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FOCAL MESANGIAL PROLIFERATIVE GLOMERULONEPHRITIS IN THE RAT CAUSED BY HABU VENOM: THE EFFECT OF ANTIPLATELET AGENTS

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Summary.—The effects of the antiplatelet agents aspirin (ASA), dipyridamole (DP) and prostacyclin (PGI_2) on glomerulonephritis following Habu snake venom have been investigated. ASA greatly increases glomerular damage and PGI_2 inhibits glomerular damage. DP suppresses glomerular proliferation. The results extend previous studies on platelet activity in this model and provide evidence that (1) glomerular injury involves a platelet/endothelial reaction and (2) glomerular proliferation is affected by an antiplatelet drug.

THE ROLE OF PLATELETS in the pathogenesis of glomerulonephritis (GN) remains unclear, despite recent evidence of platelet activation in human GN (Clark *et al.*, 1976; Parbtani and Cameron, 1979).

Habu venom causes an experimental model of focal proliferative GN in which the first ultrastructural change in the glomurulus is platelet aggregation (Cattell and Bradfield, 1977). Antiplatelet serum administration has been shown to inhibit proliferation, but not early (24 h) glomerular injury (Cattell, 1979). The effect of the antiplatelet drugs aspirin (ASA), Dipyridamole (DP) and prostacyclin (PGI_2) on this model has been evaluated to investigate further the role of platelets in GN and to see if experimental evidence supports the current use of antiplatelet therapy in human GN.

MATERIALS AND METHODS

Animals.—Inbred male Lewis rats weighing 150–300 g were used.

Habu venom.—Lyophilized Habu snake venom (Japan Snake Institute) was dissolved in physiological saline at a concentration of 1 mg/ml. GN was induced in all experimental groups by a single injection of venom *via* the penile vein (2 mg/kg body wt), rats of Group I and Group II (see below) receiving a separate batch of Habu venom.

Antiplatelet drugs.—Aspirin (Claradin, Nicholas Labs. Ltd. U.K.) dissolved in tapwater at a concentration of 2·5–50 mg/ml. Dipyridamole (Persantin; Boehringer Ingelheim) at a concentration of 5 mg/ml. Prostacyclin (a gift from the Wellcome Research Labs., U.K.) dissolved in 0·001M Sorensens glycine buffer, pH 10·4. Activity of solutions was tested *in vitro* against 0·5 ml fresh rat platelet-rich plasma (PRP) immediately before and at the end of infusions. Only solutions inhibiting ADP-induced (5 μg of a 2mM solution) platelet aggregation *in vitro* were used in infusion experiments.

Renal histology.—Left nephrectomies were performed 5 days after Habu venom injection and tissues processed for light microscopy as previously described by Cattell and Bradfield (1977).

Experimental protocols.—Group I rats received the following drugs for 7 days starting 2 days before Habu venom was given.

A ASA by daily intragastric dosage of (i) 10 mg/kg body wt (12 rats). (ii) 200 mg/kg body wt (6 rats).
B DP daily 10 mg/kg i.v. (17 rats).
C ASA 10 mg/kg by the intragastric route plus DP 10 mg/kg/day i.v. (6 rats).
D Untreated controls—venom only (29 rats).

In Group II rats the right external jugular vein was cannulated with a Portex polythene tubing catheter under ether anaesthesia. The catheters lay s.c. and emerged through a skin

incision at the back of the neck. The rats were then placed in restraining cages and solutions infused in unanaesthetized animals by syringe pump at a rate of 1 ml/h for 3 h. Habu venom was given 15 min after the infusion was started. To determine a suitable length of time for infusions the *in vitro* activity of Habu venom some hours after injection was assessed. Two rats were bled 1 h after venom (platelet counts 83 and $103 \times 10^3/mm^3$), and given 2 ml of fresh rat PRP (platelet concentration $650 \times 10^3/mm^3$) 6 h after venom, platelet counts of 412 and $320 \times 10^3/mm^3$ being reached. One h later final platelet counts were taken (200 and $220 \times 10^3/mm^3$). Two control rats not receiving venom were given equivalent amounts of fresh rat PRP and bled at the same times. The fall in platelet count was not significantly different from that in Habu-treated animals, indicating that by 6 h very little active venom was circulating.

Three groups of venom-treated animals received the following i.v. infusions:

A PGI_2 in 0·001M glycine buffer, infused at 200 ng/kg/min (10 rats).
B 0·001M glycine buffer (10 rats).
C Physiological saline (10 rats).

Assessment of effect of antiplatelet drugs

All groups were assessed by the following methods:

1. *Histological lesions.*—2000 glomeruli were counted in each nephrectomy specimen. The number of Habu-affected glomeruli, and whether they had 24-h-type cystic lesions or mesangial proliferative lesions was determined. Results were assessed using analysis of variance and *t* tests using a $\log_{10}(X+1)$ transformation of the data.

2. *Peripheral platelet counts.*—Rats were bled between 10 and 60 min after venom, as previously described by Cattell (1979).

3. In vitro *studies.*—(a) Group I. PRP was prepared as previously described (Cattell, 1979) from blood samples taken after 2 days of drug therapy. 0·5ml samples were tested for aggregation with Habu venom (20–50 μl of a 1mg/ml solution), in an EEL 169 platelet aggregometer.

(b) 0·5ml samples of PRP from normal rats was preincubated with PGI_2 (1–100 ng) for 1 min at 37°, and then tested with Habu venom (20–50 μl) as above.

RESULTS

Effect on histological lesions

Table I is a summary of the results in Group I. Injury was increased in ASA− and ASA+DP-treated rats ($P < 0·001$), being significantly greater with high-dose ASA (200 mg/kg) compared with low-dose ASA ($P < 0·04$). DP had no effect. Glomerular proliferation was not affected by ASA or ASA+DP but was inhibited by DP ($P < 0·03$).

TABLE I.—*Effect of antiplatelet drugs (Group I) on Habu glomerulonephritis*

Experimental group	No. of rats	Histology Day 5											
		P	T	P	T	P	T	P	T	P	T	P	T
I A (i)													
10 mg/kg	6	7	8	65	75	40	44	78	93	45	51	32	39
ASA	6	62	71	31	40	18	18	20	22	16	18	8	13
I A (ii)													
200 mg/kg	6	155	192	5	6	175	215	62	99	235	244	135	150
ASA													
	5	2	2	3	3	0	0	4	5	19	24	—	—
I B	6	6	6	26	32	46	50	3	5	19	19	30	32
DP 10 mg/kg	6	10	15	10	11	15	16	10	11	12	13	0	0
I C													
ASA 10 mg/kg	6	40	46	51	55	32	41	27	31	31	36	22	24
DP 10 mg/kg													
I C	6	14	15	10	11	12	12	17	20	14	14	29	33
Controls	5	2	2	20	20	11	11	3	3	5	5	—	—
No drugs	6	14	14	4	4	14	14	2	2	5	5	11	11
	6	2	2	0	0	5	5	27	27	12	15	52	79
	6	6	9	9	9	5	5	6	6	11	11	15	15

All groups were given Habu venom 2 mg/kg i.v. on Day 0.
P = number of proliferative
T = total number of Habu affected } glomeruli per 2000 glomeruli.
Each paired number represents a single nephrectomy.
T (incidence of Habu lesions) was increased ($P < 0·001$) in Groups 1 A (i), (ii) and C.
P (proliferative glomeruli) were reduced in Group I B only ($P < 0·03$).

TABLE II.—*Effect of infusions (Group II) on Habu glomerulonephritis*

Experimental group	Infusion	No. of rats	Histology Day 5 P.	T.	P.	T.
IIA	PGI_2 in glycine buffer	2	0	0	0	1
		2	0	0	0	2
		2	1	1	0	2
		2	0	0	0	0
		2	1	2	13	13
B	Glycine buffer alone	2	13	15	0	0
		2	9	9	0	0
		2	0	0	1	1
		2	4	12	1	6
		1	14	18		
		1	4	4		
C	Saline alone	2	4	5	1	1
		2	1	1	3	3
		4	19	19	2	2
			2	2	2	2
		1	2	2	2	2
		1	7	7	7	7

Rats received venom 15 min after the start of infusion. The legends are as in Table I. The total incidence (T) of Habu lesions was significantly reduced ($P = < 0{\cdot}098$) in PGI_2-infused rats. Glomerular proliferation (P) was reduced in glycine alone infused rats ($P = < 0{\cdot}05$).

Table II is a summary of the results in Group II. PGI_2 infusion reduced the incidence of injury ($P < 0{\cdot}098$). Only 1 animal showed lesions equivalent to those in the control group (13 per 2000 glomeruli). The much lower incidence of Habu lesions in Group II rats compared with those of Group I was due to a different batch of crude venom being used. Proliferation was inhibited in the group given glycine alone ($P < 0{\cdot}05$).

Peripheral platelet counts

Acute thrombocytopenia occurred 10 min after injection of venom (average platelet count $23 \times 10^3/mm^3$) in controls, and had persisted at 30 min and 1 h. No significant difference was found in the drug-treated groups.

In vitro *studies*

(a) PRP prepared from Group I rats 2 days after drug therapy was begun showed aggregation *in vitro* with Habu venom, as previously described for normal PRP preparations (Cattell, 1979).

(b) Prior incubation with PGI_2 failed to inhibit Habu-induced aggregation.

DISCUSSION

The effect of antiplatelet drugs on the 2 phases of this model, initial glomerular injury and subsequent glomerular proliferation, have been studied.

Glomerular injury

The level of damage was significantly altered by ASA and PGI_2, but not DP. ASA greatly increased the number of injured glomeruli, the enhanced effect with a high-dose regime (200 mg/kg/day) indicating that the mechanism was enhanced platelet aggregation due to inhibition of endothelial PGI_2 production (Kelton, Hirsch and Buchanan, 1978; Korbut and Moncada, 1978). In our experiments low-dose (10 mg/kg/day) ASA also increased glomerular injury, suggesting a similar effect to high-dose ASA. Precisely what the low dose of ASA is which will inhibit platelet but not vascular prostaglandin synthesis remains in dispute (Villa and de Gaetano, 1977; Masotti *et al.*, 1979) and our results suggest that in the rat 10 mg/kg is not low enough to produce a selective effect on platelets. Inter-species variation in drug metabolism may be a relevant factor (Bream, Philip and Ferguson, 1979). Further support for the above observations was obtained from the PGI_2 infusion experiments in which reduced injury after venom was found. As PGI_2 has a hypotensive as well as an anti-platelet-aggregating effect, blood-pressure measurements were performed during infusions. No hypotensive effect was found. Studies in the rat have shown that renal haemodynamics and glomerular function are not altered by PGI_2 infusions which do not affect systemic blood pressure (Baer, Kauker and McGiff, 1979). DP had no effect on glomerular injury perhaps because the damage is not only dependent on platelet aggregation, but involves a more complex interaction between platelets and endothelium.

One unexplained feature is the failure of the drugs to affect either the acute thrombocytopenia of the *in vivo* model, or venom-induced platelet aggregation *in vitro*. A lack of correlation between *in vitro* and *in vivo* results has been previously observed in other studies on antiplatelet agents (Buchanan and Hirsch, 1978). The failure of PGI_2 to prevent early thrombocytopenia *in vivo* may be similar to its failure to prevent early platelet sequestration in experimental haemodialysis in dogs (Woods *et al.*, 1978). In the light of the results presented here implicating platelets in glomerular injury, the 24 h histological material from antiplatelet-serum (APS)-treated rats (Cattell, 1979) was re-examined. Animals had previously been assessed only as + or ++ for degree of injury. Nine APS-treated rats which survived for 24 h or more after 2 mg/kg Habu venom were compared with 9 control rats, extra sections being used to provide 2000 glomeruli per rat. A total of 429 injured glomeruli were found in APS-treated rats, compared with 935 in non-APS-treated animals.

The results implicate platelets but do not prove that glomerular damage is the result of a direct venom-induced platelet reaction. The platelet activation could be secondary to endothelial damage caused by the venom. Platelet activation by Habu venom does occur *in vitro* (Cattell, 1979) and may depend on the platelet cyclo-oxygenase pathway (Ouyang and Teng, 1979). Habu venom has been shown to damage cells directly (Yoshikura *et al.*, 1966) but we have failed to produce glomerular lesions by direct renal perfusion with up to 480 μg Habu venom (unpublished observations).

Glomerular proliferation

The results with ASA are similar to those found in experimental arterial proliferation (Clowes and Karnovsky, 1977; Burns *et al.*, 1976) and are supported by the failure of drugs affecting the platelet cyclo-oxygenase pathway to prevent *in vitro* release of platelet growth factor (Cazenave *et al.*, 1978; Ihnatowycz *et al.*, 1979). DP significantly inhibited glomerular proliferation, and a similar effect of this drug has been reported on arterial proliferation (Harker *et al.*, 1976). These findings support the previously reported lack of proliferation in APS-treated rats (Cattell, 1979). The reason for suppression in the glycine infusion group is not clear, but was not due to pH, as blood pH during infusions was no different from that in the control saline-infused group. The effect with glycine has been observed in a further 12 infusions (results not presented here).

These experiments show that the lesions in this model involve a platelet/endothelial reaction, and emphasize that in this complex situation drugs such as ASA may promote rather than protect against glomerular injury. The previous evidence of inhibition of glomerular proliferation in APS-treated animals is supported by a similar effect in DP-treated rats.

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