

ROZALIA GRABOWSKA-BOCHENEK, MARIAN DRÓZDŹ, EWA KOPIECZNA-GRZEBIENIAK and MAREK PIEKARSKI

**CONCENTRATION OF SOME PROTEINASE INHIBITORS:
 α_1 -ANTITRYPSIN AND α_2 -MACROGLOBULIN IN RABBIT BLOOD
SERUM IN TWO MODELS OF EXPERIMENTAL ATHEROSCLEROSIS**

*Chair and Department of Biochemistry and Chemistry, Silesian Medical Academy
Medyków 14; 40-752 Katowice, Poland*

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In rabbits with experimental atherosclerosis induced by a cholesterol-rich diet, α_1 -antitrypsin concentration was decreased as compared with control, by 34%, whereas α_2 -macroglobulin concentration was increased by 86%. In animals fed a methionine-rich diet changes in concentration of both inhibitors involved in elastase metabolism were but slight, if any.

Degradation of elastic fibres in the walls of blood vessels is an early and consistently observed event in atherosclerosis [1]. Increased degradation of elastin is due to increased elastinolytic activity (in preparation). Among numerous inhibitors of proteolytic enzymes circulating in the blood α_1 -antitrypsin, present at the highest concentration, seems to play the most important role in elastin metabolism (in preparation); α_2 -macroglobulin is also significantly involved in these processes [3].

The aim of the present work was to check whether changes in elastinolytic activity observed in rabbits (in preparation) are dependent on changes in concentration of inhibitors of proteolytic enzymes. Experimental atherosclerosis was induced by feeding the animals either a cholesterol-rich or methionine-rich diet. The former diet is a well known atherogenic factor [4], whereas the effect of methionine, which is metabolised to homocysteine [5], has not been extensively studied.

MATERIALS AND METHODS

For experiments, male rabbits of a New Zealand strain obtained from the Central Breeding Station of Laboratory Animals, Silesian Medical Academy,

were used. The animals were divided into three groups (5 animals each): the control group was fed the standard rabbit diet; the methionine-treated group received in addition DL-methionine (0.3% of the amount of food) [5], and the cholesterol-treated group, egg paste in the amount calculated to give 0.5% cholesterol in the diet.

Serum was obtained from the blood withdrawn from the ear vein, and α_1 -antitrypsin and α_2 -macroglobulin concentration was determined by the colorimetric method using a reagent kit supplied by Boehringer Mannheim.

RESULTS

In rabbits fed a cholesterol-rich diet a statistically significant lowering of α_1 -antitrypsin concentration (119 ± 33 mg/l) was observed together with a highly statistically significant increase, by 86%, of α_2 -macroglobulin concentration (235 ± 56 mg/l) as compared with the control group (α_1 -antitrypsin, 180 ± 38 mg/l; α_2 -macroglobulin, 126 ± 38 mg/l). In animals fed a methionine-rich diet, changes in concentration of these inhibitors were slight and statistically insignificant (α_1 -antitrypsin, 182 ± 41 mg/l; α_2 -macroglobulin 145 ± 10 mg/l).

DISCUSSION

In a group of rabbits kept on a cholesterol-rich diet we have observed previously increased activity of leukocyte elastase and total elastolytic activity in blood serum and in aorta (in preparation). In the present study we have found, in addition, a significantly decreased concentration of α_1 -antitrypsin, which is the main elastase inhibitor in serum. Thus, the increased elastase activity in rabbits kept on a cholesterol-rich diet could be due to disturbed equilibrium between inhibitor and enzyme. Lowered concentration of α_1 -antitrypsin could be caused by its proteolytic degradation, by inactivation with oxidizing agents, or by its decreased biosynthesis. Proteolysis of this inhibitor can result from the action of bacterial metalloproteinases or of cathepsin B liberated during phagocytosis [6]. Susceptibility of α_1 -antitrypsin to the action of oxidizing agents is related to the presence of methionine in its active centre [3]. Atherosclerosis is considered a "free-radical disease" [7]. The free radical and autooxidative theory of atherosclerosis assumes that the processes in which free radicals are involved become disturbed while the protective mechanisms of the organism remain inadequate and unable to restore the equilibrium.

α_1 -Antitrypsin is synthesized in liver, which showed histopathological changes in rabbits fed a cholesterol-rich diet [8]; thus, in this group of animals, lowered concentration of this inhibitor could be due to its decreased synthesis.

The increased concentration of α_2 -macroglobulin in cholesterol-fed rabbits could possibly result from decreased removal of the inhibitor-proteinase complexes as a result of liver damage and/or increased α_2 -macroglobulin synthesis. It seems that macrophages may also contribute to synthesis of this inhibitor [9].

In rabbits fed a methionine-rich diet elastin underwent enhanced degradation (in preparation). However, in this group of animals, concentration of both inhibitors studied was either unchanged (α_1 -antitrypsin) or changed but slightly (α_2 -macroglobulin). In liver of those animals changes of an inflammatory character were observed, whereas in the cholesterol-fed rabbits the liver showed signs of fatty degeneration and cirrhosis [8]. Thus it seems that increased degradation of elastin in methionine-fed animals observed previously was not due to changes in concentration of the inhibitors studied.

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