Anti-inflammatory activity of copper salicylates applied to rats percutaneously in dimethyl sulphoxide with glycerol

S. J. BEVERIDGE*, W. R. WALKER†, M. W. WHITEHOUSE, * *Department of Physical Sciences, Newcastle College of Advanced Education, P.O. Box 84, Waratah, N.S.W., Australia 2298,* †*Department of Chemistry, University of Newcastle, Shortland, N.S.W., Australia 2308 and Department of Experimental Pathology, John Curtin School of Medical Research, Australian National University, Canberra, A.C.T., Australia 2600*

Copper salicylate ethanolate, applied to the shaved dorsal skin of rats in ethanol–glycerol (4:1) containing excess salicylic acid, shows both anti-inflammatory and anti-arthritic activity (Walker et al 1980). The copper is not very rapidly absorbed from this particular formulation (Alcusal). Furthermore, it is difficult to apply copper complexes formed with salicylate esters, in this ethanol–glycerol vehicle, because of solubility limitations.

This communication describes some properties of two copper-salicylate formulations in dimethyl sulphoxide (DMSO) with glycerol (Dermcusals, DCS) that show considerable anti-inflammatory activity when applied dermally to rats. This activity is also seen in adrenalectomized animals and is greater than the sum of the individual activities of the salicylate and DMSO content of the DCS preparations; the difference evidently representing the contribution of the copper content. On a mole for mole basis, Dermcusal prepared from salicylic acid is at least 3 times as potent as the corresponding ethanolic copper salicylate formulation (Alcusal).

Female Wistar or DA × Lewis rats (160–200 g) were shaved under light ether anaesthesia to expose approximately 20 cm^2 of dorsal skin immediately below the neck. Formulations were applied 2–24 h later in dimethyl sulphoxide–glycerol (4:1 v/v) once only, in a volume equivalent to 5 ml kg^{-1}. Acute paw oedema was induced 2·5 h later by inoculating each rear paw with 1 mg Na carrageenan (Marine Colloids) or 0·2 mg zymosan (Sigma Chemical Co.) in 0·1 ml of 0·15 M NaCl. The consequent increase in paw thickness was measured with a micrometer screw gauge.

Polyarthritis was induced in male DA × Lewis rats (240g) by tail inoculation with a *M. tuberculosis*/squalane adjuvant (= day 0) and scored on days 12 and 17 thereafter (Whitehouse & Walker 1978). These arthritic animals were treated once daily on days 12–15 inclusive.

Bilateral adrenalectomies were performed on male Wistar rats (150 g) via ventral mid-line incision. Animals failing to gain weight between 20 and 90 h after operation (drinking 0·15% NaCl) were discarded.

Dermcusal-A was prepared by dissolving copper hydroxide (1·0 g) in DMSO (80 ml) containing salicylic acid (7·0 g) with gentle warming, then adding glycerol (20 ml) to give a deep green clear solution (pH 4·3), stable for at least four months at room temperature (20 °C). A less acidic formulation (Dermcusal-E, pH 5·0) was prepared by dissolving copper acetate monohydrate ($Cu(OAc)_2.H_2O$, 2·0 g) in DMSO (80 ml) containing ethyl salicylate (8·5 g, Fluka AG.) and then adding glycerol (20 ml). A reference formulation of ethanolic copper salicylate (Alcusal), pH 3·7, was

* Correspondence.

Table 1. Effect of dorsally applied copper-salicylates on two acute paw oedemas in Wistar (W) and Dark Agouti × Lewis (DA × LEW) hybrid rats. All materials applied to shaved dorsal skin in vehicle (=DMSO-glycerol, 4:1 v/v), 5 ml kg^{-1} except as noted.

	% Inhibition of paw oedema induced with:							
	Carrageenan				Zymosan			
	W		DA × Lew		W†		DA × Lew	
Formulation	2h	4h	2h	4h	1h	2h	2h	4h
$Cu(OH)_2$-salicylic acid (DCS-A)	46	53	47	39	43	21	36	22
Salicylic acid (alone)	14	18	44	32	22	16	−08	−05
Vehicle	15	14	08	06	12	17	−06	−08
Cu $(OAc)_2$-ethyl salicylate (DCS-E)	36	30	49	44	54	30	56	03
Ethyl salicylate	27	30	48	46	24	25	10	−05
$Cu(OAc)_2$	04	11	N.D.		12	12	14	04
Alcusal* ×1	24	32						
×3	58	53						

* Same composition as Dermcusal except that vehicle = ethanol-glycerol (4:1 v/v) *in lieu* of DMSO-glycerol; applied once or three times.

† Because the Wistar rats gave an early peak response to zymosan oedema, only 1 and 2 hour readings are reported.

N.D.—Not determined. Negative values = increase over untreated controls (not significant).

obtained by dissolving copper hydroxide (1·0 g) in ethanol (80 ml) containing salicylic acid (7·0 g) and then adding glycerol (20 ml). All three formulations contained 0·1 M Cu with a copper : salicylate ratio of 1 : 5.

Each preparation was tested in groups of 5 rats. As the vehicle in these DCS formulations (DMSO-glycerol) also showed some anti-oedemic activity, all results were expressed relative to oedema/arthritis manifest in shaved but untreated controls.

Table 1 records some results of testing these two copper salicylate formulations in DMSO–glycerol, namely with excess acid (DCS–A) or with the ester (DCS–E). They were applied to the upper dorsum of rats, before inducing an acute paw oedema with either carrageenan or zymosan. Two different strains of rats were employed (Wistar and DA × Lewis), differing (a) in their rate of oedemic response to zymosan (Wistar giving an earlier peak response), and (b) apparent sensitivity to dermally administered salicylates.

The data (Table 1) indicates that:

(i) DCS-A was approximately three times as potent as the comparable copper salicylate (acid) formulations in ethanol–glycerol (Alcusal) in suppressing carrageenan paw inflammation.

(ii) In intact Wistar rats, both salicylic acid (7% w/v) in DMSO–glycerol and DMSO (4 ml kg^{-1}) with glycerol (1 ml kg^{-1}) alone showed insignificant anti-oedemic activity.

(iii) In DA × Lewis rats, salicylic acid alone (in DMSO-glycerol) suppressed the carrageenan paw oedema but not the zymosan paw oedema.

(iv) Copper acetate with ethyl salicylate in DMSO–glycerol (DCS–E) was approximately equipotent with the acid formulation (DCS–A) in inhibiting both the carrageenan and zymosan paw oedemas in Wistar and DA × Lewis rats.

(v) Ethyl salicylate (8·5% w/v) in DMSO–glycerol mimicked salicylic acid in inhibiting carrageenan oedema in both Wistar and DA × Lewis rats and showed some activity against the zymosan oedema in Wistar rats.

(vi) While ethyl salicylate did not affect the zymosan oedema in DA × Lewis rats (cf. salicylic acid), the addition of copper acetate (yielding DCS–E) conferred anti-oedemic activity.

(vii) Copper acetate (2% w/v) applied by itself in DMSO–glycerol was inactive in these assays.

These data shows the problems in collating drug effects on two experimental oedemas developed in different rat strains. However, these findings certainly indicate that copper salicylates must penetrate the dermis in sufficient quantity to show pharmacological activity, largely attributable to the copper content (Sorenson 1978; Walker & Beveridge 1979). In healthy adrenalectomized Wistar rats, single applications of DCS-A (= 5 ml kg^{-1}) inhibited carrageenan paw oedema

Table 2. Effect of salicylates applied externally in DMSO-glycerol, ± copper (II), on established adjuvant-induced polyarthritis in male DA × Lewis rats (± s.e.). Signs of arthritis scored on days 12 and 17 (after adjuvant inoculation).

Vehicle	Treatment* (Daily dose)	Mean increase in thickness (mm) Rear paws	Tail	Δ Infl. Forepaws**	Mean Δ Weight (g)
	× 1	2·4±0·2	1·1±0·1	4+	−10
	× 2	2·3±0·2	1·0±0·1	4+	−11
Dermcusal-A					
	× 1	1·4±0·2	0·8±0·1	3+	−07
	× 2	0·5±0·1	0·4±0·1	+	−03
Salicylic acid†					
	× 1	2·2±0·3	1·2±0·2	4+	−07
	× 2	2·2±0·2	1·5±0·2	5+	−15
Dermcusal-E					
	× 1	1·1±0·1	0·7±0·1	2+	0
	× 2	0·4±0·2	0·5±0·2	+	−04
Ethyl salicylate††					
	× 1	2·8±0·3	1·0±0·1	3+	−12
	× 2	2·7±0·2	0·9±0·2	4+	−08
No treatment	—	2·7±0·3	1·0±0·1	3+	−09

* Given on days 12–15 inclusive. Each dose = 5 ml kg^{-1}. Group of 4 rats/single dose; 3 rats/double dose (and untreated group).

** Difference in inflammation score for both forepaws, mean values: each forepaw was scored on scale 0–4+.

† Equivalent to DCS-A but without Cu.

†† Equivalent to DCS-E but without Cu.

(2 h) by 49% whereas salicylic acid applied in DMSO–glycerol caused less than 21% inhibition.

These two rat strains differ not only in their sensitivity and rate of response to individual oedemogens but also most probably in their skin permeability (facilitated by DMSO). This latter difference may be particularly critical in comparing systemic effects of topically applied agents, e.g. components of the Dermcusals, over the limited time period of oedema development.

The zymosan oedema is considered to be more complement-dependent and perhaps less sensitive to salicylates than the familiar carrageenan model of induced inflammation (Gemmell et al 1979).

When tested on DA × Lewis rats with established polyarthritis, both DCS-A and DCS-E applied dorsally for four consecutive days (5 ml kg^{-1} day^{-1}) appreciably reduced swelling of the tail and all four paws (Table 2) with no adverse effects, e.g., gross weight change, loss of agility and alertness. Double doses profoundly arrested the increase in paw/tail swelling and were also well tolerated, apart from causing some subsequent loosening of the skin at the site of application (notably without inflammation). Equivalent formulations without copper were devoid of anti-arthritic activity, when likewise applied dermally.

At 4 ml kg^{-1} given topically with glycerol, DMSO was not a significant anti-oedemic/anti-arthritic agent in our rats. Many reports suggest that DMSO does exert an anti-inflammatory effect (e.g. Brown 1971; Herschler & Jacob 1978). Only at much higher dose (12 ml kg^{-1}) did topically applied DMSO suppress the carrageenan oedema.

In the Dermcusal formulation, the primary role of the DMSO is to solubilize and promote the permeation of copper salicylate into the stratum corneum. Replacing the copper with iron (III) in this formulation did not increase the anti-oedemic activity above that shown by salicylic acid.

The copper acetate–ethyl salicylate formulation (DCS-E) falls within the pH range accepted as promoting hydration of the stratum corneum (Katz 1973), which in turn is reported to facilitate dermal absorption (Wepierre & Marty 1979).

We are indebted to Mrs D. J. Whitehouse for adrenalectomizing rats. S.J.B. would like to acknowledge financial assistance from the G.H. Duncan Research Fund, N.C.A.E.

January 3, 1980

REFERENCES

Brown, J. H. (1971) Curr. Ther. Res. 13:536–540

Gemmell, D. K., Cottney, J., Lewis, A. J. (1979) Agents Actions 9:107–116

Herschler, R., Jacob, S. W. (1978) Communication from University of Oregon Health Sciences Centre, Dept. of Surgery, Portland, Oregon (25 pp. with 59 references)

Katz, M. (1973) in: Arients, E. J. (ed.) Drug Design. Vol. IV, Academic Press, New York, pp 93–143

Sorenson, J. R. J. (1978) Prog. Med. Chem. 15:211–260

Walker, W. R., Beveridge, S. J. (1979) Inorg. Perspect. Biol. Med. 2:93–125

Walker, W. R., Beveridge, S. J., Whitehouse, M. W. (1980) Agents Actions: 10 (1) in the press

Wepierre, J., Marty, J.-P. (1979) TIPS 1:23–26

Whitehouse, M. W., Walker, W. R. (1978) Agents Actions 8:85–90

Core temperature changes following administration of naloxone and naltrexone to rats exposed to hot and cold ambient temperatures. Evidence for the physiological role of endorphins in hot and cold acclimatization

J. A. THORNHILL*, K. E. COOPER, W. L. VEALE, *Division of Medical Physiology, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada T2N 1N4*

It has been reported (Holaday et al 1978) that peripheral administration of naloxone to rats, after acute and chronic heat exposure, precipitates an increase in colonic temperature above that seen in control animals given 0·9% NaCl (saline). It was suggested that naloxone mediated its responses by antagonism of pituitary endorphins, since the drug's effect was diminished when tested in hypophysectomized rats. From that study, endorphins were proposed to possibly play a physiological role in heat adaptation.

Naloxone, however, has also been shown to cause small, but significant changes in the core temperature responses of rats kept at 22 °C (Blasig et al 1978; Thornhill et al 1978) or when exposed to the cold (Goldstein & Lowery 1975). Thus we wanted to see what effect narcotic antagonists, in relatively high but non-toxic doses used previously (Holaday et al 1978; Goldstein & Lowery 1975), would have on the core temperature of rats acclimitizing to severe changes in ambient temperature. To do this, core temperature responses of rats were measured following a single subcutaneous injection of either of two narcotic antagonists of varying durations of action—naloxone and naltrexone HCl—to groups of animals exposed to 4 °C or 38 °C for different periods of time.

Male, Sprague-Dawley rats, appoximately 300 g, were maintained at an ambient temperature (Ta) of 22 °C until the experiments began. Rats were then kept

* Correspondence.