Clearance of fibrin from glomeruli. Renal cortical fibrinolytic response after thromboplastin infusion in the rat

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Summary

1. The time sequence of glomerular fibrin deposition, renal cortical fibrinolytic response and thrombocytopenia after thromboplastin infusion in rats has been established.

2. Fibrin clearance is rapid and is associated with markedly increased cortical fibrinolytic activity.

3. Plasma fibrinolytic activity was unchanged.

4. Experiments in which the dose–response relationship between thromboplastin dose, fibrin deposition and fibrinolytic response was examined showed that fibrinolytic response increased with fibrin deposition in glomeruli.

5. The dose–response experiments also provided data which suggested that the method of measurement of cortical fibrinolytic activity in the presence of deposited fibrin measures excess rather than total plasminogen activator production.

Key words: fibrinolysis, kidney glomerulus, thromboplastin.

Introduction

Intraglomerular fibrin deposition is a prominent feature of some forms of human glomerulonephritis (Heptinstall, 1974). When deposited in an extracapillary location, fibrin is associated with crescent formation and deterioration of renal function both in human disease (Whitworth, Morel-Maroger, Mignon & Richet, 1976) and in nephrotoxic nephritis in rabbits (Naish, Penn, Evans & Peters, 1972; Thomson, Simpson & Peters, 1975). It therefore seemed to be important to investigate glomerular fibrin clearance mechanisms, both to gain further information on fibrin-mediated glomerular damage and on the factors which might influence fibrin clearance. Some evidence exists that local fibrinolytic activity is an important mechanism of glomerular fibrin clearance (Bernick & Kwaan, 1967; Silberman, Potter & Kwaan, 1971; Sraer, Delarue, Dard, de Seigneux, Morel-Maroger & Kanfer, 1975). However, no systematic study of the time course of glomerular fibrin clearance and cortical fibrinolytic activity after thromboplastin infusion has been undertaken. Equally important, no information is available on whether or not a quantitative relationship exists between glomerular fibrin deposition and cortical fibrinolytic activity response in previously normal animals. Studies of these two interrelated areas should be performed to form a basis for the interpretation of experiments to be carried out subsequently into glomerular fibrin clearance mechanisms in a number of experimental disease states.

Materials and methods

Rats

Male Wistar rats were used throughout, weighing 220–300 g.

Thromboplastin

Suspensions of various concentrations of brain thromboplastin (National Anticoagulant Control Reagents Laboratory, Withington Hospital,
Manchester, U.K.) in sterile pyrogen-free NaCl solution (150 mmol/l : saline) were used.

**Organ removal and processing**

One kidney was removed at the end of each experiment and one pole was snap-frozen in liquid nitrogen and stored at -70°C in a closed container until sectioned. Portions of liver, lung and spleen were removed 30 min after the thromboplastin infusion ceased and processed in the same way as kidney.

**Cortical fibrinolytic activity**

Cortical fibrinolytic activity was measured essentially by the technique of Todd (1959). Three 8 μm frozen sections of kidney cortex were taken on to one glass slide. Cortex was used exclusively, both because cortical blood vessels and glomerular fibrinolytic activity were to be studied and because fibrinolytic activity in medullary tissue is very marked and interferes with assessment of cortical fibrinolytic activity.

An area 2 cm x 2 cm on each slide was marked off with clear tape and a mixture of 0.2 ml of fibrinogen (Kabi) solution (10 g/l) and 0.2 ml of thrombin solution (25 units/ml of 50% glycerol) was pipetted rapidly over the section. After mixing by tilting, the fibrin film was allowed to form by incubation of sections in a moist box for 30 min at room temperature. The slides were then transferred to a 37°C incubator for 45 min. The sections were then fixed in a formaldehyde vapour for 2 min and by immersion in 10% formalin in NaCl solution (150 mmol/l) for 10 min, stained with Harris’ haematoxylin and mounted in 90% glycerol in phosphate-buffered saline (0.01 mol/l), pH 7.2.

**Assessment of extent of cortical fibrinolytic activity**

Since lysis of fibrin overlying blood vessels was apparent in normal rat kidney tissue and did not change obviously after thromboplastin infusion, it was decided to use the relationship of the degree of lysis over blood vessels and that over glomeruli as part of the basis of the assessment of cortical fibrinolytic activity. Four grades of cortical fibrinolytic activity were recognized: grade 1, lysis present over blood vessels, negligible over glomeruli; grade 2, lysis present over blood vessels and glomeruli; grade 3, confluent areas of lysis over adjacent glomeruli; grade 4, lysis over most or all of the section. The three sections of each kidney block were assessed on this scale, one to four points being assigned to each grade respectively, and the points for all three sections were totalled. Thus minimum score of three and a maximum score of 12 was possible. In areas where sections were obviously traumatized during cutting, or at the edges of sections, fibrinolytic activity was increased. If such changes were minor, they were ignored and not included in the cortical fibrinolytic activity assessment. If sections were more extensively traumatized, repeat studies were undertaken.

**Fluorescent antibody staining**

Cryostat sections (4 μm) of kidney, liver, lung and spleen were fixed, washed and stained by routine methods. The antiserum used was fluorescein-labelled rabbit anti-(rat fibrinogen) (Hoechst, Hounslow, Middlesex, U.K.). The antiserum was absorbed with pig liver powder and used in a 1:10 dilution.

The sections were viewed on Leitz Ortholux II microscope with a HBO mercury bulb, BG12 exciting filter and K510 suppression filter.

Fibrin deposition within glomeruli was graded depending on both its extent and position. Since these features were mostly constant over any one kidney section, it was relatively simple to assign a single grade to each section. The grades were assigned as follows: grade 0, no fluorescence visible; grade 1, few flecks of fluorescence; grade 2, more marked, predominantly mesangial fluorescence; grade 3, predominantly intra-capillary fluorescence, but less than 50% of capillary lumena occluded; grade 4, greater than 50% of capillary lumena occluded.

Kidney sections contained between 30 and 50 glomeruli and the whole section was inspected in each case in the assessment of cortical fibrinolytic activity and fibrin deposition. The identity of the slides was unknown to the observer.

**Platelet count**

Platelets were counted in an Improved Neubauer counting chamber in blood taken into EDTA (0.01 mol/l) (Stavem, 1974).

**Systemic fibrinolytic activity**

The activity of the euglobulin fraction of plasma was measured against a urokinase standard on fibrin in agarose plates (Jones, Naish & Aber, 1977). The results are expressed in units of urokinase/ml.

**Control results**

Results of all the variables above in thromboplastin- or saline-infused rats were compared with...
those obtained in six normal non-perfused animals.

**Experimental protocols**

All suspensions of thromboplastin or NaCl (150 mmol/l) alone in a volume of 3 ml were given into the tail vein over a 12 min period under light inhalation general anaesthesia. At the end of each experiment animals were bled by cardiac puncture and the relevant organs removed under the same anaesthesia.

**Sequential study.** A suspension of thromboplastin (1:10) was infused. The 1:10 suspension of thromboplastin was chosen as it produced complete glomerular capillary occlusion. More concentrated suspensions almost invariably caused death. Five rats were killed at the end of infusion (zero time), or at 15, 30 or 60 min, 3, 6, 12 or 24 h subsequently.

The following investigations were performed on each rat at each time interval: platelet count, cortical fibrinolytic activity, immunofluorescence staining for fibrin on kidney sections. Blood was taken from the rats killed at 30 min, 3 and 24 h for plasma fibrinolytic activity.

**Thromboplastin dose-response study.** Suspensions of 1:10, 1:20, 1:40 and 1:80 thromboplastin and NaCl (150 mmol/l) alone were infused in a volume of 3 ml. Five rats in each group were killed at zero time, 15 and 30 min after the end of infusion. Platelet count, glomerular fibrin and cortical fibrinolytic activity were measured in all rats.

**Results**

**Sequential study**

**Cortical fibrinolytic activity (Fig. 1).** Kidney sections from control uninfused rats showed a cortical fibrinolytic activity score of up to five. Cortical fibrinolytic activity rose to a maximum between zero time and 30 min after the end of thromboplastin infusion. By 60 min and thereafter it had fallen to levels little different from control values.

**Glomerular fibrin deposition (Fig. 2).** At zero time all glomeruli from all rats showed greater than 50% glomerular capillary thrombosis. Thirty minutes after the end of infusion, sections from three rats showed less than 50% capillary thrombosis; in the other two all glomerular capillary loops appeared patent and fluorescence in mesangial regions was visible. Between 1 and 6 h after infusion, a slow reduction in glomerular fibrin took place. During the time between 30 min and 3 h post-infusion, marked punctate fluorescence was visible at the luminal margin of tubular cells. No positive staining for fibrin was visible in glomeruli 12 and 24 h after infusion. At no stage was fluorescence visible within blood vessel lumina or walls.

**Platelet count (Fig. 3).** Platelet count depression was maximal during the first 30 min after thromboplastin infusion. The mean level at each time interval studied remained below the control value until 24 h.

**Plasma fibrinolytic activity (Table 1).** No significant change took place over the experimental period.

**Organ distribution of fibrin.** This protein was deposited to various degrees in the pulmonary capillaries, and within the spleen and liver. The exact location in the latter two organs was difficult to determine, but some fibrin did appear to be in liver sinusoids or Kupffer cells.
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FIG. 3. Platelet count after infusion of 3 ml of 1:10 thromboplastin. C, Control rats.

Discussion

The objectives of this study were to establish the time course of fibrin clearance and renal cortical fibrinolytic activity after thromboplastin infusion in normal rats and to attempt to determine whether or not the Todd (1959) technique measured total tissue fibrinolytic activity in the presence of fibrin deposition. The method used to measure tissue fibrinolytic activity may suffer from a number of theoretical disadvantages, particularly lack of accurate quantification, compared with that used by Giroux, Verroust, Morel-Maroger, Delarue, Delauche & Sraer (1979) in which glomeruli are isolated and lysis of radiolabelled fibrin is measured. Tissue sections were used in our study for a number of reasons. We wished to observe the fibrinolytic activity of structures other than glomeruli. It was hoped that the information gained might be used in the study of human renal biopsies. Finally, our observation that traumatized glomeruli exhibited enhanced fibrinolytic activity led us to decide that glomerular isolation might introduce a similar artifact.

The findings in relation to time are in agreement with those of Sraer et al. (1975). However, immunofluorescence staining allowed a more accurate and sensitive assessment of fibrin deposition and possible routes of clearance. Fibrin was cleared from occluded glomerular capillary loops very rapidly and this was associated with a marked increase in cortical fibrinolytic activity. Interestingly, no fibrin deposition or change in fibrinolytic activity occurred in extraglomerular blood vessels. No change in systemic fibrinolytic activity occurred during the study period, and it is likely, therefore, that local activity only is measured by the Todd (1959) technique. In addition, the possibility that the fibrinolytic activity was derived from plasma constituents in the thrombi is small, as maximum fibrin deposition occurred at zero time, whereas maximum fibrinolytic activity was present 30 min later. After glomerular capillary loop recanalization material reacting with fibrinogen antiserum appeared sequentially in the mesangium and in tubular cells. The latter observation presumably indicates that tubular handling of fibrin derivatives takes place. Similar appearances are observed in rabbit kidneys during nephrotoxic nephritis (M. H. Sindrey & P. Naish, unpublished work). Localization of material reacting with fluoresceinated antifibrinogen antiserum in tubular cells has not been reported in human glomerular disease, but our findings require the significance of urinary fibrinogen derivatives in human glomerulonephritis to be interpreted with caution. Naish, Evans & Peters (1975) found that urinary fibrinogen derivative excretion was proportional to intraglomerular fibrin deposition.

Dose–response study

Cortical fibrinolytic activity (Fig. 4). At zero time a dose-related difference appeared to exist between the thromboplastin-infused groups. At the same time saline-infused animals showed a cortical fibrinolytic activity score similar to that of the 1:10 thromboplastin-infused rats. At 15 min little difference was apparent between any of the groups. At 30 min cortical fibrinolytic activity has risen quite markedly in the 1:40, 1:20 and 1:10 thromboplastin groups.

Glomerular fibrin deposition (Fig. 4). At zero time a dose-related difference existed between each thromboplastin-infused group. No fibrin deposition was seen in any saline-infused animal. Fibrin clearance was apparent in the 1:10 thromboplastin-infused animals between 15 and 30 min. In the 1:20, 1:40 and 1:80 thromboplastin-infused rats slight increases in glomerular fibrin occurred over the study period. The location of this material was mesangial in most animals, but four showed a few flecks, probably on glomerular capillary loops.

Platelet count (Fig. 5). A thromboplastin dose-related fall in platelet count occurred at zero time, and rose towards normal with time in each group. Little change occurred in the saline-infused animals.
Glomerular fibrin clearance

Fig. 4. Cortical fibrinolytic activity (●) and intraglomerular fibrin deposition (○) at zero time, 15 min and 30 min after infusion of 3 ml of NaCl (150 mmol/l: saline), and 1:10, 1:20, 1:40 and 1:80 thromboplastin.

Table 1. Plasma fibrinolytic activity (expressed as units of urokinase/ml) in control and thromboplastin-infused rats

<table>
<thead>
<tr>
<th>Control uninfused rats</th>
<th>Time after thromboplastin infusion (min)</th>
<th>1:10 Thromboplastin-infused rats</th>
<th>1:20 Thromboplastin-infused rats</th>
<th>1:40 Thromboplastin-infused rats</th>
<th>1:80 Thromboplastin-infused rats</th>
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<tr>
<td>1.81</td>
<td>1.50</td>
<td>0.91</td>
<td>1.05</td>
<td>1.39</td>
<td>0.93</td>
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or no attention in previous studies (Silberman et al., 1971; Bergstein & Michael, 1972; Sraer et al., 1975). Bearing in mind that observed cortical fibrinolytic activity is presumably a balance between plasminogen activator production and utilization and that in acute experiments it is also a time-dependent phenomenon, a number of proposals may be made from the dose-relationship experiments reported in this paper. At zero time cortical fibrinolytic activity increased with intraglomerular fibrin deposition. However, renal sections from saline-infused animals showed similar fibrinolytic activity to 1:10 thromboplastin suspension in NaCl solution (0·15 mol/l). C. Control rats.

The amount and position of intraglomerular fibrin after thromboplastin infusion presumably depends on the degree of intravascular coagulation induced locally, the rate and route of clearance and possibly also glomerular deposition of soluble fibrin monomer complexes released from other sites of fibrin deposition. It is reasonable to propose that fibrin present in glomeruli at zero time was due to the degree of local coagulation, and our results show that a thromboplastin dose-dependent relationship exists between fibrin deposition and cortical fibrinolytic activity at this time. An extrarenal supply of soluble fibrin monomer complexes may well explain the minor increases of fibrin in the mesangium in the 1:20, 1:40 and 1:80 thromboplastin-infused rats and the continued increase in cortical fibrinolytic activity up to 30 min after the end of thromboplastin infusion. No observable increase in intraglomerular fibrin over the 30 min study period was seen after 1:10 thromboplastin infusion, probably because the large amounts of locally deposited fibrin obscured any relatively small extrarenal contribution.

The results of our experiments are derived in the non-steady state. It is possible that low-grade chronic intravascular coagulation might induce different responses in cortical fibrinolytic activity, although experiments about to be reported from this laboratory do not show this. Nevertheless, the results reported in this paper have defined the time course and dose relationships of glomerular fibrin deposition, cortical fibrinolytic activity and intravascular coagulation in normal rats. Some information on the routes of fibrin clearance has also been obtained. Importantly, evidence is presented to suggest that in the presence of fibrin the Todd (1959) technique measures excess, rather than total, tissue plasminogen activator production.

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References
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