Brit. J. Pharmacol. (1949), **4**, 132.

CONTRIBUTION ON THE THROMBOCYTOPENIC ACTION OF URETHANE IN DOGS*

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(Received December 4, 1948)

Thrombocytopenic purpura in dogs has been obtained by subcutaneous urethane administration (Cruz and Moussatché, 1948). Suppurative reactions commonly occur in the skin of the experimental animals after a few injections. These reactions, possibly due to sensitization to the drug, are undesirable in experiments on the action of urethane. The present paper deals with the results of urethane administration by vein, a route much more suitable for controlling the toxic doses.

METHODS

Our routine haematological technique has been described in a previous paper (Cruz, Silva, and Mello, 1945). We have estimated bleeding time according Duke's method (1910), coagulation time by Lee and White's method (1913), and clot-retraction by Macfarlane's method (1939). Urethane was used as a 60 per cent solution in distilled water.

RESULTS

When urethane is administered in high doses (0.4 g. or higher per kg. body weight) the animals usually eliminate faeces and urine immediately after the injections and show somnolentia followed by sound sleep for 10–15 minutes. This slight anaesthetic effect is of short duration, and animals become normal again after a drowsy period of about half an hour.

No striking differences were observed between the action of urethane administered subcutaneously and intravenously so far as the time of the establishment of the purpuric picture or pathological findings are concerned.

The results of effective doses (0.4–0.5 g. per kg. body weight) administered to 5 dogs are shown in Table I. In 4 dogs platelets disappeared from the circulation between 6–9 days when bleeding time was already very much prolonged and the other signs of purpura were also present. The animals were sacrificed a few days later, and the haematological findings revealed a picture of severe anaemia. In one of these dogs (426–20) platelets obtained by fractioned centrifugation of 50 c.c. of normal dog's blood were injected daily soon after the urethane administration in order to observe any eventual interference in the pathological picture. This preliminary experiment was completely negative. In another dog (494–16) urethane was stopped after seven daily injections and blood changes were followed. Confirmation of other authors (Duke, 1911; Firket, 1922; and Tocantins, 1938) and our previous experiments (Silva and Cruz, 1945) concerning platelet regeneration have been verified. Normal platelet volume was attained 3–5 days after the beginning of the platelet regeneration, which only started 20 days after the absence of platelets in the peripheral blood. In one splenectomized dog (466–19) the evolution and the pathological picture were very similar to those of normal dogs treated with urethane (Table I).

In order to ascertain the optimal doses to obtain the picture of purpura, higher and smaller doses than those described have been tried. As higher doses, we tried eight intravenous injections, each 0.4 g. per kg. body weight, within a period of 36 hours (dog 437–3) as well as two daily injections of a slightly higher dose (0.5 g. per kg.) (dog 439–23). Both these animals died from acute urethane intoxication in 2–4 days before presenting any signs of purpura (Tables II and III).

A smaller dose was tried in dog 496–26 for a period of 45 days without any signs of purpura or other

* Aided by a grant from Dr. Guilherme Guinle.

TABLE I

Dog No.	Weight (kg.)	Dose of urethane per kg. (g.)	Day of experiment	Red blood cells (millions per cu. mm.)	Haemoglobin (g./100 c.c.)	Haematocrit (%)	Reticulocytes (%)	Leucocytes (thousands per cu. mm.)	Platelets (c.c. per 100 c.c.)	Bleeding time	Coagulation time	Observations	Post-mortem examination
425–10 ♀	4.6	0.4	0	5.0	10.65	35	0.5	10.5	0.40	3′ 25″	4′ 30″	Killed on 7th day	Few petechiae on intestine. Scarce petechiae on kidneys, lungs, and stomach.
			6*	—	5.0	—	—	—	0	25′	4′		
			7	1.8	3.8	12	0.1	10.2	0	35′	4′		
426–20 ♂	7.8	0.4	0	4.7	9.8	29	0.2	12.0	0.70	5′	4′ 30″	Killed on 10th day	Numerous petechiae on intestine and heart. Few petechiae on lungs, kidney (cortical zone), and skin. Bloody stools.
			4	3.0	5.6	19	0.1	10.0	0.50	4′	4′		
			9*	—	3.0	—	0.1	—	0	30′	4′		
			10	1.4	2.2	9	1.0	10.5	0	20′	5′		
462–22 ♀	5.6	0.4	0	—	9.2	—	0.5	8.8	0.35	3′	2′ 40″	Killed on 10th day. Bloody stools on 9th day	Numerous petechiae on small intestine and heart. Few petechiae on lung and kidney. Blood in the lumen of intestine.
			5	—	6.2	—	—	—	0.25	3′ 40″	4′		
			8*	2.4	4.5	16	0.1	1.4	0.15	3′	4′ 30″		
			9	2.0	4.0	14	0	1.1	0.01	30′	4′		
			10	1.8	3.8	13	0	0.2	0.01	35′	4′		
466–19 ♂ (Splenectomized 24 days before)	7.5	0.4	0	4.8	9.4	33	0.5	13.8	0.25	3′ 40″	5′	Killed on 10th day	Many petechiae on small intestine, lungs, kidney, stomach, heart, and urinary bladder. Scarce petechiae on skin.
			4	4.6	9.2	29	0.2	12.0	0.12	3′	5′		
			8	4.0	8.2	28	0.2	10.5	0.05	6′	4′ 30″		
			9*	—	7.4	—	—	—	0	30′	5′		
			11	2.5	5.0	18	0.1	10.8	0.01	60′	4′		
			15	1.0	1.8	7	0	1.8	0	60′	5′		
494–16 ♂	12.4	0.5	0	7.0	14.2	44	0.5	12.4	0.40	4′	3′ 40″	Died on 52nd day. From 7th until 12th days bloody stools	Scarce typical purpuric lesions on small intestine.
			4	6.8	14.2	46	0.2	12.0	0.40	4′	5′		
			7*	5.8	11.8	36	0.2	10.8	0.05	20′	5′		
			9	5.0	9.0	31	0.1	—	0	35′	4′		
			12	2.0	4.5	13	0.1	10.5	0.05	28′	4′		
			19	2.0	3.8	14	0.1	10.5	0.01	25′	4′		
			31	—	5.4	—	—	—	0.02	5′ 40″	6′		
			33	—	6.0	—	—	—	0.05	5′	4′		
			34	—	5.6	—	—	—	0.15	5′ 30″	4′ 30″		
			35	3.0	6.0	21	4.0	12.4	0.40	4′	5′		
			36	—	—	—	—	—	0.38	5′	4′		
			39	—	7.4	—	1.0	—	0.35	4′	4′		

* Urethane stopped

TABLE II

DOG 439–23♂ 9.4 KG.

Two intravenous injections of 0.5 g. urethane per kg. body weight daily (injections morning and afternoon)

Hours of exp.	Haemoglobin (g./100 c.c.)	Leucocytes (thousands per cu. mm.)	Platelets (c.c. per 100 c.c.)	Bleeding time	Coagulation time
0	13.2	16.2	0.35	4′	3′ 30″
31	13.4	—	0.35	3′ 35″	4′
55	13.0	11.6	0.38	4′	4′
81	14.2	10.8	0.36	5′	3′ 40″

NOTE.—Death at about 96 hr. After the first five injections, somnolentia for about 15–20 minutes. From the 6th injection, inappetence and the somnolentia became more prolonged. No blood in stools. At autopsy only great hyperaemia in intestines and central nervous system.

TABLE III

DOG 437–3♀ 7.4 KG.

Eight intravenous injections (each 0.4 g. per kg.) in the period of 36 hours

Hours of exp.	Haemoglobin (g./100 c.c.)	Platelets (c.c./ 100 c.c.)	Bleeding time	Coagulation time
0	11.40	0.40	5′	4′
3	11.10	0.35	6′	4′
7	12.80	0.42	4′	4′
10	12.80	—	5′	—
14	13.60	—	5′	4′ 30″
24	13.80	0.35	5′	4′
31	14.40	—	6′	—
37	14.40	0.42	5′	4′ 30″

NOTE.—Slight somnolentia, defaecation, and urination after the 1st injection. After the 2nd and 3rd injections somnolentia for about 15 minutes. After the 4th injection great excitation and sound sleep for about 2 hr.; after the 6th injection sound sleep. Bloody stools between 7th and 8th injections. Death around 45th hr. At autopsy conspicuous hyperaemia of intestinal mucosa. No purpuric lesions.

haematological manifestation. Appreciable loss of weight was observed in this animal (Table IV).

As we expected, the coagulation time was normal at all times in these dogs submitted to urethane. The bleeding time was inversely proportional to the platelet numbers. Bleeding times as high as 1 hr. 20 min. have been observed in severe cases. Clot-retraction was proportional to the platelets number. Skin fragility to mechanical trauma was observed in all animals after purpura had been established.

TABLE IV

DOG 496–26♂ 12.0 KG.

Daily administration of 0.2 per kg. intravenously for 45 days

Day of experiment	Haemoglobin (g./100 c.c.)	Leucocytes (thousands per c.c.)	Platelets (c.c. per 100 c.c.)	Bleeding time	Coagulation time
0	11.4	11.0	0.25	4′	5′
7	10.8	14.0	0.28	5′	4′
21	11.1	7.8	0.22	5′	4′
39	10.5	—	0.15	5′	4′ 30″
45	10.8	10.5	0.28	4′	3′ 40″

NOTE.—Urethane was not administered on 15th and 19th days of the experiment. Slight somnolentia of short duration after injection from 35th day on. Urethane stopped on 45th day.

Intestinal haemorrhages, manifested by the presence of blood in faeces, were very conspicuous during the period of haemoglobin drop, as has been described in the anaemia produced in dogs by oestradiol benzoate or anti-platelet serum (Cruz, Silva, and Mello, 1945).

SUMMARY

Administration of urethane by vein produces in dogs a similar purpura picture to that obtained with subcutaneous injections. For experimental investigation of the mechanism of purpura, intravenous injections are safer and more reliable because abscess formation, so commonly observed when the subcutaneous route is employed, does not occur.

The best dose of urethane for the production of purpura in dogs by intravenous administration is 0.4 g. per kg. body weight per day. Higher doses (1 g. per kg. body weight) killed the animals by acute intoxication in a few days before purpura was established. A smaller dose (0.2 g. per kg. body weight) did not produce purpura after daily administration for 45 days.

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