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MECHANISMS OF DEVELOPMENT OF THIORIDAZINE RETINOPATHY

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Several drugs (amiodarone, gentamicin, perhexiline, deferoxamine), while differing in their therapeutic action, may have side effects on the retina. This list of drugs also includes others widely used in clinical practice: the antimalarial and antirheumatoid preparation chloroquine and the psychotropic drug thioridazine (Melleril, Sonapax) which, on long-term administration gives rise to retinopathy in man [5, 7, 9, 11]. Despite much research into the study of chloroquine and thioridazine retinopathy, the mechanism of development of these pathological processes is not yet clear. The writers' previous ultrastructural investigations of degenerative processes in the retina [2, 10], reproduced by injection of chloroquine and thioridazine, showed that under the influence of these drugs the most damage is sustained by the outer segment (OS) of the photoreceptors, which are extremely sensitive to lipid peroxidation (LPO) due to their high content of polyunsaturated fatty acids. Considering the existing view that one of the leading mechanisms of destruction of photoreceptor membranes is LPO [4], it was natural to suggest that the preparation may have a damaging action on the retina through a mechanism of peroxidation. However, in relation to chloroquine, we showed [3] that LPO is not the initiating mechanism in the development of the retinopathy induced by this drug. As regards thioridazine, no definite information on the effect of this drug on LPO processes could be found in the literature.

The aim of this investigation was to study the effect of thioridazine on LPO processes in the retina of experimental animals. Comparative electron-microscopic, electrophysiological, and biochemical investigations were carried out on the retina in vivo (rats and rabbits) and in vitro (on model systems).

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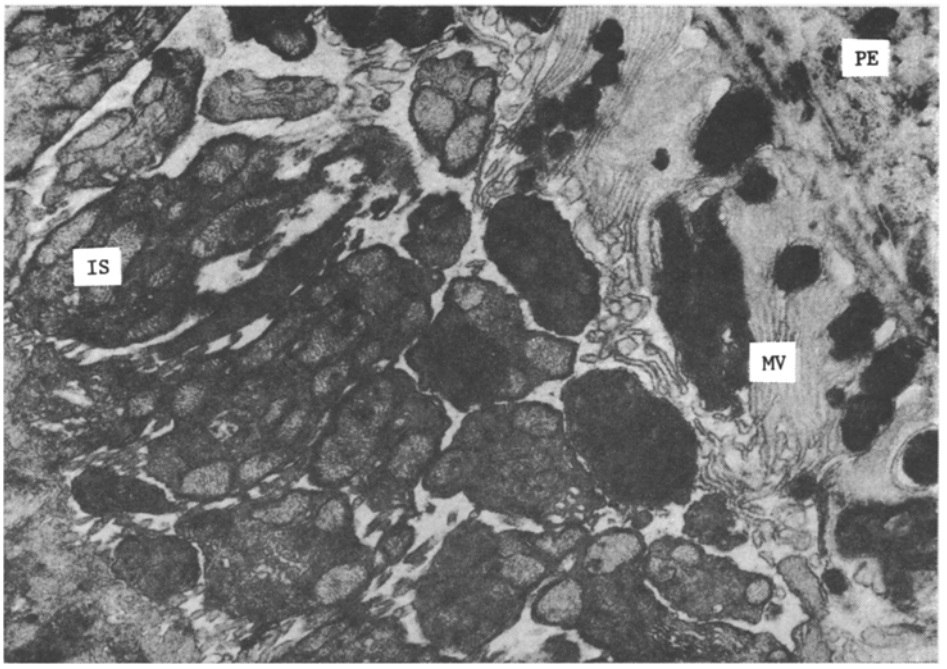

Fig. 1. Retina of rat receiving thioridazine for 15 months in a dose of 150 mg/kg. Outer segments of photoreceptors are absent. IS) Inner segment, MV) microvilli of pigmented epithelial cells, PE) pigmented epithelium.

EXPERIMENTAL METHOD

Altogether 45 Wistar albino rats weighing 180-200 g, 5 chinchilla rabbits weighing 2-2.5 kg, and 5 frogs were used. The chronic experiments were carried out on rats. The animals were divided into three groups: 1) animals receiving no drugs (control), 2) animals receiving thioridazine (Sonapax, from Polfa, Poland), 3) animals receiving thioridazine and dibunol (2,6-di-tert-butyl-4-methylphenol). Dibunol is an antioxidant which prevents the development of LPO. Each group consisted of 15 animals. The drugs were given to the animals perorally on alternate days for 15 months, dibunol in a dose of 20 mg/kg and thioridazine in a dose of 150 mg/kg. The electroretinogram (ERG) was recorded by the usual method, using flashes of light from an IFK-10 lamp. The electrophysiological investigations were carried out in the course of 11 months after the beginning of administration of the drug. Thioridazine in a concentration of 5 mM was given by intravitreal injection into the right eye of the rabbit, the left eye served as the control. The animals were killed 24 h after injection of the drug. For electron-microscopic investigations the rat's retina was removed 1, 2, 7, 8, 9, 11, and 15 months after the beginning of the experiment, the rabbit's retina after 24 h. The retina was fixed in 3% glutaraladehyde in 0.1 M phosphate buffer, pH 7.4, and postfixed in 1% OsO_4 solution in the same buffer. The tissue was embedded in the epoxide resin "Epon." Sections cut on the LKB-III ultramicrotome were examined in the JEM-100B electron microscope. Retinas from rabbits and frogs were used for the biochemical investigations. To induce LPO in the experiments in vitro, the following system was used: Fe^{++} ions in a concentration of 5 μM and ascorbic acid in a concentration of 0.5 mM. The course of the process was judged from accumulation of malonic dialdehyde (MDA) in the test system [1]. The protein concentration was determined by the microbiuret method [6].

EXPERIMENTAL RESULTS

The electron-microscopic investigation showed that membranous inclusions of lysosomal origin characteristic of this form of retinopathy appeared in the cytoplasm of the pigmented epithelial cells of rats receiving thioridazine for 11 months [2]. Only the initial stages of degeneration of OS were observed in the photoreceptor cells. However, 15 min after the beginning of thioridazine administration marked destruction of the photoreceptor cells was observed: OS were virtually completely absent (Fig. 1), whereas inner segments and pigmented epithelial cells were preserved. No morphological differences could be found between the groups of animals receiving thioridazine alone and a combination of thioridazine with dibunol.

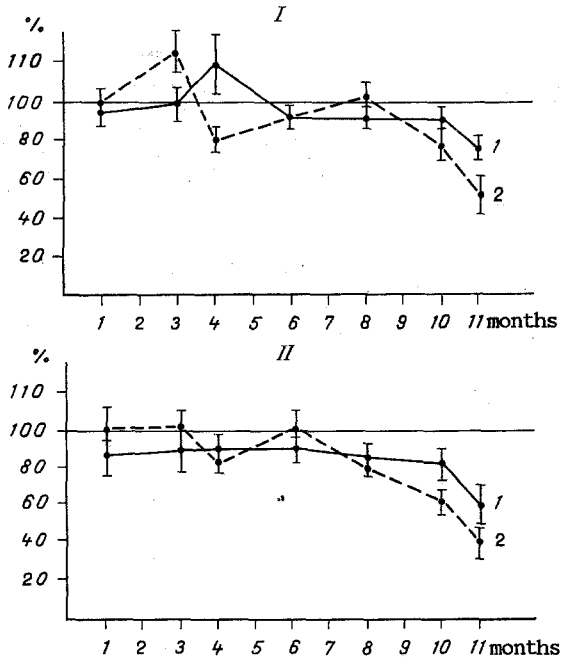

Fig. 2. ERG a (I) and b (II) waves of rats during chronic administration of thioridazine (1) in a dose of 150 mg/kg and thioridazine together with dibunol (2). Amplitude of a wave in control rats taken as 100%.

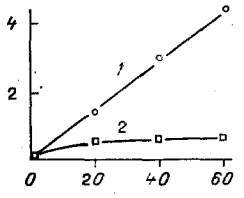

Fig. 3. Effect of thioridazine on LPO induced in rabbit retina by system of Fe^{++} (15 μM) ascorbic acid (0.5 mM). Abscissa, time (in min); ordinate, MDA concentration (in nmoles/mg protein). 1) Without thioridazine; 2) in presence of thioridazine (5 mM). Protein concentration in sample 1.7 mg/ml.

In rats receiving thioridazine for 10 months no disturbances of retinal function were observed, but by the 11th month a reduction in the amplitude of the b wave (to 60%) and, to a lesser degree, of the a wave (75%) took place compared with the control (Fig. 2: a, b). Administration of dibunol together with thioridazine aggravated the inhibitory action of this compound on retinal electrical activity (Fig. 2: a, b).

The results of the electron-microscopic and electrophysiological investigation show that dibunol had no protective action on the development of thioridazine retinopathy in the experimental animals. According to the results of the biochemical tests, in experiments on rabbits into whose vitreous body thioridazine (5 mM) was injected, no increase in MDA accumulation in the retinas of the experimental animals could be observed after 24 h compared with the control. In the experiments in vitro, to study the effect of thioridazine (in concentrations of 5 and 0.5 mM) on LPO induced by the Fe^{++} + ascorbic acid system revealed no prooxidant action of thioridazine. Addition of the preparation to a homogenate of retinas isolated from rabbits' and frogs' eyes led to a decrease in the rate of MDA accumulation, and the greater the concentration of thioridazine used, the more marked was this decrease. After injection of 5 mM thioridazine, virtually total inhibition of the LPO process was observed (Fig. 3), so that it can even be said that thioridazine has an antioxidant action in the systems studied.

To discover whether the specific binding of MDA with thioridazine takes place, appropriate experiments were carried out, and they showed that no such binding exists. The results of experiments to study the effect of thioridazine on LPO processes indicate that the drug cannot stimulate LPO, i.e., it is not a compound with pro-oxidant activity.

The absence of a protective action of the antioxidant dibunol against thioridazine retinopathy in rats, the absence of MDA accumulation in the rabbits' retinas, and inhibition of LPO in vitro by this drug are thus evidence that the damaging action of thioridazine on the retina is not based on LPO. Comparison of the effect of thioridazine and chloroquine on the retina revealed a similarity, expressed by their action on photoreceptor ultrastructure and the absence of LPO initiation in the retina. As a rule, attention is concentrated in the literature on differences in the action of these preparations on the retina. It is noted that chloroquine has a damaging action on ganglion cells and thioridazine on visual cells [8]. The similarity in the action of thioridazine and chloroquine which we found can probably be attributed to the fact that both these drugs belong to the same class of so-called amphoteric cation-active compounds [12].

Considering the continuous renewal of OS of the photoreceptors, in the basal part of which they are formed, and the shortening of the length of OS of the photoreceptors observed under the influence of thioridazine and chloroquine, it can be tentatively suggested that the damaging action of these drugs on the retina is based on a disturbance of the renewal of the photoreceptor discs. This problem requires further study.

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