C. Bendotti, V. Velkov, et al., J. Pharm. Pharmacol., 33, No. 1, 33 (1981).
9. D. Luttinger, M. Freedman, L. Hamel, et al., Eur. J. Pharmacol., 107, No. 1, 53 (1984).
10. P. A. Martorana and R. E. Nitz, Arzneim.-Forsch., 29, No. 6. 946 (1979).
11. S. Nomura, J. Shimizu, M. Kinjo, et al., Eur. J. Pharmacol., 83, No. 3-4, 171 (1982).
12. R. D. Porsolt, M. Le Pichon, and M. Jalfre, Nature, 266, No. 5604, 730 (1977).
13. R. D. Porsolt, G. Anton, N. Blavet, and M. Jalfre, Eur. J. Pharmacol., 47, No. 4, 379 (1978).
14. R. D. Porsolt, A. Bertin, N. Blavet, et al., Eur. J. Pharmacol., 57, No. 2/3, 201 (1979).
15. J. Zebrowska-Lupina, Psychopharmacology, 71, No. 2, 169 (1980).

MECHANISMS OF RETINAL DAMAGE IN CHLOROQUINE RETINOPATHY

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KEY WORDS: retina; chloroquine; lipid peroxidation

Chloroquine, which is widely used in clinical practice for the treatment of malaria and autoimmune diseases, if used for a long time can cause degeneration of the retina [4, 6, 8]. Previously the authors showed [3, 9] on a model of chloroquinine-induced retinal degeneration that the outer segments (OS) of the photoreceptors are most vulnerable to the action of chloroquine and were destroyed virtually completely. According to other investigators certain antimalarial drugs and, in particular, primaquine, exert their damaging action on the malarial parasite by a mechanism of lipid peroxidation (LPO) [5]. Considering that the phospholipids of the membranes of OS contain mainly polyunsaturated fatty acids, which are extremely sensitive to peroxidation, it might be supposed that chloroquine retinopathy is also connected with LPO. No definite data are yet available, however, on chloroquine and its effect on LPO.

This paper describes comparative electron-microscopic, electrophysiological, and biochemical investigations of the effect of chloroquine on LPO in vivo (rats, rabbits) and in vitro (on model systems).

EXPERIMENTAL METHOD

Wister albino rats and pigmented rabbits were used. The rats were divided into three groups: animals of group 1 (control) received no drugs, animals of group 2 received chloro-

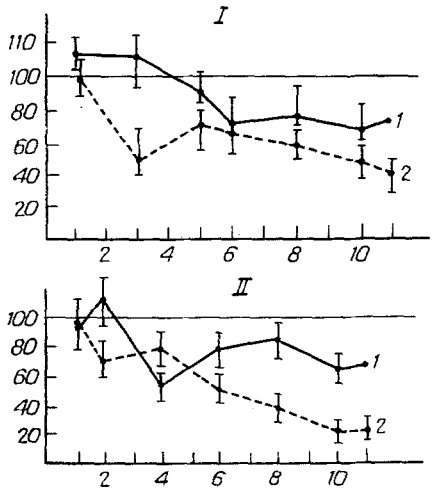

Fig. 1. Change in amplitude of a (I) and b (II) waves on ERG of rats during chronic administration of chloroquine. Abscissa, duration of administration of drug (in months); ordinate, amplitude of wave in % of control. 1) Chloroquine; 2) chloroquine + ionol.

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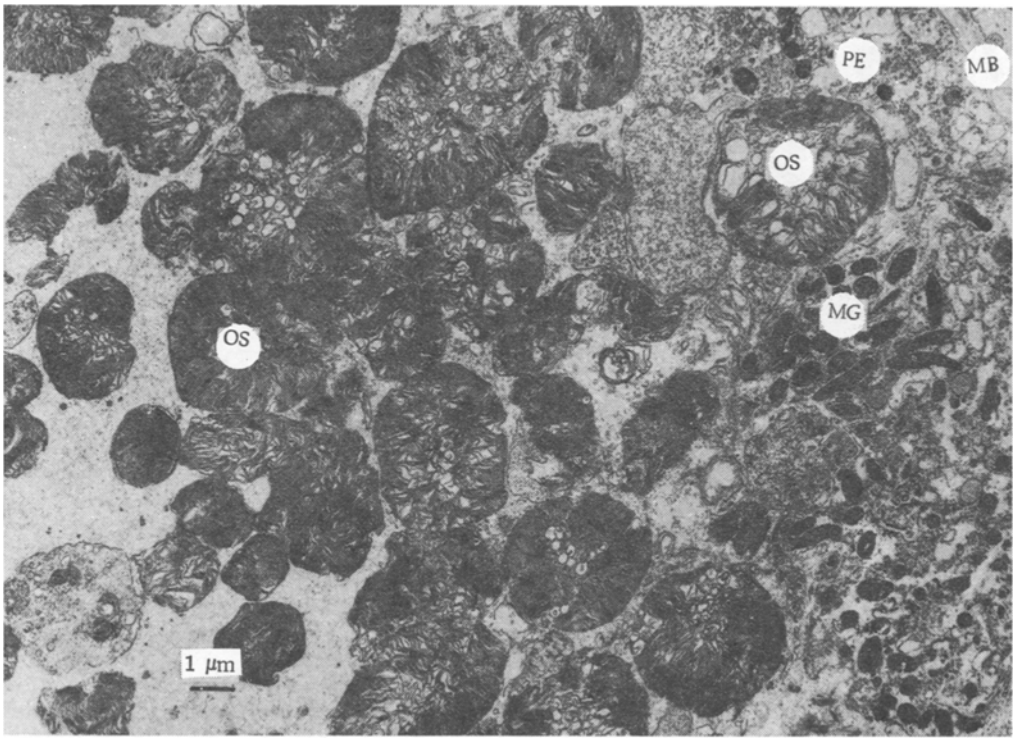

Fig. 2. Pigmented epithelium and outer segmetns of retina of rabbit 1 day after intravitreal injection of 5 mM choloquine. MB) Brunch's membrane; MG) melanin granules; OS) Outer segments of photoreceptors; PE) pigmented epithelium.

quine, and those of group 3 received chloroquine plus the antioxidant ionol. Each group consisted of 15 animals. The drugs were given perorally on alternate days for 11 months: ionol in a dose of 20 mg/kg and chloroquine in a dose of 120 mg/kg. The electroretinogram (ERG) was recorded in intact animals, using flashes from a ISK-10 lamp. Chloroquine was injected intravitreally into the right eye of the rabbit in concentrations of 0.5 and 5 mM, and the left eye was used as the control. The animals were killed 24 h after injection of the drug.

The retina of the rats were removed for electron-microscopic investigation 1, 2, 7, 8, 9, and 11 months after the beginning of the experiments, and the rabbit retina was removed after 1 day. The retina was fixed in 3% glutaraldehyde solution made up in 0.1 M phosphate buffer (pH 7.4), followed by postfixation in 1% OsO_4 solution, made up in the same buffer. The tissue was embedded in the epoxide resin Epon. Sections cut on the LKB-III Ultramicrotome were examined in the IEM-100B electron microscope. The retina was taken from the control group of rats and from the groups of rats receiving chloroquine for biochemical investigation 1, 6, 7, 8, and 11 months after the beginning of injection of the drug.

The concentration of primary LPO products was determined by measuring absorbance at 232 nm of a solution of lipids in a mixture of methanol and heptane (5:1 by volume) and estimated as the ratio of absorbance at 232 nm to absorbance at 206 nm.

Cardiolipin liposomes were obtained by suspending cardiolipin in 0.1 M K-phosphate buffer, pH 7.4. The cardiolipin concentration in the medium was 2.5 mg/ml. LPO was induced by UV radiation (DRK-120 high-pressure mercury vapor lamp) and a system containing Fe^{++} ions and ascorbic acid in concentrations of 15 mM and 0.5 mM, respectively. The velocity of LPO was estimated from the accumulation of malonic dialdehyde (MDA) in the incubation medium [1] and the protein concentration was determined by the microbiuret method [7].

EXPERIMENTAL RESULTS

The morphological picture in the rats receiving chloroquine was similar to that described by the authors previously [3, 9]: first to respond to the drug were the ganglion cells, (GC) — many membrane inclusions of lysosomal origin were observed in their cytoplasm as early as after 1 month. In rats receiving chloroquine plus ionol, no differences were found in the

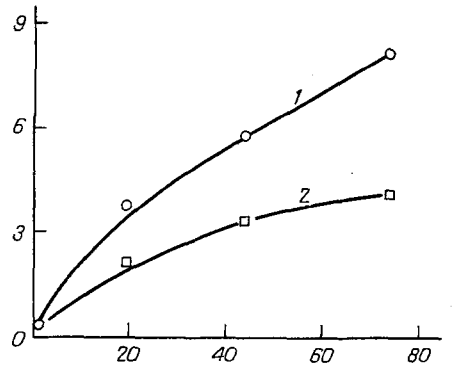

Fig. 3. Effect of chloroquine on LPO of rabbit retinal tissue homogenate, induced by a system of Fe^{++} + ascorbic acid. Abscissa, incubation time (in min); ordinate, MDA level (in nmoles/mg protein). 1) Control; 2) chloroquine (5 mM). Concentrations in sample: protein 1.4 mg/ml, Fe^{++} 15 μM, ascorbic acid 0.5 mM.

distribution of inclusions in the retina compared with animals receiving chloroquine alone. The cytoplasm of the ganglion cells 11 months after the beginning of administration of chloroquine to the rats of both groups was filled with membrane inclusions, and the width of the outer nuclear layer (ONL) was reduced to 5-6 nuclei in a row, whereas in the control at these same times the width of ONL was 11-12 nuclei in a row. This is clear evidence of degradation of ONL in rats receiving chloroquine, both alone and in combination with ionol. The initial stages of degeneration of OS were observed in the photoreceptors 11 months after the beginning of administration of the drug.

Electroretinography showed that in rats receiving long-term chloroquine injections a permanent decline of retinal function gradually developed: the amplitude of the *a* and b waves of the ERG was reduced (Fig. 1). Changes in electrical activity of the rat retina developed as early as by the 4th month of peroral administration of chloroquine, and they gradually increased in severity until the end of the experiment, when the values of the amplitudes of *a* and b waves were only about 60% of the control (Fig. 1). Simultaneous administration of chloroquine and ionol likewise did not prevent depression of the electrical activity of the retina. Rats of this group actually showed a rather greater decrease in amplitude of the *a* and b waves on the ERG (to 40% of the control) than animals receiving chloroquine alone (Fig. 1). The antioxidant ionol thus had no protective action on the development of chloroquine retinopathy in the experimental animals.

The results of the biochemical tests showed that during 11 months of administration of chloroquine the quantity of primary LPO products in the rat retina did not increase.

Experiments on the rabbits showed that 24 h after intravitreal injection of 0.5 mM chloroquine the ultrastructure of their retina was virtually unchanged. Injection of 5 mM chloroquine, however, gave rise after 24 h to considerable damage to all layers of the retina, including the pigmented epithelium (Fig. 2). The outer segments of the photoreceptors were disoriented and fragmented. A parallel spectrophotometric determination of the content of primary LPO products in these same retinas after 24 h showed that chloroquine, in concentrations of 0.5 and 5 mM did not bring about an increasing level of primary LPO products.

Experiments on the model system of UV-irradiation-induced peroxidation of cardiolipins indicated that the introduction of chloroquine in concentrations of 0.5 and 5 mM slowed the rate of photo-oxidation, and the effect was more marked with the higher concentration of chloroquine. Since UV light is an active traumatic factor, it had to be discovered whether the chloroquine itself was destroyed in the course of UV irradiation. Judging by the adsorption spectra, chloroquine is stable relative to UV irradiation. Meanwhile, chloroquine possesses considerable optical density in the regions of absorption of of cardiolipins. The possibility of an optical effect of screening of the light absorbed by cardiolipins cannot therefore be ruled out. To rule out the possible screening action of chloroquine another system of LPO induction was used, namely Fe^{++} + ascorbic acid. Addition of chloroquine in this case also reduced the velocity of cardiolipin peroxidation. Moreover, in a concentration of 5 mM, chloroquine suppressed LPO completely. The study of the action of chloroquine on LPO in the rabbit retina, induced by a mixture of Fe^{++} + ascorbate, showed that addition of chloroquine to the retinal homogenate led to a marked decrease in the velocity of LPO (Fig. 3). Experiments in vitro showed that chloroquine exhibits an inhibitory action on LPO.

Meanwhile a comparative study of the action of chloroquine on the rat retina with or without the addition of ionol showed that this antioxidant did not delay the development of chloroquine retinopathy in rats, but instead, intensified it. Ionol protected neither the lysosomal apparatus of GC nor the photoreceptors from injury. In animals receiving chloro-

quine + ionol, not only was its positive effect on the ERG not found, but there was actually some worsening of its parameters.

The absence of an increase in the content of diene conjugates in lipid extracts of the rat retina throughout the period of chloroquine administration may be evidence of the absence of correlation between the appearance of membrane inclusions and degeneration of the photoreceptors, on the one hand, and LPO on the other.

Experiments in vitro showed also that chloroquine (5 mM), while inducing ultrastructural lesions in the retina, not only did not potentiate LPO in different systems, but considerably inhibited it.

The absence of a protective effect of the antioxidant ionol on chloroquine retinopathy in rats, the absence of an increase in the content of primary LPO products in experiments on rats and rabbits, and also the inhibition of LPO by chloroquine in vitro are evidence that LPO is not the inducing mechanism of damage to the retinal cells in chloroquine retinopathy. The possibility cannot be ruled out that the pathogenetic action of chloroquine may take place by a different mechanism: for example, it may have some effect on renewal of the photoreceptor disks of OS. This is shown by the morphological features of damage to the photoreceptor cells observed in chloroquine retinopathy (shortening of OS of the photoreceptors) [9], which differ from the ultrastructural picture on the retina observed by the present authors following the injurious action of light on the retina [2].

LITERATURE CITED

1. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
2. M. V. Zueva and T. A. Ivanina, Vest. Oftal'mol., No. 4, 48 (1980).
3. T. A. Ivanina, M. V. Zueva, and M. N. Lebedeva, et al., Morphological Aspects of Ophthalmology [in Russian], Moscow (1983), pp. 23-25.
4. G. B. Arden and H. Kolb, Br. Med. J., 1, 270 (1966).
5. A. Clark and W. B. Cowden, Oxidative Stress, London (1985), pp. 131-147.
6. A. Elman, A. E. R. Gulliberg, and E. Nilsson, Scand. J. Rheum., 5, 161 (1976).
7. A. G. Gornall, C. I. Bardawill, and M. M. David, J. Biol. Chem., 177, 757 (1949).
8. P. Henkind and N. F. Rothfield, New Engl. J. Med., 269, 433 (1963).
9. T. A. Ivanina, M. V. Zueva, M. N. Lebedeva, et al., Graefes Arch. klin. exp. Ophthalmol., 220, 32 (1983).

STIMULATING EFFECT OF COCAINE IN RATS DEPENDING ON SPECTRUM OF BLOOD SERUM ESTERASE ACTIVITY

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KEY WORDS: cocaine; rat blood serum; esterase activity

One pathway of cocaine metabolism in rats is its hydrolytic degradation by blood serum esterases, including cholinesterase (ChE), with the formation of polar metabolites which do not pass through the blood-brain barrier: the methyl ester of ecgonine, benzoylecgonine, ecgonine itself, etc. [8]. Direct positive correlation has been demonstrated between the rate of disappearance of ^{14}C-labeled cocaine from the plasma and the level of ChE activity in man [5]. One of the factors determining individual sensitivity to xenobiotics is the heterogeneity of the enzyme systems involved in their biotransformation. Polymorphism of the blood serum esterases and ChE in animals and man has been sufficiently well studied [1, 3, 6, 7, 9]. However, the role of multiple forms of these enzymes in the manifestation of the pharmocologic effects of cocaine is not clear.

The aim of this investigation was to study correlation between individual behavioral effectiveness of cocaine and the blood serum esterase spectrum in rats.

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