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Inhibitory Effect of Dextran 40 upon Thrombin-induced Fibrin Deposition in Rat Lungs

CHARLES DIFFANG, LENNART RAMMER and TOM SALDEEN

From the Department of Forensic Medicine, University of Uppsala, Uppsala, Sweden

ABSTRACT

The effect of dextran 40 upon thrombin-induced fibrin deposition in rat lungs was studied. A quantitative method employing isotope-labelled fibrinogen was used to determine the amount of fibrin in the lungs. It was shown that pretreatment with dextran 40 suppressed the accumulation of fibrin in the lungs after injection of thrombin. This was not due to redistribution of the fibrin to other organs but appeared to be a result of decreased intravascular coagulation. It seemed unlikely that the finding could be explained by an effect of dextran upon fibrinolysis.

INTRODUCTION

Dextran has been widely used in the prophylaxis and treatment of post-traumatic shock, owing to its plasma expanding and rheological properties. It is known that in post-traumatic shock disseminated intravascular coagulation often occurs, with resultant microembolisation and damage to organs, especially the lungs (19). The effect of dextran upon this post-traumatic pehnomenon, the microembolism syndrome, is unknown, however.

Dextran has been shown by both *in vivo* and *in vitro* methods to have inhibitory effect on platelet aggregation (9, 23), but its effect upon the accumulation of fibrin in organs has not yet been studied.

In the present investigation a quantitative method employing isotope labelled fibrinogen was used to determine the effect of dextran upon thrombin induced fibrin deposition in rat lungs.

MATERIAL AND METHODS

Experimental animals. 121 adult rats of both sexes of a Wistar strain, largely resistant to the dextran anaphylactoid reaction (11), and weighing 150–250 g, were used. The animals had free access to food (Anticimex rat pellets) and tap water.

Labelled fibrinogen. Human fibrinogen (AB Kabi) prepared by the glycine method described by Blombäck & Blombäck (6) was used. Earlier studies have shown that human fibrinogen and rat fibrinogen behave similarly after thrombin injection (3, 18) and, as human fibrinogen has the greater stability of the two, it was preferred for these experiments. The fibrinogen was labelled with ^{125}I, using the iodine monochloride method (12) or the electrolytic iodination method of Rosa et al. (17). The labelled preparations had a coagulability of 90–93 % of the protein and the iodination was calculated to be <0.5 atoms per molecule fibrinogen. The freeze-dried preparation was dissolved in sterile, distilled water shortly before use and run through an ion-exchange column (Dowex 1-X8, 50–100 mesh) to eliminate free ^{125}I. Of the fibrinogen solution, containing about 0.8 mg protein and 5 μCi/ml, 2,5 ml per kg body weight was injected into a tail vein under ether anaesthesia 48 hours before the experiment.

Dextran. Rheomacrodex® (AB Pharmacia), 10% in saline, with a mean molecular weight of 40 000 and >90 % between 10 000 and 80 000 was used. An infusion of 1 g Rheomacrodex/kg body weight was given in 15 min into a catheter in the left jugular vein.

Thrombin. Bovine thrombin (Topostasin®, Roche) was dissolved in saline shortly before use, the solution containing 100 NIH units/ml. An amount of 250 NIH units/kg body weight was injected in 5 min into the jugular vein catheter.

Fibrinolysis inhibition. Tranexamic acid (AMCA, Kabi), 0.1 g/kg body weight, was injected intravenously; or epsilonaminocaproic acid (EACA, Epsikapron®, Kabi), 1.0 g/kg body weight, was given intraperitoneally.

Collection of blood and organ samples. The abdomen was opened under ether anaesthesia and blood was taken into plastic Ellermann tubes by puncture of the aorta at the bifurcation. 1 ml of blood was collected for radioactivity measurement and 2 ml of blood were taken into a plastic tube containing 0.5 ml of trisodium citrate solution to which had been added 20 mg AMCA/ml for fibrinogen determination. About 0.5 ml of blood was collected into EDTA tubes for platelet count and microhaematocrit determination. One lung, one kidney and a piece of liver were quickly removed, cleaned with filter paper, weighed and put into plastic Ellermann tubes for radioactivity measurement. The radioactivity of blood, dissolved fibrinogen and organ samples was measured in a well-type counting system (Picker Nuclear, Autowell II).

Calculation of the fibrin content of organs. The follow-

ing formula was used for calculating the fibrin content of the organs of each individual rat (7):

$$F = \frac{1}{Q} \cdot (T_{exp} - T_c)$$

where

F = fibrin content in mg/g tissue,

Q = mean relative specific radioactivity (cpm × 10^3/mg) of plasma fibrinogen from control rats (Rheomacrodex or saline infused rats respectively),

T_{exp} = ^{125}I-radioactivity per gram tissue in the experimental rat, and

T_c = mean ^{125}I-radioactivity per gram tissue in the respective control group.

The radioactivity in an organ is derived from fibrin, plasma and the extravascular space. It has previously been shown (7) that after this type of thrombin infusion the changes of the plasma and extravascular radioactivities are of subordinate importance and therefore the radioactivity of the control rats was used as a measure of the sum of these two radioactivities.

Determination of coagulation factors. Fibrinogen was determined according to the method of Nilsson & Olow (15). Haematocrit was measured in triplicate in microhaematocrit tubes after centrifugation at 12 000 *g* for 5 min.

The platelets were counted according to Björkman (5), using phase contrast microscopy. The fibrinolytic activity of plasma and resuspended euglobulin precipitate on unheated bovine fibrin plates and the euglobulin clot lysis time were determined by the method described by Nilsson & Olow (16). Plasma was placed in citrate solution not containing AMCA.

Morphological studies. Lung, liver and kidney samples were fixed in 10% neutral formalin. Paraffin sections 5 μ thick were stained with haematoxylin-eosin and Mallory's PTAH method for demonstration of fibrin.

Statistical analysis. Conventional statistical methods according to Snedecor (20) were used and the results are given as mean values ±S.D. Differences between the groups were tested with Student's *t*-test at the 5% level.

EXPERIMENTS AND RESULTS

Experiment I

Forty-eight hours before the experiment ^{125}I-labelled fibrinogen was injected. Under ether anaesthesia a polyethylene catheter was introduced into the left external jugular vein for infusions. Rheomacrodex or saline was infused in 15 min. Two hours later thrombin was injected in 5 min. AMCA was injected 15 min before the thrombin infusion. The following groups were studied:

I. Rheomacrodex + thrombin
   The animals were sacrificed 5, 20, or 40 min after the thrombin injection.

II. Saline + thrombin
   The animals were sacrificed 5, 20, or 40 min after the thrombin injection.

III. Rheomacrodex, sacrificed 2 h after infusion.

IV. Saline, sacrificed 2 h after infusion.

V. Rheomacrodex + AMCA + thrombin
   The animals were sacrificed 40 min after the thrombin injection.

VI. Saline + AMCA + thrombin
   The rats were sacrificed 40 min after the thrombin injection.

The results of the experiment are shown in Table I. In saline infused rats the thrombin injection gave rise to a marked increase in the radioactivity in the lungs 5 min after the injection, corresponding to 2.6 mg fibrin/g tissue. The fibrin content rapidly decreased and after 40 min fibrin was no longer detectable in the lungs. In the liver and kidney smaller amounts of fibrin were found. In the Rheomacrodex infused rats the accumulation of fibrin in the lungs amounted to 1.5 mg/g tissue at the 5 min interval, which was significantly lower than that of the saline-infused rats ($p<0.05$). The fibrin content in the kidney was also significantly lower in this group. In the two groups pretreated with AMCA prior to the thrombin injection the fibrin content was practically unchanged at 40 min compared with the 5 min interval in the two groups given thrombin alone. In the rats given AMCA and thrombin the fibrin content in the lungs was significantly lower following pretreatment with Rheomacrodex than following saline pretreatment ($p<0.05$).

Morphological studies revealed that the fibrin was deposited in the pulmonary arterioles and capillaries after thrombin injection in rats pretreated with saline and with Rheomacrodex.

The fibrinogen concentration fell during the 5 minutes following the thrombin injection from 233 to 162 mg/100 ml in the saline pretreated group and from 211 to 161 mg/100 ml in the group pretreated with Rheomacrodex ($p>0.05$). The haematocrit was about 36–38%; it showed no change with time and no difference between Rheomacrodex and saline infused rats.

The euglobulin clot lysis times were 127±27 and 107±57 min in the Rheomacrodex and saline pretreated control groups, respectively, and the fibrinolytic activities of resuspended euglobulin precipitate 57±37 and 90±37 mm^2 in these two groups. The differences were not statistically significant.

Table I. *Blood and organ ^{125}I radioactivities (cpm $\times 10^3$/g) of rats killed at various intervals after a 5-minute thrombin injection. Mean ±S.D.*

*=significantly different from saline-pretreated group ($p<0.05$)

Group	Sacrifice after (min)	Blood	Lung	Liver	Kidney	No. of animals
Rheomacrodex+thrombin	5	5.25±0.78	13.71±2.47	3.54±0.59	3.46±0.87*	6
Saline+thrombin		6.32±1.77	22.38±9.36	3.72±0.93	4.88±1.08	5
Rheomacrodex+thrombin	20	4.79±0.84		3.53±0.84	6.70±1.84*	5
Saline+thrombin		5.43±0.78	17.55±5.46	3.89±0.55	9.21±1.88	6
Rheomacrodex+thrombin	40	7.92±2.48	2.98±0.81	2.12±0.72	3.30±1.08	7
Saline+thrombin		8.18±1.10	3.03±1.54	2.30±0.41	4.02±0.89	6
Rheomacrodex+AMCA+ thrombin	40	5.03±0.63	15.16±2.32*	3.22±0.60	7.64±3.02	6
Saline+AMCA+thrombin		5.64±1.37	21.26±6.24	3.31±0.70	9.94±4.55	6
Rheomacrodex	0	8.30±0.88*	3.11±0.43	1.17±0.17	1.43±0.31	8
Saline		9.99±1.05	3.10±0.70	1.02±0.50	1.45±0.37	7

Experiment II

To study the changes in platelet count after the thrombin injection in Rheomacrodex and saline pretreated rats, the following experiment was performed.

Rheomacrodex (20 rats) or saline (17 rats) was infused in 15 min into the jugular vein. EACA was given intraperitoneally 15 min before the thrombin injection. Thrombin was injected in 5 min, 2 hours after the Rheomacrodex or saline infusion. Blood for platelet count was taken from the tip of the tail 5 min before the thrombin injection and 1 hour after its termination.

In the saline group the platelets fell from 985±135 to 512±192×10^3/mm^2 (a decrease of 473±237) and in the Rheomacrodex group from 839±226 to 547±211×10^3/mm^2 (a decrease of 292±248). The difference between the decrease in individual rats in the two groups was statistically significant ($p<0.05$).

Experiment III

The possible trapping of fibrin in organs (*a*) and the possible changes in serum radioactivity (*b*) caused by Rheomacrodex infusion per se were studied in these experiments.

(*a*) ^{125}I-fibrinogen was injected 48 hours before the experiment. Seven rats were injected with Rheomacrodex and 7 with saline in 15 min and then sacrificed immediately for radioactivity measurements. The fibrin content in one lung from each animal was determined by homogenization and centrifugation of the organ and isolation of the labelled fibrin (pellet) from water-soluble tracer (supernatant) (8). In the other lung the radioactivity was determined as described above.

No accumulation of radioactivity was detectable in the lungs or the other organs.

(*b*) ^{125}I-fibrinogen was injected 48 hours before the experiment. Rheomacrodex was injected into 8 animals and saline into 7 animals. The rats were sacrificed 2 hours later and blood was collected.

The results are presented in Table II. No detectable differences in serum radioactivity were found between the two groups.

DISCUSSION

This study has thus shown that pretreatment with dextran 40 suppresses the accumulation of fibrin in the lungs after injection of thrombin in rats. This result is not due to redistribution of the fibrin to the other organs accumulating fibrin, i.e. the liver and kidney (10), but appears to be due to decreased intravascular coagulation. The rats pretreated with dextran had a smaller consumption of platelets after the thrombin injection. The difference in the fibrin content of the lungs can be calculated to correspond to a difference in fibrinogen consumption of about 15 mg/100 ml. The observed difference in the latter was 21 mg/100 ml and although it is not statistically

Table II. *Plasma (P), fibrinogen (F) and serum (P–F) ^{125}I-radioactivity (cpm $\times 10^3$/ml) and the ratio between serum and plasma [(P–F)/P] ^{125}I-radioactivity in rats killed 2 hours after Rheomacrodex or saline infusion. Mean ± S.D.*

	P	F	$P-F$	$\frac{P-F}{P}$	n
Rheomacrodex	13.10±1.27	9.45±0.93	3.65±0.70	0.279±0.044	8
Control (saline)	15.68±2.19	11.44±1.35	4.24±0.88	0.269±0.031	7
Significance	$p<0.01$	$p<0.005$	$p<0.1$	ns	

ns = not significant, n = number of animals.

significant it corresponds well to the anticipated value.

There are several possible explanations for decreased coagulation after pretreatment with dextran 40.

It is possible that an effect on the platelet aggregation might be of importance. Dextran 40 has been shown to inhibit ADP (9, 14, 23) induced platelet aggregation, and also to inhibit platelet activation (surface topography) by thrombin (4).

Fibrinogen and thrombin may be displaced from the intravascular to the extravascular space by steric exclusion through dextran (13).

The slight difference in the plasma fibrinogen level i.e. the substrate concentration, is, however, an unlikely explanation, since the plasma fibrinogen level in both groups lies within the optimal fibrinogen concentration for clotting (22).

Although dextran has not been found to have antithrombin effects in *in vivo* systems (1), a direct effect upon the conversion of fibrinogen to fibrin *in vivo* cannot be excluded. An effect on the fibrin polymerisation is, however, unlikely, since dextran rather has an accelerating effect on this end stage in the coagulation process (1, 21).

Dextran administration results in a coarser and looser fibrin network which is more susceptible to fibrinolysis (2, 21). The effect of fibrinolysis on the accumulation of fibrin in dextran pretreated rats has been expecially investigated in the present study. As early as 5 min after the thrombin injection the rats pretreated with dextran had a lower uptake of fibrin in the lungs, and the same suppression of the fibrin accumulation in the lungs by dextran pretreatment was found irrespective of whether the rats were given AMCA or not. Furthermore, no significant difference in fibrinolytic activity was observed in saline and dextran pretreated control rats. An effect upon the fibrin lysis is thus an unlikely reason for the lower uptake of fibrin in the lungs of dextran-pretreated rats.

Another mechanism that might be of importance is the rheological effects of dextran. Although the haematocrit was normal in the dextran-pretreated rats and the increase in cardiac output should be largely normalized at 2 hours after the dextran infusion used in the present study, an improved capillary perfusion during the process of intravascular coagulation might influence the magnitude of the coagulation.

A possibility that has not been wholly excluded is that the dextran infusion might give rise to a very small formation of fibrin and that after lysis of these deposits, circulating fibrin degradation products could exert anticoagulant effects during the infusion of thrombin. No accumulation of fibrin was detectable in the lungs after the dextran infusion and no increase in serum radioactivity was observed at the time point of the thrombin infusion, but these changes might have been so small that they escaped detection by the methods used.

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Address for reprints:

Tom Saldeen, M.D.
Department of Forensic Medicine
University of Uppsala
Uppsala
Sweden