COMPARISON OF TETRADECYL SULPHATE OF SODIUM WITH OTHER SCLEROSANTS IN RATS

W. K. BLENKINSOPP

From the Department of Pathology, St. Mary's Hospital Medical School, London, W.2

Received for publication November 9, 1967

THE 2 sclerosants widely used in the treatment of varicose veins of the leg are tetradecyl sulphate of sodium (Fegan, 1963) and ethanolamine oleate (Dodd and Cockett, 1956; Foote, 1960). Tetradecyl sulphate of sodium is also used in the treatment of haemorrhoids (Cowett, 1948; Wright, 1953) and in this situation its competitor is 5 per cent phenol in almond oil (Graham-Stewart, 1962; Clark, Giles and Goligher, 1967). Hitherto no comparison of the effects of these 3 sclerosants appears to have been published, despite their very extensive use. The present work, in rats, reports on their efficiency as vein sclerosants, the mortality following intravenous injection, and the incidence of ulceration following intradermal or subcutaneous injection. A detailed study of the effect on the veins of injection of tetradecyl sulphate of sodium is reported elsewhere (Blenkinsopp, in preparation).

MATERIAL AND METHODS

Male albino rats weighing 150–180 g. were used throughout; before injection of sclerosant they were anaesthetised by intraperitoneal injection of chloral hydrate solution.

The sclerosants used were 3 per cent and 1 per cent tetradecyl sulphate of sodium (TSS), 5 per cent ethanolamine oleate, and 5 per cent phenol in almond oil. As phenol in almond oil proved almost entirely ineffective as a sclerosant, the mortality following its intravenous injection and the ulceration following its intradermal or subcutaneous injection were not studied.

Intravenous and perivenous injection of sclerosant.—The femoral vein was exposed by skin incision over the lower thigh and the injection was made into or around its distal part. Forty-eight veins were exposed to perivenous injection of 0·1 ml. of sclerosant; 70 veins were given a 1 sec. exposure to sclerosant by i.v. injection; and 104 veins were given an exposure of longer than 1 sec. to sclerosant by i.v. injection (Table I). The volume of ethanolamine and phenol injected was 0·1 ml., the longer exposure was 60 sec., and the rats were killed 4 weeks after injection; the volume of TSS injected was 0·1–0·4 ml., the longer exposure was 3–60 sec., and the rats were killed between 6 days and 1 yr, after injection.

When the rats were killed, each injected vein was excised with the adjacent nerve, artery and muscle, fixed in formol-saline, and processed for histology. Transverse paraffin sections (5 μ) were cut at 1 mm. intervals, and at each level 1 section was stained with haematoxylin and eosin, 1 with haematoxylin-elastic-van Gieson, and 1 by the Gordon-Sweets method for reticulin.

Mortality following intravenous injection of sclerosant.—Seventy-four rats were each given a slow i.v. injection of 3 per cent TSS or ethanolamine (during 20 sec.) into 1 exposed femoral vein. The volumes injected and the deaths which ensued are given in Table II. All the deaths occurred within 2 hr. of injection, and the survivors were killed at intervals from 1–28 days after injection. A full post-mortem examination was made of each animal, including microscopy of the liver, spleen, kidney, adrenal, brain and 3 lobes of lung.

Intradermal injection of sclerosant.—Fifteen rats were given an i.d. injection into each ear of 0·05 ml. of sclerosant: 10 ears were injected with 3 per cent TSS, 10 with 1 per cent TSS, and 10 with ethanolamine; the injection sites were examined daily for 14 days after injection.

Ten other rats were given an i.d. injection into each ear of 0·05 ml. of 3 per cent TSS (5 rats) or ethanolamine (5 rats); 1 rat in each group was killed at 1, 2, 4, 8 and 14 days, and paraffin sections of the formalin-fixed lesions were examined.

Subcutaneous injection of sclerosant.—Forty-eight rats were each given 3 s.c. injections of sclerosant: 0·6 ml. into the back of the neck (where the subcutaneous tissue is thick), 0·2 ml. into the inner aspect of the right thigh (where the subcutaneous tissue is thin), and 0·1 ml. into the inner aspect of the left thigh (Table III). The injection sites were examined daily for 14 days after injection.

Ten other rats were similarly injected with 3 per cent TSS (5 rats) or ethanolamine (5 rats); 1 rat in each group was killed at 1, 2, 4, 8 and 14 days, and paraffin sections of the formalin fixed lesions were examined.

RESULTS

Intravenous and perivenous injection of sclerosant

Examination of sections of the veins showed that "good" and "bad" results could be distinguished. A "good" result was characterised by almost complete occlusion of the vein lumen by fibrous tissue, leaving only narrow capillary channels with a total cross-sectional area of less than 5 per cent of the original lumen; a "poor" result consisted of a patent channel of more than 10 per cent of the original lumen (and was usually 80 per cent or more).

In a previous study of TSS (Blenkinsopp, unpublished), it was found that varying the volume of sclerosant used, varying the exposure time between 3–60 sec., and killing the rats between 6 days and 1 yr. after injection, made no difference to the degree of occlusion of the vein lumen. In order to simplify the presentation of the results these variations are not specified in the present paper. The results (Table I) show that intravenous injection of 3 per cent TSS was more effective than 1 per cent TSS, and phenol and ethanolamine were much less effective. Perivenous injection of all 4 sclerosants was almost entirely ineffective.

TABLE I.—*Comparison of the Effects of the Sclerosants on Veins*

	I.v. injection 1 sec. exposure		I.v. injection 3–60 sec. exposure		Perivenous injection	
Sclerosant	No. of "good" results	Total No. of results	No. of "good" results	Total No. of results	No. of "good" results	Total No. of results
3 per cent TSS	17	20	37	37	1	8
1 per cent TSS	8	14	28	35	0	8
Ethanolamine	3	20	5	16	0	16
Phenol	0	16	1	16	0	16

Comparison between sclerosants using the χ^2 test and the data for all i.v. injections gave:
3 per cent TSS was better than 1 per cent TSS, $P < 0{\cdot}005$
1 per cent TSS was better than ethanolamine, $P < 0{\cdot}0005$
Ethanolamine was better than phenol, $P < 0{\cdot}025$

Mortality following intravenous injection of sclerosant

The mortality following i.v. injection of TSS or ethanolamine is given in Table II. No pathology other than congestion of the heart and lungs was found in animals which died. In rats killed up to 8 days after injection the lungs showed a slight increase in the number of alveolar macrophages. One rat killed 2 days after 0·4 ml. 3 per cent TSS had massive recent hepatic necrosis, but no changes were found in the large blood vessels to account for this. No other abnormalities were found.

TABLE II.—*Mortality of Rats Following Intravenous Injection of 3 per cent TSS and Ethanolamine*

Volume of sclerosant (ml.)	3 per cent TSS		Ethanolamine	
	No. of deaths	No. injected	No. of deaths	No. injected
0·6	6	6	6	6
0·4	2	16	13	16
0·3	0	6	1	12
0·2	0	6	0	6

Intradermal injection of sclerosant

Following i.d. injection of 0·05 ml. of sclerosant into each ear, ulceration occurred at all of 10 sites of injection of ethanolamine, at all of 10 sites of injection of 3 per cent TSS, and at 4 of 10 sites of injection of 1 per cent TSS.

The histological appearance of the ears after i.d. injection of sclerosant was the same whether TSS or ethanolamine had been used. The epidermis, dermis, subcutis and underlying muscle were dead at 24 hr. and showed a moderate infiltration with polymorphs. At 4 days most of the polymorphs were replaced by mononuclear cells and fibroblasts; there was reticulin formation and foreign body giant cells were present in the dead muscle; the epithelium at the margin of the ulcer showed moderate hyperplasia. At 8 days the dead tissue was replaced by granulation tissue and the necrotic epithelium was shed, and at 14 days most of the ulcerated surface had been re-covered by epithelium. Sections of the ulcers stained with Martius Scarlet Blue showed no " fibrinoid " material, and sections stained with Biebrich Scarlet for eosinophils or with polychrome methylene blue for mast cells showed no evidence of participation by these cells.

Subcutaneous injection of sclerosant

The results of s.c. injection of sclerosant are given in Table III. The first ulcers appeared on the 3rd day, and the number of ulcers reached a maximum on the 10th day.

TABLE III.—*Number of Sites Showing Ulceration following Subcutaneous Injection of Sclerosant*

Sclerosant	No. of sites showing ulceration			
	0·6 ml. injected	0·2 ml. injected	0·1 ml. injected	Total
3 per cent TSS	6 (16)	9 (16)	3 (16)	18 (48)
1 per cent TSS	2 (16)	6 (16)	1 (16)	9 (48)
Ethanolamine	9 (16)	6 (16)	2 (16)	17 (48)

Number of sites injected is given in parentheses

The histological appearances after s.c. injection were the same whether TSS or ethanolamine had been used, and were the same as those following intradermal injection, except that in some cases ulceration did not occur.

DISCUSSION

Perivenous injection of TSS, ethanolamine oleate, or 5 per cent phenol in almond oil produced little or no occlusion of the vein, and i.v. injection of phenol

was equally ineffective (Table I). When given by i.v. injection, ethanolamine produced satisfactory sclerosis in some veins but was significantly inferior to 1 per cent TSS, which in turn was significantly inferior to 3 per cent TSS. On this experimental evidence the sclerosant of choice is 3 per cent TSS.

Comparison of the mortality following intravenous injection of 3 per cent TSS with that following i.v. injection of ethanolamine indicated that both had a very wide safety margin. Of 16 rats given 0·4 ml. 3 per cent TSS, 2 died; this dose is equivalent to 4·8 g. in 160 ml. for a 70 kg. man on a weight-for-weight basis. Reiner (1946) found that the LD50 for 20 g. mice given intravenous TSS was 1·8 mg. in 0·1 ml. (equivalent to 6·3 g. in 350 ml. for a 70 kg. man). These amounts are enormously larger than the usual maximum dose in man, which is 0·03 g. TSS in 1 ml. However, the mortality in rats given an i.v. injection of ethanolamine was greater than that in rats given 3 per cent TSS (Table II).

TSS has been shown to be remarkably free from systemic toxic effects. Reiner (1946) gave mice 0·1 mg. i.v. once then i.p. 12 times in 3 weeks, and found no abnormality apart from peritonitis. Benaglia, Robinson, Utley and Cleverdon (1943) gave rats up to 0·87 g./kg., and rabbits, monkeys and dogs 0·1 g./kg., orally in 24 hr., and found no appreciable toxicity apart from some inhibitory effect on the growth rate. Smyth, Seaton and Fischer (1941) fed TSS in 25 per cent aqueous solution by stomach tube to rats and estimated the LD50 at 0·13 g./kg. They also gave rats 0·15 g./kg. daily in the drinking water for 30 days and noted no toxic effects. The solitary instance of hepatic necrosis found in the present study is difficult to explain; this lesion has not been seen in about 2000 rats in which the same anaesthetic technique has been used, but its isolation suggests that it is unlikely to be attributable to TSS. Apart from this finding the absence of toxic effects agrees with the other reported work. In man, ethanolamine has been known to produce a severe allergic response (Foote, 1944), and 1 fatality has occurred following its injection (Shelley, 1939), although Truman (1942) found no evidence of an allergic response in guinea-pigs injected with ethanolamine, and in the present study no toxic effects were observed.

The present report confirms Reiner's (1946) finding in animals that ulceration is very likely to occur if injection of sclerosant is made into the skin, and Merlen (1949) commented on this in man. Reiner (1946) found no lesion after the subcutaneous injection of 0·1 ml. of TSS in concentrations of up to 5 per cent in rabbits, and Merlen (1949) considered that in clinical use subcutaneous injection produced no appreciable reaction; however, Reiner (1946) examined his animals only up to 48 hr. after injection, and in the present work ulceration was not seen until the 3rd day and did not achieve a maximum incidence until the tenth day, when it was found at 44 of 144 sites.

SUMMARY

The sclerosants tetradecyl sulphate of sodium (TSS), 5 per cent ethanolamine oleate and 5 per cent phenol in almond oil were compared in rats.

The sclerosant activity of perivenous injection of all 3 sclerosants, and of i.v. injection of the phenol, was virtually nil. Intravenous injection of ethanolamine produced some sclerosis, but was significantly inferior to 1 per cent TSS, which in turn was significantly inferior to 3 per cent TSS.

Intradermal injection of 3 per cent TSS or ethanolamine was always, and s.c. injection was frequently, followed by ulceration.

The mortality following i.v. injection was slightly higher from ethanolamine than from 3 per cent TSS on a volume for volume basis, but in clinical practice the safety margins of both are extremely wide.

Intravenous injection of 3 per cent TSS or ethanolamine was free from systemic toxic effects, although one rat showed an unexplained liver necrosis after injection of 3 per cent TSS.

I am grateful to Miss Jennifer Gock Chew for technical assistance.

REFERENCES

BENAGLIA, A. E., ROBINSON, E. J., UTLEY, E. AND CLEVERDON, M. A.—(1943) *J. ind. Hyg. Toxicol.*, **25**, 175.

CLARK, C. G., GILES, G. R. AND GOLIGHER, J. C.—(1967) *Br. med. J.*, ii, 12.

COWETT, M. P.—(1948) *Am. J. Surg.*, **76**, 770.

DODD, H. AND COCKETT, F.—(1956) ' The Pathology and Surgery of Veins of the Lower Limb. ' Edinburgh (Livingstone).

FEGAN, W. G.—(1963) *Lancet*, ii, 109.

FOOTE, R. R.—(1944) *Lancet*, ii ,390. (1960) ' Varicose Veins: A Practical Manual. ' 3rd Ed. Bristol (Wright).

GRAHAM-STEWART, C. W.—(1962) *Br. med. J.*, i, 213.

MERLEN, F.—(1949) *Bull. Soc. fr. Phlébol.* **3**, 23.

REINER, L.—(1946) *Proc. Soc. exp. Biol. Med.*, **62**, 49.

SHELLEY, H. J.—(1939) *J. Am. med. Ass.*, **112**, 1792.

SMYTH, H. F., SEATON, J. AND FISCHER, L.—(1941) *J. ind. Hyg. Toxicol.*, **23**, 478.

TRUMAN, S. R.—(1942) *J. Lab. clin. Med.*, **27**, 586.

WRIGHT, G. L.—(1953) *Am. Surg.*, **19**, 799.