Analgesic and Local Anesthetic Activity of Dimethyl Sulfoxide

By RALPH W. MORRIS

To provide data with which to settle some of the current controversy over the clinical claims for dimethyl sulfoxide (DMSO) specific pharmacologic tests were performed. DMSO is "relatively nontoxic" to rodents but appears to be more toxic as the phylogenetic order is ascended. Considerable irritation results from the introduction of DMSO into the conjunctival sac of the rabbit. No topical or nerve block anesthetic activity was demonstrated. Infiltration anesthetic potency of DMSO was so low as to be of no therapeutic importance. No analgesia, free of sedation, was found. Topical application of trypan blue with DMSO produced considerable dermal staining but no distribution of the dye as contrasted with the rapid systemic absorption and distribution of the dye when given subcutaneously with DMSO. No evidence was obtained in these experiments which could support clinical claims made to date.

CONSIDERABLE controversy has been aroused by recent claims of striking clinical activity by dimethyl sulfoxide (DMSO) in certain musculoskeletal injuries and inflammations, including acute and chronic subacromial bursitis, acute musculoskeletal trauma, osteoarthritis, rheumatoid arthritis, gouty arthritis, scleroderma, and Dupuytren's contracture (1, 2). In an attempt to resolve some of the controversy specific pharmacologic tests were selected to determine whether the reported activity in man could be demonstrated in laboratory animals. Special consideration was given to testing for DMSO activities most nearly associated with the therapeutic claims of "local analgesia," "penetrant carrier," and "relatively nontoxic" (1).

EXPERIMENTAL

DMSO, certified reagent grade, Fisher Scientific Co., was kept in tightly sealed bottles since DMSO is very hygroscopic, rapidly picking up 70% of its own weight in water while evolving as its heat of solution 60 cal./Gm. at 20° (3). Analgesic activity was made relative to several standard analgesics (*i.e.*, morphine sulfate, meperidine hydrochloride, and codeine phosphate). Analgesic activity was determined in Carworth Farms (CF-1) mature female albino mice by the classical hot plate method, essentially that of Ohlsson (4), and by the Bianchi and Franceschini (5) modification of the Haffner tail clamp method (6). The DMSO was administered intraperitoneally, 0.01 ml./Gm. of mouse body weight, in at least 4 doses between 5.5 and 11.0 Gm./Kg., diluting with 0.9% saline whenever necessary. Design was such that the sample size was at least 10 and usually 20 mice per test dose of each drug. Three types of local anesthetic activity were investigated: topical anesthesia by the classical corneal response in rabbits, infiltration anesthesia by the guinea pig intracutaneous wheal technique of Bulbring and Wajda (7), and nerve block anesthesia by a modification of the frog sciatic nerve-gastrocnemius muscle preparation of Sinha (8). Three to 6 guinea pigs with 6 response sites per guinea pig were used for each dose of drug in the intracutaneous wheal test. Procaine and lidocaine hydrochlorides served as standards.

The effects of DMSO on membrane permeability were determined by noting the latent periods required for the adsorption, distribution, and subsequent discoloration of connective tissues from topically and subcutaneously administered trypan blue in mice. All median response doses (*i.e.*, ED_{50} and LD_{50}) and their 95% confidence limits were determined by the method of Litchfield and Wilcoxon (9), whereas all other statistical analyses were performed by the methods of Snedecor (10).

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RESULTS AND DISCUSSION

Toxicity.—A primary concern with the introduction of any new substance into human pharmacology is the precision with which projections of acute human toxicity can be made from tests on laboratory animals. Table I summarizes the available data on the acute lethality of DMSO as obtained from the literature (11, 12) and experiments recently completed in this investigator's laboratory. From Table I it would appear that a variably increasing lethality to DMSO occurs as the phylogenetic order is ascended.

Ventricular fibrillation and death occurred within 3 min. after the administration of 1.65 Gm./Kg. of 100% DMSO into the marginal ear vein of the rabbit. The LD_{50} for intravenous DMSO in female albino rabbit was 1.34 Gm./Kg. based on 3 doses between 1.10 and 1.65 Gm./Kg. given to a total of 28 rabbits. Female rabbits appear to be more sensitive to DMSO than are rodents, but are about equally sensitive as male dogs (Table I). DMSO

TABLE I.—ACUTE LETHALITY OF DIMETHYL SULFOXIDE

Animal	Rt. of Administration[a]	LD_{50} in Gm./Kg. (95% Confidence Limits)
Chicken	Oral	13.74[b]
Mouse		
	i.v.	5.75[c]
	i.p. (male)	10.10 (9.22–11.06)
	i.p. (female)	9.95 (9.35–10–59)
	Oral	21.40[c]
	Oral	21.98[b]
Rat		
	i.v.	5.36[c]
	Oral	28.30[c]
Rabbit	i.v. (female)	1.34 (0.96–1.88)
Dog		
	i.v. (male)	2.40[c]
	i.v. (male)	1.50[d]

[a] Routes of administration: i.v., intravenous; i.p., intraperitoneal. [b] From Brown *et al.* (11). [c] From Wilson *et al.* (12). [d] Preliminary value based on intravenous titration in 4 dogs.

might therefore be said to be "relatively nontoxic" (1) when relating chickens or rodents to rabbits and dogs. To date human toxicity following topical application consists of warmth, erythema, itching, local urticaria, increased skin pigmentation, dermatitis, fatigue, and lethargy (2). This observed topical toxicity plus the relative ease with which DMSO is said to be absorbed through the epidermis (1) should arouse considerable concern over the potential systemic toxicity of DMSO in man if it were applied *via* a route of administration (*e.g.*, parenteral) or to hyperpermeable tissue (*e.g.*, inflamed or open wounds) thereby facilitating an even more rapid and extensive absorption and distribution than by the topical route. There has been a recent report of a death possibly related to an acute allergic reaction to DMSO (13). Such reports must be thoroughly investigated, however.

Analgesia.—The unusual term "local analgesia" as used by Rosenbaum *et al.* (1) does not give recognition to the striking pharmacologic difference between analgesics and local anesthetics. Therefore, specific tests were made to determine if DMSO was a local anesthetic, an analgesic, or both. (Table II.) The analgesic ED_{50} for DMSO and morphine sulfate in mice are 6700 and 15 mg./Kg., respectively, using the Haffner tail clamp method 20 min. after the intraperitoneal administration of the test solutions. The other analgesics tested are all less potent than morphine which is in keeping with their known clinical potency. Attempts to determine DMSO analgesic activity in mice by the classical hot plate method were unsuccessful because DMSO produced extensive sedation thereby making the mouse incapable of performing the test response (*i.e.*, jumping to the lip of a 10-in. high glass cylinder sitting on a thermostatically controlled hot plate maintained at 60 ± 0.5°). On the basis of this observation a sedative rather than analgesic ED_{50} for subcutaneously administered DMSO was determined as being 6.92 Gm./Kg. Criteria for the sedative end point was the presence of a thoroughly depressed, immobile, but not ataxic mouse which was still capable of righting itself from the dorsal to the ventral positions within a 30 sec. test period (*i.e.*, neither asleep nor anesthetized). Such similarity in the DMSO sedative and analgesia ED_{50} values seriously question whether the failure of the mice to respond to the Haffner tail clamp stimulus might not be equally as well explained on the basis of sedation as analgesia. This possibility is presently being explored.

TABLE II.—CENTRAL NERVOUS SYSTEM ACTIVITY OF DIMETHYL SULFOXIDE IN THE MATURE FEMALE ALBINO MOUSE

Pharmacologic Activity	ED_{50}, mg./Kg. (95% Confidence Limits)	No. Doses	Total Sample Size
Sedation, s.c.			
DMSO	6920 (3980–12040)	2	20
Analgesia, i.p. Haffner tail clamp			
DMSO	6700 (6200–7240)	5	110
Morphine sulfate	11.6 (9.47–14.21)	5	110
Meperidine HCl	38.3 (31.39–46.73)	3	90
Codeine phosphate	34.5 (28.51–41.75)	4	100
Dextropropoxyphene HCl	36.7 (31.75–42.43)	3	60

TABLE III.—LOCAL ANESTHETIC ACTIVITY OF DIMETHYL SULFOXIDE

	ED_{50} in % Soln. (95% Confidence Limits)
Topical	
Rabbit (female):	
DMSO	Inactive[a]
Infiltration	
Guinea pig (female):	
DMSO	51.0 (38.5–67.6)
Lidocaine HCl	1.17 (0.90–1.52)
Procaine HCl	0.84 (0.62–1.13)
Sciatic nerve block	
frog: DMSO	Inactive[a]

[a] No anesthesia produced by maximum dosage used, a 100% solution.

Local Anesthesia.—No alterations in the pupillary response to penlight illumination or to touch with a glass probe were noted during 2 hr. test periods following the instillation into the conjunctival sac of the rabbit of 0.25 ml. of DMSO solutions, varying from 30 to 100%. In contrast to the nonirritant report of Brown (11) all 24 female albino rabbits that received DMSO, irrespective of the per cent concentration, experienced considerable conjunctival inflammation and limbic corneal vasodilatation. The irritation and vasodilatation cannot be solely the consequence of the heat of solution (*i.e.*, counter-irritant activity) since even 30% solutions (well below the 62% equillibrium point of DMSO–water mixture) produced irritation and vasodilatation. The duration of the severe irritation (30.8 ± 20.7 min.) does not appear to be dose dependent; however, the experiments were not designed to provide statistical evaluation of such a regression function. A positive corneal reflex to touch with a glass probe (*i.e.*, an eyelid movement) was present before and throughout the 90 min. period following the introduction of all per cent solutions of DMSO into the conjunctival sac. In all 24 rabbits tested with DMSO, blockade of the corneal reflex was produced after 90 min., the blockade persisting for the remainder of the test period (*i.e.*, 120 min. in 19 rabbits and 165 min. in 5 rabbits). No explanation of this delayed blockade of the corneal reflex is possible, since the experiments were not designed with this question in mind.

The ED_{50} of DMSO as an infiltration anesthetic was 51.0% solution compared to the ED_{50} of 1.17 and 0.84% solutions for lidocaine and procaine hydrochlorides, respectively. (Table III.) No evidence of nerve block anesthesia was obtained when 100% DMSO was tested on each of four sciatic nerve-gastrocnemius muscle preparations; therefore, further nerve block testing was discontinued.

Membrane Permeability.—Topical application of 1% solutions of trypan blue in either distilled water or DMSO solutions varying from 50–100% to the shaved scapular area of the mouse failed to induce systemic absorption and distribution of trypan blue, although extensive staining at the site of application occurred with the solutions containing

DMSO. However, the administration of 1% trypan blue in solution with 50–100% DMSO facilitated the speed with which subcutaneous injections of 1% trypan blue were absorbed and distributed to the connective tissues in the ears, tail, and paws of mice. Trypan blue in DMSO solutions were significantly more rapidly distributed than 1% trypan blue in distilled water (*i.e.*, 6.9% more rapidly in 50% DMSO and 3.9% faster in 100% DMSO: each with $p < 0.001$). After 24 hr. all mice that had received topical administration of 1% trypan blue, with or without DMSO, showed no signs of the typical bluish discoloration of connective tissues. On the other hand, the previously noted statistically significant effect of subcutaneously administered DMSO on the absorption and distribution of trypan blue solutions were even more evident after 24 hr., *i.e.*, all visible connective tissues were dark blue after trypan blue in 100% DMSO, moderately blue in 50% DMSO, and pale blue when in distilled water.

All animals receiving DMSO, regardless of dosage, very rapidly (*i.e.*, usually within 2 or 3 min.) gave strong evidence in their expired air of extremely rapid DMSO metabolism, *i.e.*, to dimethyl sulfone in mice, guinea pigs, rabbits, and dogs (11, 14); to dimethyl sulfide in cats (15); and to unspecified metabolites in man (1, 2, 14). Shortly thereafter similar evidence could also be found in the urine, feces, and saliva.

The rapidity with which DMSO is metabolized jeopardizes precise determinations of chronic toxicities and confounds acute toxicities, especially when the DMSO is administered by any route other than the intravenous. Even DMSO data by the intravenous route of administration would have to be considered misleading if one accepts the literature reports that DMSO induces perivascular inflammation (12) and extensive protein denaturation as evidenced by the rapid development of hemolytic anemia (12, 15). No evidence of perivascular inflammation was seen in any of the 13 rabbits that survived the 1.10 Gm./Kg. dose of DMSO during the acute toxicity tests or during the 6 hr. of intravenous administration of DMSO in each of 4 dog carotid artery blood pressure preparations (16).

Unequivocal evaluation of the toxicologic and pharmacologic data for DMSO is therefore rather difficult. First, the physical properties, especially the heat of solution (3, 11), confounds attempts to attach chemical and/or biological mechanisms to observed pharmacologic responses. Second, the biochemical instability of DMSO in the systemic circulation is so great that an uncertain number of potentially and variably toxic metabolities are very rapidly produced. Third, determining precise values for such an impotent agent as DMSO makes for rather inaccurate estimates of its very subtle activities. Fourth, the public and scientific controversy attendant to the plethora of DMSO lay articles published to date[1] creates an atmosphere in which strictly objective evaluations by all parties is difficult. Recent statements by the Food and Drug Administration, U. S. Department of Health, Education, and Welfare (17–19), and in *Medical Letter* (2, 13) have introduced some stability into the very controversial therapeutic merits of DMSO.

On the basis of the experiments reported here, no evidence was obtained in support of the therapeutic claims of "local analgesia," "penetrant carrier," or "relatively nontoxic" in humans (1).

REFERENCES

(1) Rosenbaum, E. E., Herschler, R. J., and Jacob, S. W., *J. Am. Med. Assoc.*, **192**, 309(1965).
(2) *Medical Letter*, No. 11, 42 (May 21, 1965).
(3) Ranky, W. O., and Nelson, D. C., "Organic Sulphur Compounds," Karasch, N., ed., vol. 1, The Macmillan Co., New York, N. Y., 1961, Chap. 17, pp. 170–182.
(4) Ohlsson, L., *Acta Pharmacol. Toxicol.*, **9**, 322(1953).
(5) Bianchi, C., and Franceschini, J., *Brit. J. Pharmacol.*, **9**, 280(1954).
(6) Haffner, F., *Deut. Med. Woschshr.*, **55**, 731(1929).
(7) Bulbring, E., and Wajda, I., *J. Pharmacol. Exptl. Therap.*, **85**, 78(1945).
(8) Sinha, H. K., *ibid.*, **57**, 199(1936).
(9) Litchfield, J. T., and Wilcoxon, F., *ibid.*, **96**, 99 (1949).
(10) Snedecor, G. W., "Statistical Methods," 5th ed., The Iowa State University Press, Ames, Iowa, 1962, Chap. 2, pp. 35–65.
(11) Brown, V. K., Robinson, J., and Stevenson, D. E., *J. Pharm. Pharmacol.*, **15**, 688(1963).
(12) Willson, J. E., Brown, D. E., and Timens, E. K., *Toxicol. Appl. Pharmacol.*, **7**, 104(1965).
(13) *Medical Letter 7*, No. 20, 80 (September 24, 1965).
(14) Williams, K. I. H., Whittemore, K. S., Mellin, T. N., and Layne, D. S., *Science*, **149**, 204(1965).
(15) Distefano, V., and Borgstedt, H. H., *ibid.*, **144**, 1137 (1964).
(16) Morris, R. W., *J. Pharm. Sci.*, **50**, 438(1961).
(17) Food and Drug Administration, U. S. Department of Health, Education, and Welfare Statement, H46, November 11, 1965.
(18) Food and Drug Administration, *Federal Register*, November 25, 1965.
(19) Food and Drug Administration, U. S. Department of Health, Education, and Welfare Statement, H88, December 3, 1965.

[1] Lay articles have appeared in *Life, Time, Newsweek, Science and Mechanics, Confidential, Man's World, Saturday Evening Post, Medical World News, Chemical Week, Pageant, Mechanix Illustrated, Science Digest, etc.*