CONCENTRATION OF SOME PROTEINASE INHIBITORS: 
$\alpha_1$-ANTITRYPsin AND $\alpha_2$-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

Presented at 25th Meeting of the Polish Biochemical Society; September, 1989

In rabbits with experimental atherosclerosis induced by a cholesterol-rich diet, 
$\alpha_1$-antitrypsin concentration was decreased as compared with control, by 34%, whereas 
$\alpha_2$-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 
$\alpha_1$-antitrypsin, present at the highest concentration, seems to play the most 
important role in elastin metabolism (in preparation); $\alpha_2$-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 
centration 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Serum was obtained from the blood withdrawn from the ear vein, and $\alpha_1$-antitrypsin and $\alpha_2$-macroglobulin concentration was determined by the colorimetric method using a reagent kit supplied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 $\alpha_1$-antitrypsin concentration (119 ± 33 mg/l) was observed together with a highly statistically significant increase, by 86%, of $\alpha_2$-macroglobulin concentration (235 ± 56 mg/l) as compared with the control group ($\alpha_1$-antitrypsin, 180 ± 38 mg/l; $\alpha_2$-macroglobulin, 126 ± 38 mg/l). In animals fed a methionine-rich diet, changes in concentration of these inhibitors were slight and statistically insignificant ($\alpha_1$-antitrypsin, 182 ± 41 mg/l; $\alpha_2$-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 elastinolytic activity in blood serum and in aorta (in preparation). In the present study we have found, in addition, a significantly decreased concentration of $\alpha_1$-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 $\alpha_1$-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 $\alpha_1$-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 antioxidative 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.

$\alpha_1$-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 $\alpha_2$-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 $\alpha_2$-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 ($\alpha_1$-antitrypsin) or changed but slightly ($\alpha_2$-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.

REFERENCES