SHORT COMMUNICATION

EFFECT OF LOW-DENSITY LIPOPROTEIN PREPARATIONS ON PLASMIN

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SUMMARY

1. Crude preparations of β-lipoprotein inhibited the fibrinolytic and caseinolytic activity of plasmin: they were contaminated by α₂-macroglobulin and α₁-antitrypsin, known inhibitors of plasmin.

2. Purified β-lipoprotein, free from α₂-macroglobulin and α₁-antitrypsin, did not inhibit plasmin. It is concluded that β-lipoprotein does not have antiplasmin properties.

Key words: plasmin, β-lipoprotein, plasmin inhibitors.

Human plasma contains a number of inhibitors of the proteolytic enzyme plasmin including α₂-macroglobulin, α₁-antitrypsin (Ganrot, 1967; Schwick, Heimburger & Haupt, 1967) and Cl inactivator (Ratnoff, Pensky, Ogston & Naff, 1969).

It has been reported that preparations of β-lipoprotein (low-density lipoprotein) inhibit plasmin (Riding & Ellis, 1964; Skrzydlewski, Niewiarowski & Skrzydlewska, 1966), and Sarkar (1961) concluded that low-density lipoprotein contributed to the decreased fibrinolytic activity he noted in the circulating blood of atherosclerotic subjects. We have re-examined the inhibition of plasmin by β-lipoprotein preparations and conclude that β-lipoprotein itself does not function as an antiplasmin.

METHODS

Preparation of crude β-lipoprotein

Blood was obtained from healthy donors and allowed to clot at room temperature for 2 h; the serum was separated by centrifugation at 1500 g for 5 min. Crude β-lipoprotein was separated from the serum by the method of Burstein & Samaille (1958): 1/200 vol. of dextran sulphate (10 g/100 ml) and 1/20 vol. of CaCl₂ (1 mol/l) were added to fresh serum and after 15 min the resulting precipitate was separated by centrifugation at 6000 g for 10 min and suspended in sodium chloride (0·16 mol/l) to the original serum volume or dissolved in sodium bicarbonate.

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ate (0·12 mol/l). Immunoelectrophoretic studies indicated that these crude preparations contained at least five different proteins.

**Preparation of purified β-lipoprotein**

A mixture of low- and very-low-density lipoprotein was isolated from human serum by the method of Burstein, Scholnick & Morfin (1970) using precipitation by dextran sulphate and MgCl₂, dissolution of the precipitate in sodium bicarbonate (0·12 mol/l) and repeated precipitation by MgCl₂ (final conc. 0·1 mol/l). The final washed precipitate was dialysed against Tris–saline buffer (pH 7·7), then against BaCl₂ (0·005 mol/l) in NaCl (0·17 mol/l), the precipitate was removed by centrifugation and finally dialysed against Tris–saline buffer. α₂-Macroglobulin, α₁-antitrypsin and Cl inactivator were absent from these purified preparations when they were examined by double diffusion in agar gels against antisera specific for these proteins. The preparations were not, however, free of contaminant proteins; immunoelectrophoresis against anti-human serum indicated the presence of two proteins other than β-lipoprotein. The concentration of β-lipoprotein, as assayed by radial immunodiffusion, varied from 10 to 20 times that observed in whole serum; the highest concentration of β-lipoprotein in the purified preparations was 48 mg/ml.

**α₂-Macroglobulin**

This was prepared by the method of Harpel (1970) from human plasma obtained from healthy volunteers.

**Measurement of plasmin inhibition**

Plasmin (0·2 ml; Kabi, Stockholm), diluted 1 in 10 in borate–saline buffer (pH 7·4), was incubated with 0·1 ml of β-lipoprotein, or with α₂-macroglobulin (5–50 mg/100 ml) or, as controls, with similar concentrations of albumin and 0·4 ml of borate–saline buffer for 10 min at 25°C. After the addition of 1·5 ml of casein (2 g/100 ml) in borate–saline buffer, incubation at 37°C was started. At 2 min and 3 h, aliquots were mixed with an equal volume of trichloroacetic acid (10 g/100 ml). The acid-soluble, tyrosine-like material liberated was measured with the Folin–Ciocalteu phenol reagent.

The inhibition on plasmin activity of fibrin plates was determined by applying 30 μl aliquots of mixtures of plasmin and β-lipoprotein or albumin, which had been incubated together for 10 min at 25°C, to fibrin plates prepared from human fibrinogen (Grade L, AB Kabi, Stockholm 200 mg/100 ml). After incubation at 37°C for 24 h the area of lysis was estimated from the product of two diameters at right angles to each other. Dilutions of a standard plasmin preparation, similarly incubated, were also applied to the fibrin plates and the areas of lysis produced plotted; the residual activity in the presence of β-lipoprotein was obtained by extrapolation.

**Assay of α₂-macroglobulin, β-lipoprotein, Cl inactivator and antithrombin III**

Each was performed by single radial immunodiffusion (Mancini, Carbonara & Heremans, 1965). Antisera to α₁-antitrypsin, α₂-macroglobulin, Cl inactivator and β-lipoprotein were obtained from Hoechst Behringwerke A.G. Antiserum to antithrombin III was obtained from Nyegaard and Co. A.S., Oslo.

β-Lipoprotein was also estimated by the method of Walton & Scott (1964) using reagents obtained from BDH Chemicals Ltd, Poole.
RESULTS

Preparations of crude β-lipoprotein were capable of inhibiting the caseinolytic activity of purified plasmin (Table 1); two further preparations inhibited 71% and 100% of the fibrinolytic activity of plasmin on fibrin plates. The preparations were not homogeneous and at least five separate proteins were demonstrable on immunoelectrophoresis against anti-normal human serum. On double diffusion against specific antisera the concentrates contained no Cl inactivator, but α₂-macroglobulin and α₁-antitrypsin were present in quantities detectable by the specific antisera. For example, using single radial immunodiffusion for quantitation, four crude β-lipoprotein concentrates contained 7–20 mg of α₂-macroglobulin/100 ml. The β-lipoprotein content of these concentrates was 8–23 mg/ml. They inhibited 41–69% of the caseinolytic activity of a standard plasmin preparation: the amount of inhibition was not, however, related to the β-lipoprotein concentration. When plasmin inhibition was compared with the α₂-macroglobulin concentration and the known antiplasmin activity of purified α₂-macroglobulin it was found that this agent could account for approximately half the inhibiting activity of each concentrate.

More highly purified β-lipoprotein at a concentration of 24 mg/ml showed no antiplasmin activity in a caseinolytic assay in contrast to the crude concentrates (Table 1) and also failed to inhibit the activity of plasmin on fibrin plates. These purified preparations were free of immunologically detectable quantities of the major antiplasmins contaminating the crude concentrates (α₂-macroglobulin and α₁-antitrypsin).

One of the proteins present in both crude and purified preparations of β-lipoprotein was that detected by antiserum to antithrombin III. A purified preparation, which contained β-lipoprotein in a concentration approximately 20 times that normally found in whole serum, was contaminated with antithrombin III in a concentration 0-6 times that in whole serum.

DISCUSSION

The principal antiplasmins in human plasma are α₂-macroglobulin and α₁-antitrypsin (Ganrot, 1967; Schwick et al., 1967). Cl inactivator has also been identified as a potential inhibitor of
plasmin (Ratnoff et al., 1969). The present study does not support previous claims that β-lipoprotein acts as a further plasma antiplasmin, since our purified preparation of β-lipoprotein failed to inhibit the caseinolytic and fibrinolytic activity of plasmin. The crude preparations of β-lipoprotein, which, as reported by other investigators (Riding & Ellis, 1964; Skrzydleowski et al., 1966), are capable of inhibiting plasmin, were found to contain both α₂-macroglobulin and α₁-antitrypsin. The α₂-macroglobulin alone in the crude β-lipoprotein preparations could account for half of the inhibitory activity, as based on experiments with purified α₂-macroglobulin and plasmin.

Both crude and purified preparations of β-lipoprotein also contained small quantities of antithrombin III. It has been suggested that this material may have antiplasmin activity (Schwick et al., 1967). Despite the presence of this agent in the purer β-lipoprotein preparations, however, no antiplasmin activity was detectable.

We conclude that β-lipoprotein should not be considered a natural inhibitor of plasmin.

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REFERENCES


