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Influence of various compounds on the degradation of hyaluronic acid by a myeloperoxidase system

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Abstract

Myeloperoxidase in the presence of 0.7 mM hydrogen peroxide degrades hyaluronic by a mechanism which involves iron. Degradation is enhanced in the presence of chloride ion, which is attributed to the formation of hypochlorous acid. Myeloperoxidase-dependent degradation of hyaluronic acid is inhibited by superoxide dismutase, desferrioxamine, iodide ion, bromide ion, mannitol, histidine and various antiinflammatory agents. The destructing agent is presumably the hydroxyl radical.

Key words: Myeloperoxidase; Free radicals; Iron; Hypochlorous acid; Anti-inflammatory agents

1. Introduction

Many factors have been recognized which activate the polymorphonuclear leucocytes (PMN) to produce tissue damage [1,2]. Perturbation of the PMN membrane which starts the oxidative metabolism of the cells, i.e., the respiratory burst, is essential for this activation [3]. The respiratory burst is associated with the release and further generation of the highly toxic and reactive oxygen-containing species H_2O_2, $O_2^{\cdot -}$, $^1O_2$, $OH^{\cdot}$ and HOCl into the phagosome with the ingested foreign material as well as into the extracellular space [4–9]. During phagocytosis some

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myeloperoxidase (MPO) is also released from the PMN into the extracellular space where the enzyme mediates the iodination of plasma proteins [10].

Myeloperoxidase, H_2O_2 and a halide form a potent antimicrobial and cytotoxic system. It is suggested that release of MPO and H_2O_2 from polymorphonuclear leucocytes results in a peroxidase-mediated leucocytic injury which may be an important feature of the inflammatory response [11]. The release of radicals and enzymes into the surrounding environment by phagocytic cells is a potential cause of damage to the tissues and chronic inflammatory disease results when this damage gets out of hand. The destructive effects depend on the formation of hydroxyl radicals [12].

A complex of hydrogen peroxide with MPO has been described [13] which has been classified as peroxidase Compound II [14]. On addition of increasing concentrations of H_2O_2 to MPO it has been shown that three compounds are formed: compounds I, II and III. When using H_2O_2 concentrations below 0.5 mM, Compound II is formed, which upon further addition of H_2O_2 is convened to Compound III [15]. Compound III is also formed on addition of $O_2^{\cdot-}$ to MPO [16]. Evidence has been presented that superoxide anion radicals are generated from hydrogen peroxide in the presence of MPO and that it is conceivable that $O_2^{\cdot-}$ is generated in the reduction of Compound I to Compound II as well as in the reduction of Compound II to the native enzyme [17].

Studies on the formation of hydroxyl radicals have been performed using the superoxide-forming enzyme xanthine oxidase. The substrates used were methional [18] as well as deoxyribose, benzoate or α-keto-γ-methiolbutyric acid [19]. In the latter cases iron was used as a catalyst. Hyaluronic acid has also been used in studies on the formation of $OH^{\cdot}$ by xanthine oxidase and the decrease in the viscosity of a solution of the acid was related to the extent of formation of hydroxyl radicals [20].

In the present study, hyaluronic acid has been used as substrate. The production of $OH^{\cdot}$ by MPO in a system containing high concentrations of H_2O_2 and NaCl and with iron as a catalyst has been investigated. In addition, the influence of various compounds on the formation of the hydroxyl radicals as well as their effect on the radicals themselves has been studied.

2. Experimental

2.1. Materials

Myeloperoxidase (EC 1.11.1.7) was isolated from granulocytes from healthy donors [21] and had a UV-absorption ratio A_{430}/A_{280} of 0.82. Superoxide dismutase was obtained from Sigma Chemical Co., St. Louis, USA.

The potassium salt of hyaluronic acid, which had been isolated from human umbilical cord, was purchased from Sigma Chemical Co. Analysis of Cu and Fe in a solution of 1.5 mg hyaluronic acid per ml in 0.1 M phosphate buffer by means of atomic absorption spectroscopy showed a content of 0.07 μg Cu/ml and 0.35 μg Fe/ml. The buffer contains 0.08 and 0.09 μg/ml of Cu and Fe, respectively. A solution of 1.6 M NaCl in buffer had a content of 0.35 μg Fe/ml analysed by use of polarography with a hanging mercury drop electrode. Analysis of Cl^- by potentiometric titration with silver nitrate showed that the hyaluronic acid solution contains 80 μM and the buffer < 3 μM Cl^-.

Desferrioxamine was obtained from Ciba-Geigy, Switzerland, mannitol, reinst, from Merck Schuchard, Germany, 6-chloro-3-hydrazinopyridazine (ABC 907) from Maybridge Chemical Co. Ltd., UK, α-histidine, *p*-hydroxybenzoic acid and *p*-hydroxyacetanilide (Paracetamol) from Fluka, Buchs, Switzerland. Salicylic acid, acetylsalicylic acid and diethylaminoaceto-2,6-xylidide (Lidocaine) were provided by AB Astra, Sweden. 2-(4-Isobutylphenyl) propionic acid (Ibuprofen) and 4-butyl-1, 2-diphenyl-3,5-pyrazolidinedione (Phenylbutazone) were purchased from Sigma Chemical Co., USA.

2.2. Methods

A mixture of 1375 μl 0.1 M phosphate buffer, pH 6.9, (compounds studied were dissolved in the buffer), 2000 μl of a freshly prepared solution of hyaluronic acid in buffer (1.5 mg/ml), 500 μl of a 1.6-M solution of NaCl in buffer and 25 μl of a 6.9 μM solution of MPO is thoroughly shaken. The reaction is started by adding 100 μl of a 0.028-M solution of H_2O_2 in buffer. 3.0 ml of this mixture are immediately transferred to an Ostwald viscosimeter with a outflow time of 28.4 s for the phosphate buffer. The outflow time of the mixture was measured 1,3,5,7,9 and 15 min after the addition of the H_2O_2 solution. All samples were maintained at 25° in a thermostat. The specific viscosity was calculated according to the formula

$$\eta_{sp} = \frac{\text{outflow time reaction solution}}{\text{outflow time solvent}} - 1 \text{ [22].}$$

The values given in figures and tables are $1/\eta_{sp}$ at various times after the addition of H_2O_2 from which the $1/\eta_{sp}$ value for the substrate is subtracted.

3. Results

Addition of MPO, NaCl and H_2O_2 to a solution of hyaluronic acid reduces the viscosity of this solution to a high degree. Omission of the enzyme as well as using a boiled solution of the enzyme results in a considerable decrease in the degradative effect. When NaCl is omitted, the degradative effect is lowered to a certain degree (Fig. 1). On omission of H_2O_2 from the system, no change in the viscosity could be observed.

Replacement of NaCl (200 mM) with KI or KBr at a concentration above 2.0 mM inhibits the degradative effect almost completely. Even in the presence of NaCl, these two halides inhibited the degradative effect. As can be seen in Table 1, iodide has a strong inhibitory effect at a concentration of 5.0 μM. Almost complete inhibition of the degradative effect was observed at concentration of 50.0 μM. Bromide ion does not show such a pronounced effect. At a concentration of 200.0 μM, the extent of degradation is still 70%. Almost complete inhibition occurred at a concentration of 2.0 mM when 200 mM NaCl was present. It was observed that the reaction mixture became coloured when iodide was added indicating liberation of I_2 during the process.

As degradation of hyaluronic acid by the superoxide anion-generating enzyme, xanthine oxidase, is inhibited by the $OH^{\cdot}$-scavenger mannitol, superoxide dismutase, or the iron-complexing compound desferrioxamine [20,23–25], the influ-

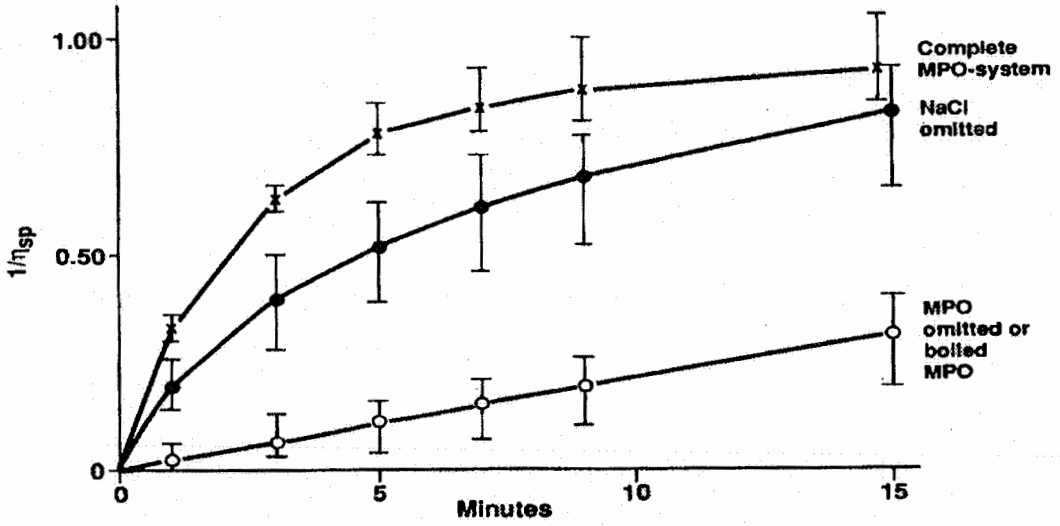

Fig. 1. The degradative effect on hyaluronic acid by the MPO-system and the influence of MPO and NaCl on this system. The mean values and ranges of six separate determinations are given.

ence of these compounds on the MPO-system was studied. As can be seen in Fig. 2, a concentration of 2.0 mM mannitol inhibits the degradative effect of the MPO-system by about 50%. At a concentration of 200.0 mM the inhibition is complete. In Fig. 3, the effect of superoxide dismutase and desferrioxamine is demonstrated. When 10.0 μg superoxide dismutase was added, there was almost complete inhibition of the degradative effect by the complete MPO-system. Similar results were obtained when 0.2 mM desferrioxamine was added. This chelator eliminates the contaminating iron which is present at a concentration of 4.5 μM in the reaction mixture [26]. The low degradative activity seen when the enzyme was omitted from the reac-

Table 1
The viscosity ($1/\eta_{sp}$) of a solution of hyaluronic acid after degradation by a MPO-system containing various concentrations of KI and KBr

Conc. μM	KI		KBr	
	$1/\eta_{sp}$	%	$1/\eta_{sp}$	%
0.0	0.62 (0.62,0.62)	100	0.71 (0.09,0.72)	100
5.0	0.17 (0.14,0.19)	27	0.65 (0.59,0.71)	92
10.0	0.09 (0.08,0.10)	15	0.61 (0.58,0.63)	86
50.0	0.05 (0.04,0.06)	8	0.56 (0.54,0.57)	79
200.0	0.02 (0.01,0.02)	3	0.50 (0.48,0.51)	71

Reaction times in minutes. Mean values and range of duplicate experiments. The ($1/\eta_{sp}$) seen after addition of the compound in percent of the control is also given.

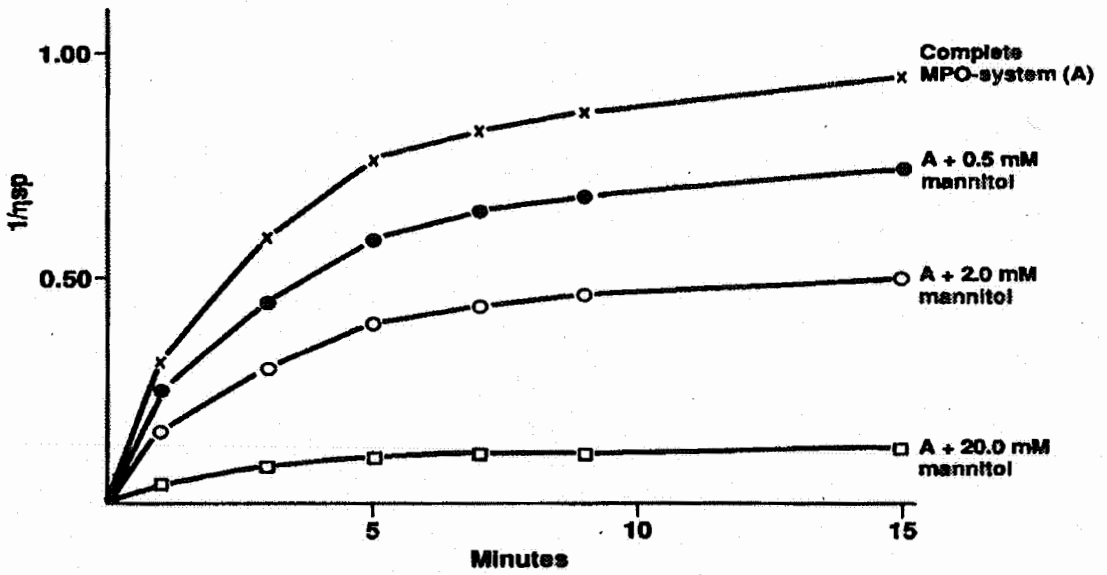

Fig. 2. Influence of mannitol on the degradation of hyaluronic acid by the MPO-system. The mean values of two separate determinations are given.

tion mixture, was almost complete inhibited on addition of either superoxide dismutase or desferrioxamine (Fig. 3).

The results from the studies on the influence of various compounds on the degradation of hyaluronic acid by the use of the MPO-system show that one group of these agents for the most part acts in the same manner as mannitol. In Table 2 it is shown that these compounds inhibit the viscosity-decreasing effect of the MPO-system in the order paracetamol > lidocaine > salicylic acid > *p*-hydroxybenzoic acid > ibuprofen > acetylsalicylic acid. A difference was seen in the inhibitory pattern at low concentrations of paracetamol and *p*-hydroxybenzoic acid compared to the other compounds. These two compounds with a hydroxyl group in the *para*-position have initially a low inhibitory effect on the degradation of hyaluronic acid, but an increase of the inhibition could be seen during the following minutes. However, at higher concentrations of these two compounds, the degree of inhibition remained constant during the experimental period as for the other compounds in this group.

Another group of compounds — histidine, phenylbutazone and ABC 907 — inhibit the degradation of hyaluronic acid by the MPO-system in quite a different manner. When these compounds were added to the system, a high degree of inhibition was initially observed, but was followed by a decrease in inhibition during the following minutes. As can be seen in Table 3, the increase in the degradation at a certain concentration of the compounds is the same as that seen when the enzyme is omitted from the reaction mixture. The results show also that ABC 907 initially

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