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ABSORPTION, EXCRETION, AND BIOTRANSFORMATION OF DIMETHYL SULFOXIDE IN MAN AND MINIATURE PIGS AFTER TOPICAL APPLICATION AS AN 80% GEL*

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ABSTRACT

The absorption, excretion, and biotransformation of dimethyl sulfoxide (DMSO) 80% gel, DEMASORB®, was studied in man and in miniature pigs. DMSO 80% gel (15 cc, t.i.d.) was applied topically to the elbows of human subjects and allowed to remain there for 30 minutes after each application. Under these conditions, daily absorption of DMSO 80% gel ranged from 25 to 40% of the total dose. DMSO 80% gel (15 g, t.i.d.) was completely absorbed within 4 hours after application to the shaved backs of miniature pigs. Both man and miniature pig transformed DMSO to dimethylsulfone ($DMSO_2$) and dimethylsulfide (DMS). DMSO and $DMSO_2$ were excreted in the urine, whereas DMS was eliminated in the expired air. In man, the relative amounts of DMSO and $DMSO_2$ in the plasma were similar to those found in the urine. The biological half-life of $DMSO_2$ in both the plasma and urine of man was 2.5 to 3 days. Urinary excretion of DMSO plus $DMSO_2$ ranged from 9 to 35% of the dose in both man and miniature pigs; only 1.6% of the dose was present in the feces of miniature pigs. Whereas $DMSO_2$ was the main excretory product in the urine of man, DMSO was the major component in the urine of miniature pigs.

Dimethyl sulfoxide (DMSO) has been used to facilitate the percutaneous absorption of drugs (1, 2, 3) and as a medication for the treatment of pain associated with various joints of the body (4). The present investigation has studied the metabolism and percutaneous absorption of a DMSO 80% gel preparation, DEMASORB®. This formulation of DMSO has properties that allow it to be readily applied and localized to an affected area of the body. Miniature pigs were employed as an animal model for preclinical studies since pig skin bears some resemblance to human skin, although the two are also distinctly different (5), and because of the increased use of swine in biomedical research (6). Data are also presented for the absorption and metabolism of DMSO 80% gel in man.

MATERIALS AND METHODS

Studies in man. Studies were conducted at Lankenau Hospital, Philadelphia, Pa. under the supervision of Dr. John J. Blizzard. DMSO 80% gel (15 cc, equivalent to 16.5 g) was applied t.i.d. around the elbow of normal male subjects to cover an area of approximately 250 cm^2. Samples of DMSO 80% gel were contained in individual jars. The exact weights of a jar and its contents, and of a plastic glove were determined. The contents of the jar was then applied by a gloved hand to the elbow of a subject, then the glove and the empty jar were reweighed together. Thus, the exact amount of gel applied each time could be determined. The drug remained on the skin for 30 minutes, then the unabsorbed portion was wiped off with tared paper toweling. Reweighing of the toweling revealed how much of the applied gel had been absorbed. The application of DMSO 80% gel t.i.d. was continued for 8 days. Urine was collected daily at 4-hour intervals, except from midnight to 8 AM, when an 8-hour collection was made. A sample of plasma was obtained once daily at 2 PM. Concentrations of DMSO and dimethylsulfone ($DMSO_2$) in urine and plasma were determined by gas chromatography.

Samples of expired air were collected from human subjects for the determination of volatile metabolites by having them breathe into a bag equipped with a check valve, permitting air to flow in only one direction. Each collection was carried out for 5 minutes. The contents of the bag were evacuated by an air pump through a solution of methanol (200 ml) cooled in a Dry Ice bath. A fixed volume of the expired air (28.3 l) was passed through the methanol solution, then the volume of air remaining in the bag was pumped out through a gas meter. The fixed

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volume plus the volume remaining in the bag represented the air expired in the 5-minute interval. The methanol solution was also analyzed by gas chromatography for the presence of dimethylsulfide (DMS).

Studies in miniature pigs. Male miniature pigs (7 to 9 kg) were obtained from Vita Vet Laboratories, Marion, Indiana. About 15 g of DMSO 80% gel was applied t.i.d. to the shaved back of each pig to cover an area of approximately 250 cm^2. The first application of drug on the first day contained 50 μCi of DMSO-^{14}C (New England Nuclear Corporation) with a specific activity of 3.33 μCi per gram of 80% gel. Drug application continued for 3 days, the drug being left on the skin to allow for total absorption after each application. The miniature pigs were housed individually in stainless steel metabolic cages that allowed for the separate collection of urine and feces. A commercial pig diet was supplied. Excreta were collected daily for 8 days and then the animals were sacrificed. After the animals were sacrificed, the interior surfaces of the metabolism cages were rinsed with 1 N HCl to recover any DMSO-^{14}C 80% gel that may have been transferred from the pig to the cage. These measurements indicated that less than 0.1% of the dose of DMSO-^{14}C 80% gel was present inside the cages. Concentrations of DMSO and $DMSO_2$ in urine were determined by gas chromatography; excretion in the feces was determined by liquid scintillation counting. A sample of feces was homogenized in 2 to 3 volumes of methanol. A weighed portion of the homogenate was digested in 1 ml of 0.5 N NaOH for 16 hours at 80° C, bleached with 30% hydrogen peroxide, neutralized with 0.2 ml of 2-ethylhexanoic acid, and counted in 15 ml of Bray's scintillation fluid (7). The pigs were necropsied on the eighth day after the initial application of DMSO-^{14}C 80% gel. Tissues removed from various organs were homogenized in 2 to 3 volumes of methanol. An aliquot (0.5 ml) of each tissue homogenate was digested in 2 ml of NCS solubilizer (Amersham/Searle) followed by the addition of 15 ml of a toluene scintillation fluid containing, per liter of toluene, 4 g of 2,5-diphenyloxazole and 0.2 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene. To determine whether any volatile metabolites were excreted, a sample of air expired by an anesthetized pig (pentobarbital, 30 mg/kg, i.p.) through an endotracheal tube was passed through a trap containing methanol and analyzed by gas chromatography. All measurements of radioactivity were made with a Packard Tri-Carb liquid scintillation spectrometer, Model 3380. Counting efficiency was determined by the use of external standardization.

Gas chromatography. DMSO, DMS, and $DMSO_2$ were detected gas chromatographically by a modification of the method of Wallace and Mahon (8). The Victoreen Model 4000 gas chromatograph that was used was equipped with 2 columns (6 feet × 1/8 inch diameter stainless tubing) packed with 20% carbowax (20 MM) on

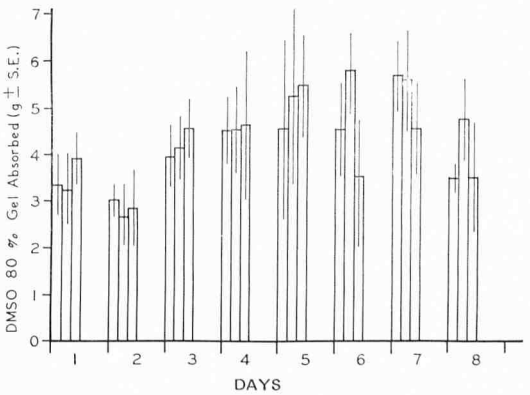

FIG. 1. Absorption of DMSO 80% gel in man. Each of the three subjects received 15 cc of DMSO 80% gel, t.i.d. Each bar is the amount of gel absorbed after a single application.

Chromasorb W. For the measurement of DMSO and $DMSO_2$, the system was operated isothermally at an oven temperature of 170°C, with the temperatures of the injector and the flame ionization detector set at 210 and 220°, respectively. For the measurement of DMS, the system was operated isothermally at an oven temperature of 40° C, with the temperatures of the injector and the flame ionization detector set at 180 and 210°C, respectively.

Each urine and plasma sample was diluted with 4 volumes of methanol. After the precipitated protein and other insoluble substances had been removed by centrifugation, 2 μl of the supernatant fluid from each sample was injected into the gas chromatograph for the determination of DMSO and $DMSO_2$. Similarly, a volume of the methanol solution (2 to 4 μl) used to trap DMS from the expired air was injected into the gas chromatograph. Authentic DMSO, $DMSO_2$, and DMS were used as standards. These compounds under the conditions described above, had retention times of 2, 6, and 0.5 minutes, respectively. The amount of each compound in the sample was determined by integrating the area under the peak produced by that compound. The lower limit of sensitivity of this method for DMSO and $DMSO_2$ was about 10 μg per ml of urine or plasma.

RESULTS

Percutaneous absorption in man. During the 30 minutes that DMSO 80% gel remained on the skin of human subjects, 25 to 40% of the topically applied drug was absorbed per day, as shown in Figure 1. The total amount absorbed was similar in all subjects. The greatest differences were found for the subjects from day to day rather than from application to application on any one day.

Plasma levels of DMSO and $DMSO_2$ in man.

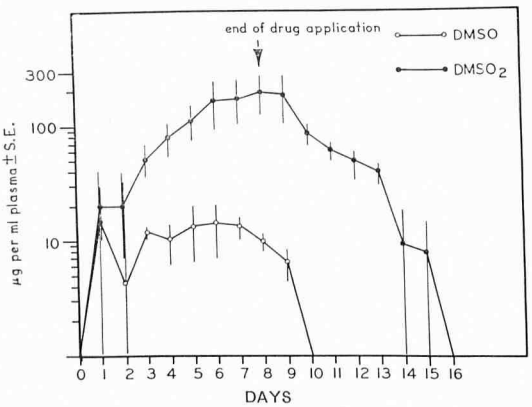

FIG. 2. Levels of DMSO and $DMSO_2$ in the plasma of humans. Each of the three subjects received 15 cc of DMSO 80% gel, t.i.d.

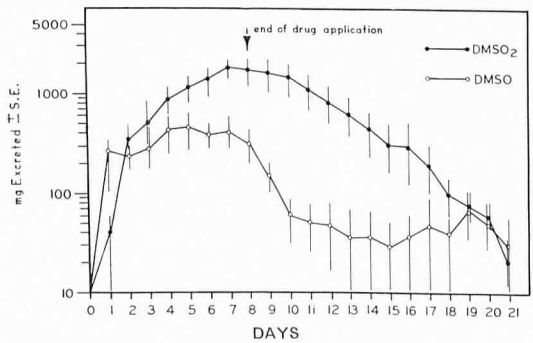

FIG. 3. Excretion of DMSO and $DMSO_2$ in the urine of humans. Each of the three subjects received 15 cc of DMSO 80% gel, t.i.d.

Figure 2 shows the average levels of DMSO and $DMSO_2$ in the plasma. The concentrations of $DMSO_2$ were greater than those of DMSO; the levels of both declined and were no longer detectable after 9 and 15 days, respectively. During the interval of 8 to 13 days, the half-life of $DMSO_2$ in the plasma was estimated as 2.5 to 3 days.

Urinary excretion in man. Figure 3 shows the average daily excretion of DMSO and $DMSO_2$ in the urine. The combined excretion of DMSO and $DMSO_2$ in the urine of three subjects ranged from 9.1 to 30.7% of the dose. $DMSO_2$ was the predominant urinary metabolite. The excretion of DMSO remained fairly constant throughout the period of drug application, but declined immediately after drug application had ceased. Only small amounts of DMSO were present in urine 2 days after the cessation of drug application and for the remainder of the collection period. $DMSO_2$ was excreted in increasing amounts relative to DMSO during the period of drug application; its excretion continued at a high level for 24 to 48 hours after drug application had ceased, then decreased gradually. Based on the slope for the curve of the excretion of $DMSO_2$ (Fig. 3), the half-life for its elimination is 2.5 to 3 days, the same as that observed for the plasma.

Metabolites exhaled by man. The Table shows the average daily amount of DMS expired by human subjects. DMS was present in the expired air during the entire period of drug application and was still being excreted 24 hours after the cessation of drug application. Sampling of the air expired by subjects was restricted to three 5-minute collections each day; no attempt was made to calculate the total amount of DMS excreted per day.

Absorption and excretion in miniature pigs. Each application of DMSO 80% gel to miniature pigs appeared to be totally absorbed within 4 hours. The urinary excretion of DMSO and $DMSO_2$ by miniature pigs is shown in Figure 4. Total urinary excretion ranged from 19.3 to 35.4% of the dose. Interestingly, DMSO was the predominant urinary metabolite in miniature pigs, whereas in man it was only a minor component. The excretion of DMSO diminished rapidly after the cessation of drug application and

TABLE

DMS in the expired air of man

Samples were taken for 5-minute intervals during each of the three periods in which DMSO 80% gel was present on the skin. Each figure for DMS expired is an average of figures for the three collection periods each day. The number of subjects participating in the test on each day is shown in parentheses.

Time of application	DMS expired
days	*mg/min ± S. E.*
1	14.9 ± 2.4 (3)
2	10.1 ± 2.6 (2)
3	6.7 (1)
4	9.2 (1)
5	9.3 (1)
7	10.2 ± 2.3 (2)
8 (last dose)	5.8 (1)
9	10.7 (1)

was no longer detected after 3 days, but the excretion of $DMSO_2$ was still pronounced 5 days after drug application had ceased. An average of only 1.6% of the dose was excreted in the feces of miniature pigs during 6 consecutive days of sample collection. As was the case in man, substantial amounts of DMS were detected in the air expired by miniature pigs, but these amounts were measured only qualitatively.

Residual DMSO and/or its metabolites in tissues of miniature pigs. Since the recovery of radioactivity in the urine and feces of miniature pigs constituted less than 30% of the total dose, the following tissues were examined for residual radioactivity: lung, kidney, heart, skin, liver, brain, testes, gall bladder, spleen, bone marrow, pancreas, lymph node, diaphragm, whole blood, bile, salivary, thyroid, thymus, and pituitary glands. No radioactivity was detected in any of these tissues. A trace amount of radioactivity was present in the skin in the area to which the DMSO-^{14}C 80% gel had been applied. Thus, the major portion of the dose given to miniature pigs appeared to be excreted in the expired air as DMS.

DISCUSSION

The present data for miniature pigs can be compared with those from earlier studies in rodents, dogs, and rabbits. Unaltered DMSO is the major urinary constituent excreted by miniature pigs, with $DMSO_2$ being the minor constituent; however, large amounts of DMS are excreted in the expired air. Conversion of DMSO to $DMSO_2$ has been demonstrated in rats, guinea pigs, and rabbits (9). Since rats, dogs, and guinea pigs excreted more than 50% of a topically administered dose in the urine (9, 10), in contrast to a maximum of 30% for miniature pigs, there appears to be a quantitative difference in the excretory patterns of these animal species. The available data from rats, rabbits, dogs, and miniature pigs indicate that fecal elimination of DMSO, its metabolites, or both, constitutes a very minor pathway (0.5 to 2% of the dose); the reabsorption of biliary products in the intestinal tract may, in part, account for this finding (10).

In man, Gerhards and Gibian (11) reported that DMSO and $DMSO_2$ were excreted in the urine after intravenous or topical administration of DMSO. The excretion of DMSO in the urine

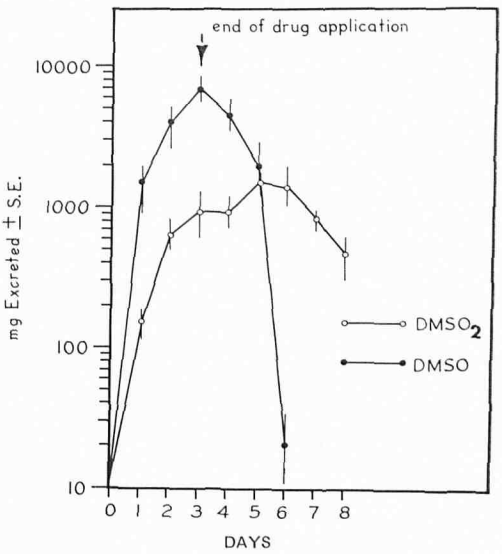

FIG. 4. Excretion of DMSO and $DMSO_2$ in the urine of miniature pigs. Each of the four animals received 15 g of DMSO 80% gel, t.i.d.

diminished rapidly and the drug was not detectable 24 hours after its administration, but $DMSO_2$ was detectable in the urine 96 hours after administration of DMSO. Kolb *et al.* (10) reported that about 40% of the dose was eliminated in human urine during the first week after topical administration of 50% DMSO; DMS was detected in the expired air during the first 6 hours after administration but none could be demonstrated after 24 hours. Thus, the results of the present studies in man show a metabolic disposition for DMSO 80% gel similar to that described previously for unformulated DMSO.

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