SHORT COMMUNICATION

THE EFFECT OF DEFIBRINATION ON NEPHROTOXIC SERUM NEPHRITIS IN RABBITS

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SUMMARY

1. Nephrotoxic serum nephritis was induced in rabbits.
2. Treatment with Arvin to produce defibrination provided structural and functional protection to the glomeruli.

Key words: nephrotoxic serum nephritis, Arvin.

Administration of heparin before the induction of nephrotoxic serum nephritis in rabbits decreases the incidence of crescent formation and glomerular sclerosis (Halpern, Milliez, Lagrue, Fray & Morard, 1965). Anticoagulation with a dicoumarol also decreases the amount of intraglomerular fibrin deposition (Vassalli & McCluskey, 1964), and it has been postulated that fibrin deposition may be an important factor in the pathogenesis of some features of glomerular damage in this disease. It is known, however, that heparin has a number of other effects apart from that of anticoagulation, such as anticomplementary and histamine binding activity. Neither heparin nor dicoumarols directly inhibit the conversion of fibrinogen into fibrin but act at earlier stages in the coagulation cascade.

To investigate the role of fibrin deposition more precisely, and eliminate, as far as possible, effects on other pathogenetic mechanisms, the circulating amount of fibrinogen in animals given the disease has been decreased to extremely low values by the intravenous administration of Arvin. This causes the intravascular formation of unstable fibrin polymers which are sequestered mainly in the lungs and reticulo-endothelial system and undergo rapid lysis with no apparent harmful effect on renal or other organ function. As the only significant effect of Arvin that has been shown to occur is one of defibrination, its effects on nephrotoxic serum nephritis may be expected to indicate only that part played by fibrin deposition in the pathogenesis of the glomerular damage.

MATERIALS AND METHODS

The experiment was divided into two parts: in each six treated and six untreated animals were used. The animals were New Zealand white rabbits weighing between 2 and 2.5 kg. They were

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fed on a normal pellet and water diet. The disease was induced in all animals by the intravenous injection of 2 ml of sheep anti-rabbit glomerular basement membrane antiserum. In the first experiment the treated rabbits were defibrinated 48 h before the administration of antiserum. Arvin (0-5 unit/kg) was injected intravenously followed 1 h later by 1 unit/kg. Defibrination was maintained by twice-daily intravenous injection of Arvin (1 unit/kg). In the second experiment Arvin injections were not started until day 7 of the disease, but the induction and maintenance of defibrination was the same as in the first experiment. Untreated animals were injected intravenously with the same volume of 0·15 M-NaCl at the same time as the Arvin.

Venous blood samples were taken from all animals before the start of the experiment and at the time of killing; blood urea and serum albumin concentrations were estimated by routine AutoAnalyzer methods.

The effectiveness of defibrination was tested by the clot quality test (Reid, Chan & Thean, 1963).

All surviving animals were killed on day 13 of the disease. At this time, portions of kidney were taken for histological examination. These were fixed in 15% formol-saline, 5 μm sections were cut and stained with Haematoxylin and Eosin, phosphotungstic acid, Haematoxylin, and periodic acid–Schiff reagent. Further portions were taken for immunofluorescent examination, these being snap-frozen in liquid nitrogen, sectioned on a Slee cryostat at 4 μm, and stained with fluorescein-conjugated antisera to rabbit IgG, C3 and fibrin.

In assessing histological changes in the glomeruli, three features were recognized. These were, first, crescent formation, defined as a proliferation of capsular cells, two or more cells thick extending for not less than one-third of the glomerular circumference; secondly, capsular adhesion formation, defined as a cellular bridge between capsule and glomerular tuft at a point distant from the glomerular hilus; thirdly, glomerular disorganization, defined as the absence of any discernible capillary loops. At least fifty glomeruli were inspected for each feature.

The amount of intraglomerular fibrin deposition was graded from grade 0 to 3. Grade 0 represented no fluorescence, grade 1 minor focal deposits, grade 2 more generalized focal deposition and grade 3 generalized coalescent fluorescence. Between forty and 120 glomeruli were evaluated in each section.

Those who carried out the biochemical and histological tests did not know which treatment the animals had received.

RESULTS

Deaths

In the untreated group three rabbits died, two on day 10 and one on day 13 of the disease. Two animals in the group treated from days 0 to 13 died, one on the first day and one on day 12 of the disease. Renal tissue was available for histological examination from all of these animals except from that dying on day 1.

Defibrination

The clot quality tests showed adequate defibrination in all rabbits receiving Arvin but normal clots in those receiving saline.

Blood urea

The blood urea values before the induction of the disease were between 42 and 82 mg/100 ml;
the mean values in each group were not significantly different. On the day of killing, the mean value on the untreated animals was 304.7 mg/100 ml, and in the treated animals 116.6 mg/100 ml. This difference was significant (0.025 > P > 0.01). The difference between the mean value of the two treated groups was not significant [day 7–13, 132 ± 70.7 (SD) mg/100 ml, day 0–13, 88.5 ± 32 (SD) mg/100 ml, 0.4 > P > 0.2].

Serum albumin

The serum albumin concentrations in all animals before the experiments were between 2 and 3.1 g/100 ml; the mean values in each group were not significantly different. On day 13, the values had fallen, but there was no significant difference between the treated and untreated animals.
Histological appearances (Fig. 1)

Fig. 1 shows the percentage incidence of each feature, expressed as a mean for each group. There was a much lower incidence of crescent formation in the treated groups, and a higher incidence of glomeruli in which there were no histological changes. The incidence of glomerular disorganization was hardly different in any group. Adhesion formation was commoner in both treated groups.

Immunofluorescence (Fig. 1)

The deposition of rabbit IgG and C3 in a linear distribution along the glomerular basement membrane was the same in all rabbits. Fibrin deposition was markedly different in the treated and untreated groups. Fig. 1 shows the percentage incidence of each grade, expressed as a mean for each group. In both treated groups there is a higher percentage of glomeruli showing no fibrin deposition and a lower incidence showing grade 3 deposition, than in the untreated rabbits. There is a higher incidence of grade 1 and 2 changes in the treated animals (representing only minor degrees of fibrin deposition).

DISCUSSION

Defibrination has been shown in these experiments to give a significant degree of structural and functional protection to glomeruli from the effects of nephrotoxic antibody. Thus in both of the treated groups there was a significantly lower blood urea concentration, a lower incidence of crescent formation and a higher incidence of glomeruli showing none of the changes assessed. These milder structural abnormalities were also associated with less intraglomerular fibrin deposition. Since there was no significant difference in the outcome in the two treated groups it seems likely that the protection afforded by defibrination was related to events occurring after day 7.

These experiments support the hypothesis that intraglomerular fibrin deposition is closely related to the development of crescents and reduced glomerular filtration in nephrotoxic serum nephritis.

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REFERENCES

