QUENCHING OF CHEMILUMINESCENCE OF SINGLET OXYGEN IN THE NaClO + H_2O_2 REACTION IN THE PRESENCE OF ANTICATARACT AGENTS

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Among the causes of opacity of the lens, one of the foremost is intensification of singlet oxygen production [5, 8]. It may be formed as a result of photosensitization of psoralens, phenothiazines, and other drugs [3] or in the chemical reaction of H_2O_2 + NaClO [4]. There has been research into the study of quenching of singlet oxygen by preparations, some of which are regarded as promising for the treatment of cataract. This research has been undertaken with the use of systems for the photochemical production of singlet oxygen [7]. The screening of new drugs requires simple, economical and rapid methods of assessment of the quenching of singlet oxygen.

The writers showed previously that the ability of certain chemicals to extinguish singlet oxygen can be judged from chemiluminescence of the NaClO + H_2O_2 reaction [1]. This paper gives the results of a study of some known anticataract agents.

EXPERIMENTAL METHOD

Singlet oxygen was formed in the reaction of H_2O_2 + NaClO. Its production was monitored by measuring chemiluminescence recorded by means of a model 1251 chemiluminometer ("LKB-Wallach," Finland). The signal was recorded and then analyzed by computer (model M19, Olivetti, Italy). The technique was fully described in [1]. A number of drugs were investigated: Senkatalin (manufactured by the Indian firm of International) with technical aid from the Japanese firm "Takeda," Baineiting (produced by the Ukhan Pharmaceutical Combine, China), Quinax (from "Alcon," Belgium), Catachrom-OFTAN (made by "Star," Tampere, Findland), and Vita-Iodurol Triphosadenine (made by Laboratoires H. Faure, France). Amino acids of Soviet origin and of the analytically pure grade also were used. Utilization of the drugs in the presence of H_2O_2 and (or) NaClO was monitored spectrophotometrically relative to the characteristic absorption peaks of the compounds at $\lambda > 300$ nm, using a Hitachi 332 spectrophotometer (Japan).

EXPERIMENTAL RESULTS

A typical curve of chemiluminescence during interaction between H_2O_2 and NaClO is illustrated in Fig. 1. A flash of very weak luminescence lasted only a few seconds and was inhibited in the presence of typical quenchers of singlet oxygen, namely NaN_3 or histidine Conversely, ethanol, a scavenger of the HO*-radical, did not affect the kinetics of luminescence (Fig. 1).

The measurements showed that in the presence of Vita-Iodurol Triphosadenine, Catalin, and Baineiting the intensity of chemiluminescence of the chemical reaction described above decreased (Table 1). This effect for the last two preparations was described previously in [2]. However, a problem requiring separate discussion is that of the interaction of

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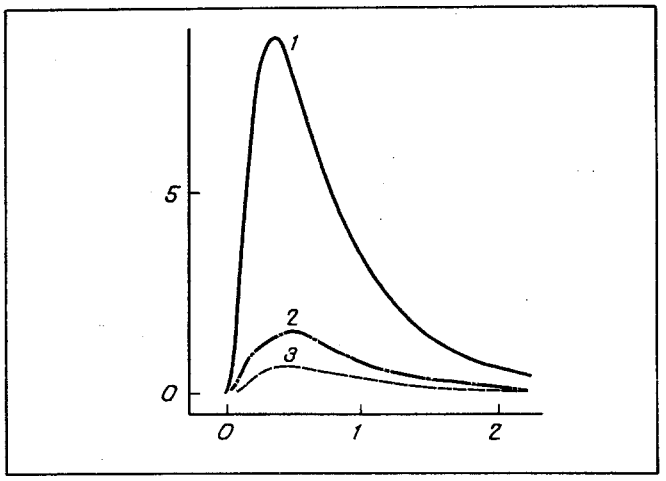

Fig. 1. Quenching of chemiluminescence of singlet oxygen in reaction of NaClO + H_2O_2 in the presence of different anticataract agents. 1) Control, 2) 5 mM histidine, 3) 5 mM NaN_3. Concentration of H_2O_2 1.5%, of NaClO 5 mM. Ordinate, intensity of chemiluminescence (in relative units); abscissa, time (in sec).

TABLE 1. Effect of Various Anticataract Agents on Chemiluminescence of the Reaction H_2O_2 + NaClO

Preparation	Dilution of original therapeutic form which inhibits chemiluminescence of reaction H_2O_2 + NaClO by 50%.
Vita-Iodurol Triphosadenine	1:228
Catalin	1:42
Baineiting	1:23
Catachrom-OFTAN	Only 49% is obtained at 1:20
Quinax	1:4

the preparations studied with either H_2O_2 or NaClO, which were present in the reaction mixture. Thus, the anticataract agent Vita-Iodurol Triphosadenine contains iodine ions, which react immediately with H_2O_2, so that it was impossible to estimate by our method the quenching of singlet oxygen. Catachrom-OFTAN also is very quickly broken down in the presence of hydrogen peroxide: during incubation for 10 min in 1.5% H_2O_2 the optical density of this preparation, measured at 410 nm, was reduced by 90%. This drug also is unstable during the reaction with NaClO: during incubation for 3 h with 5 mM sodium hypochlorite the optical density of the preparation decreased by 40%. Another preparation, namely Quinax, is relatively stable in a 1.5% solution of H_2O_2, but loses its color virtually instantly on mixing with NaClO. Senkatalin and Baineiting also are relatively stable in H_2O_2 and also in NaClO. The decrease in optical density of these preparations at 436 and 440 nm respectively in a solution of 1.5% H_2O_2 during incubation for 20 min did not exceed 10% of the initial value. Senkatalin, which decomposes in H_2O_2, forms products under these circumstances which do not affect chemiluminescence as effectively as the original therapeutic form. Hypochlorite had no appreciable action on either preparation during incubation for 20 min.

The remaining anticataract agents studied possess the property of inhibiting the development of very weak luminescence in the chemical reaction described above, possibly due to their interaction with H_2O_2 and (or) with NaClO Interaction of a number of anticataract agents with H_2O_2 and (or) with the hypochlorite ion, which we found, may be interesting for the pharmacotherapy of cataract. We know, for instance, that age-related opacity of the lens is accompanied by an increase in the H_2O_2 concentration in the aqueous humor — the fluid bathing the lens on all sides [6]. On the other hand, in a previous study [1] we found myeloperoxidase in the lens of healthy subjects and patients with cataract. The possibility

therefore cannot be ruled out that both H_2O_2 and NaClO can modify the anticataract agents as they penetrate into the anterior chamber of the eye and the various layers of the lens.

The use of the technique of screening quenchers of singlet oxygen described above is therefore suitable only for preparations which do not react with H_2O_2 or with hypochlorite. The list of such preparations includes Senkatalin and Baineiting. A different plan of investigation is needed for the other preparations.

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EFFECT OF PROTAMINE ON BLOOD LIPOPROTEINS IN A MODEL OF ATHEROSCLEROSIS

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A high content of protamines, which are proteins with marked alkaline properties, is found in male generative cells, where they replace the histones of chromatin during maturation of spermatozoa. As a pharmacological preparation, protamines isolated from fish sperm are wisely used in clinical practice for the treatment of bleeding. It has been suggested that alkaline proteins, including protamines, can prevent the development of atherosclerosis [6, 8]. However, the effect of protamine in models o the early stages of atherosclerosis in studies of the protein—lipid composition of lipoproteins, has been found to be ambivalent. The changes found have been regarded by some workers as promoting, but by others as preventing atherogenesis [4, 5].

The aim of this investigation was to compare the action of protamine on quantitative changes in lipoproteins in the early and late periods of hypercholesterolemia (HChE).

EXPERIMENTAL METHOD

The experimental animals were 40 rabbits weighing about 3 kg, divided into six groups. Animals of group 1 received cholesterol in their diet daily for 1 month in a dose of 0.5 g/kg body weight. Animals of group 2, besides cholesterol, received a 1% solution of protamine (10 mg/kg) by intraperitoneal injection. Animals of group 3 received cholesterol for

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