Comparison of the inhibitory activity and concentration of alfa$_1$-antiprotease in normal human sera[1]

B. Miszczuk-Jamska and W. Pajdak

Department of Clinical Chemistry, Medical Academy Cracow, Kopernika 17, 31-501 Cracow (Poland), 4 April 1978

Summary. Inhibitory activity and concentration of alfa$_1$-antiprotease were simultaneously determined in sera of 80 blood donors. The lack of the coincidence of these 2 parameters was observed in about half the subjects tested.

The serum glycoprotein alfa$_1$-antiprotease (α_1-AT) is a well-known polyspecific inhibitor of proteolytic enzymes such as: elastase, neutral leukocytic protease, plasmin, kallikrein[2,3]. 70–90% antiprotease activity of human serum alfa$_1$-antiprotease is believed to combine with proteolytic enzymes in one-to-one proportion[4–7]. However, Crawford[8], Johnson et al.[9] and Kress et al.[10] found that alfa$_1$-antiprotease binds 2 particles of trypsin. α_1-AT-protease complex is inactive toward natural and synthetic substrates. It is most probable that the active centre of the enzyme is blocked by the inhibitor. The immunological properties of such a complex remain unchanged. The close coincidence of α_1-AT deficiency and the incidence of pulmonary emphysema were found by Laurell and Eriksson[11]. This stimulated very extensive studies on alfa$_1$-antiprotease also in liver diseases[12–15], rheumatoid arthritis[16], duodenal and gastric diseases[17]. However, in most of these papers the authors determined either the concentration of α_1-AT (as protein)[15–17], or its inhibitory activity[9]. The aim of this study was to verify if these 2 parameters are sufficiently parallel to be determined alternatively, in healthy people.

Materials and methods. This study was carried out on 80 healthy blood donors. The serum alfa$_1$-antiprotease concentration was determined, using the radial immunodiffusion method of Mancini[18] with Partigen immunodiffusion plates (Behringwerke). Inhibitory (antitrypsin) activity was determined fluorometrically using N-α-benzoyl-DL-arginine-β-naphthylamide. HCL (BANA, Koch-Light) according to a slightly modified method of Reinharz et al.[19]. Antitrypsin activity of serum was expressed as the amount of trypsin inhibited by 1 ml of serum. The trypsin preparation was standardized by soybean trypsin inhibitor and the factor calculated to be 0.86. It was included in the final calculations.

Results and discussion. The mean alfa$_1$-AT concentration in sera of 80 blood donors was 189.1±35.5 mg/100 ml. The lowest value was 105 mg/100 ml, the highest 255 mg/100 ml. The mean serum trypsin inhibitory capacity was 1.02±0.2 units/ml. The values for these 2 parameters are plotted on the graph.

Data show that in about half of the tested blood donors the α_1-AT concentration was not in agreement with serum trypsin inhibitory capacity. This discrepancy may result from the fact that α_1-AT concentration (determined immunologically) comprises both free and bound alfa$_1$-antitrypsin. It might be consistent with the findings of Woolcock et al.[20]. They found veno-arterial differences of protease inhibitory activity, but the concentration of α_1-AT was the same in venous and arterial blood. They suggested that this is connected with binding of alfa$_1$-antitrypsin by lung proteases. It should be also kept in mind that α_1-AT is the main, but not the only, protease inhibitor in serum. In addition, at least in pathological conditions, α_1-AT produced may partially fail to react as protease inhibitor even in healthy people, as can be seen from the results. The situation is therefore not quite clear. In conclusion, we think that simultaneous determination of serum α_1-AT concentration and inhibitory activity is more informative and adequate.

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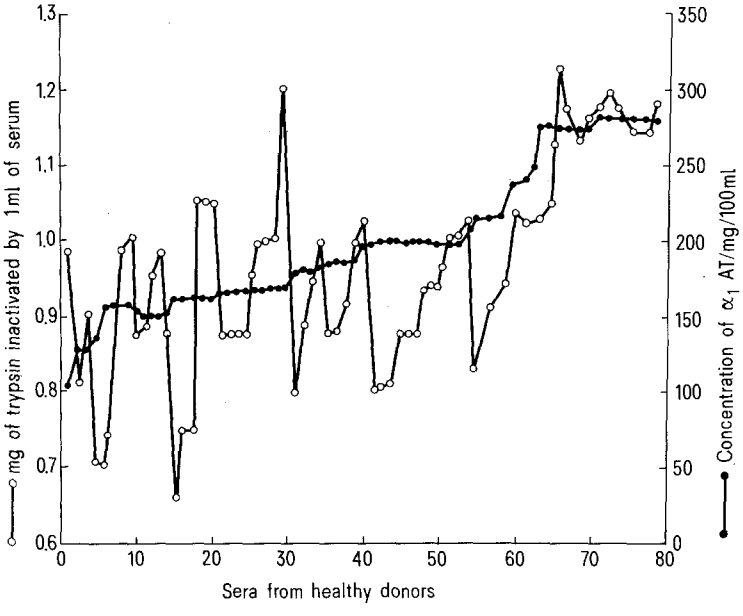

Comparison of serum alfa$_1$-antitrypsin concentration and its inhibitory activity in 80 healthy blood donors.

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Effect of acetylsalicylate on surgical bleeding, postoperative mortality and allograft survival in rats undergoing heart transplantation[1]

I. Reyers, G. Di Minno[2], M.B. Donati and G. de Gaetano

Laboratories of Cardiovascular Clinical Pharmacology and Haemostasis and Thrombosis Research, Istituto di Ricerche Farmacologiche 'Mario Negri', via Eritrea 62, I-20157 Milan (Italy), 17 April 1978

Summary. 18 rats were treated with L-ASA before heart transplantation and daily thereafter until death or rejection. 22 animals acted as controls. A significantly higher post-operative mortality rate, without any significant modification of the transplant survival time, was found in L-ASA-treated group.

It has been suggested that blood platelets may play an important role in rejection of transplanted organs[3–11]. For this reason, several drugs inhibiting platelet function have been associated with conventional treatment in experimental and human transplantation[4,5,7,10,11]. In a previous study[12], we observed that pretreatment of rats with lysine acetylsalicylate (L-ASA) resulted in a significant increase of peroperative mortality following binephrectomy and kidney transplantation. The effect of this drug on the survival of transplanted kidneys could not be evaluated, since the animals were only treated before surgery.

The purpose of the present study was to investigate the effect of L-ASA pretreatment on peroperative mortality of rats receiving heart transplants, this surgical operation being simpler than kidney transplantation. In addition, animals were treated daily until rejection occurred, in order to assess whether L-ASA had any beneficial effect on survival of transplanted hearts.

Materials and methods. 40 outbred CD male rats (Charles River, Italy) (300–350 g b. wt) were randomly allocated to either the control (18 animals) or treated group (22 animals). The animals received 2 i.p. injections of 400 mg/kg b. wt lysine acetylsalicylate (Flectadol, Maggioni, Milan, Italy) or saline 20 h and 1 h before surgery; thereafter animals in the treated group were given 400 mg/kg L-ASA daily p.o. until death or rejection of the heart allograft. Usually 1 control and 1 treated rat were operated on the same day; a few times, an additional treated rat received the transplant to replace animals which died shortly after surgery.

Hearts from outbred CD rats were transplanted according to Van Bekkum et al.[13]. At the end of the operation, all the animals were given an intradermal injection of isotonic saline solution (0.7% of b. wt). Rejection time was taken as the day on which the palpable beat of the transplanted heart ceased; this was confirmed immediately by sight at autopsy.

Packed cell volume, total haemoglobin, leucocyte differential count and platelet counts were measured by routine techniques both before and 24 h after surgery. Leucocytes and platelets were counted every 2nd day thereafter, until death or rejection. Autopsy was carried out on animals which died or were killed shortly after rejection. Histological examination was made of recipients hearts in situ and of transplanted hearts. 3 additional control and 4 treated rats underwent sham operation and were followed the same way as the transplanted animals.

Results. 1 rat in the control group and 7 in the treated group died within 24 h of heart transplantation. Haemorrhage was found in the peritoneal cavity in the control and in 5 treated animals. 1 treated animal died of intestinal occlusion and 1 of congestive heart failure in situ.

3 control and 6 treated rats died between 2 and 7 days after surgery. The apparent causes of death in these animals were: congestive heart failure in situ (1 control and 2 treated), infectious complications (2 controls and 2 treated) and peritoneal haemorrhage (2 treated). No animals died during the 2 months after the 1st postoperative week.

The overall mortality rate was thus significantly higher ($p < 0.05$) in the treated than in the control group (table 1). No rejection episode was recorded in either group during the 1st 6 post-operative days. 11 out of the 14 surviving rats in the control group and 7 out of the 9 in the treated group rejected between 7 and 20 days after transplantation; the individual survival times were 7, 8, 8, 8, 10, 11, 13, 14, 18, 18 and 19 days (median value 11 days) for control and 7, 7, 8, 9, 16, 16, 20 days (median 9 days) for treated rats respectively. The remaining animals (3 controls and 2 treated)

Table 1. Mortality of rats following heart transplantation

Time after transplantation (days)	Control (n = 18)	L-ASA-treated (n = 22)	p*
1	1	7	0.088
2–7	3	6	0.313
Overall	4 (22.1%)	13 (59.0%)	0.041

* χ^2-test, 2-tailed.