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Dimethyl Sulfoxide, a Convenient Solvent of 7,12-Dimethylbenz(a)anthracene for Intravenous Injection[1]

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SUMMARY

Dimethyl sulfoxide is a convenient solvent of 7,12-dimethylbenz(a)anthracene and related polycyclic aromatic hydrocarbons for i.v. injection. As judged by its ability to induce both adrenal necrosis and mammary tumors, this solution is at least as active as fatty emulsions of 7,12-dimethylbenz(a)anthracene now in general use. While the preparation of emulsions is time consuming and requires special equipment, the solution can be made quickly and economically in every laboratory.

INTRODUCTION

For the study of various biological actions of DMBA,[2] i.v. injectable fatty emulsions of this aromatic hydrocarbon are commonly used (4–6). However, the processing of stable lipid emulsions is rather complicated and expensive (12). While we were searching for a good solvent of 7-hydroxymethyl-12-methylbenz(a)anthracene, a weakly lipid-soluble hydrocarbon (14), it occurred to us that DMSO could also be used to prepare solutions of DMBA for i.v. injections.

In the present paper a simple method to produce a DMSO solution of DMBA will be described. Subsequently, a comparison will be made between the efficacy of this solution and an emulsion of DMBA which is presently in general use.

MATERIALS AND METHODS

Female Sprague-Dawley rats (Holtzman Farms, Madison, Wis.), with a mean initial body weight of 165 g (age, about 50 days), kept *ad libitum* on a standard diet (Purina laboratory chow, Ralston Purina Co., St. Louis, Mo.) and tap water, were used throughout the experiments.

Preparations used were the following: (*a*) 15% cottonseed oil-water emulsion containing lecithin, a nonionic emulsifier, and 5 mg of DMBA/ml as described by Schurr (12); (*b*) DMBA (Eastman Organic Chemicals, Rochester, N. Y.) dissolved by gentle heating (in a dark incubator at approximately 70° for 10 min) in DMSO (40 mg/ml). The solution was then immediately poured into bottles made of dark brown glass and each with a capacity of 10 ml, and for additional protection against light the containers were covered with aluminum foil. In addition to these precautions, the bottles were kept, except for the duration of the injections, in complete darkness at room temperature.

DMBA in both vehicles was injected into the jugular vein under light ether anesthesia.

The experiments in which adrenal necrosis was assessed were terminated 3 days after administration of DMBA by killing the animals with chloroform. The adrenal changes were graded at autopsy in terms of an arbitrary scale in which 0 represents no lesion; 1 represents just detectable, small necrotic foci, mostly in 1 adrenal; 2 represents coalescent partial necrosis in both adrenals, or complete unilateral adrenocorticolysis; and 3 represents bilateral complete necrosis involving the 2 inner layers of the adrenal cortex. The lesions were also verified histologically on paraffin-embedded sections (5 μ) stained with hematoxylin-phloxine.

In the tumor-induction experiment, the animals were examined at weekly intervals for palpable mammary tumors. This series was terminated when every rat had developed at least 1 tumor. For the sake of clarity, more technical details will be given in the description of the corresponding experiments.

RESULTS

Comparison of 2 Vehicles at Different Dose Levels of DMBA. A comparison was made between the adrenocorticolytic efficacy of DMBA given in the emulsion or dissolved in DMSO at 2-, 4-, and 6-mg dose levels. Table 1 shows that 2 mg of DMBA administered in emulsion are not sufficient to cause adrenal necrosis. However, when the same amount of DMBA dissolved in DMSO was given, 2 out of 10 rats developed very mild adrenal lesions. At the 4- and 6-mg dose levels, Schurr's emulsion and the DMSO solution were equally effective in producing severe bilateral adrenocorticolysis in every animal.

Chronological Comparison between Schurr's Emulsion and DMSO Solution of DMBA. Five mg of DMBA were injected i.v. in the form of Schurr's emulsion and in DMSO solution, respectively, to 7 groups of 5 to 15 animals each. At intervals of 6 hr, beginning 24 hr after the injection of DMBA, 1 group receiving the hydrocarbon in either of these vehicles was killed. Table 2 shows that the earliest time at which macroscopically detectable adrenal lesions were present was 36 hr.

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[2] The abbreviations used are: DMBA, 7,12-dimethylbenz-(a)anthracene; DMSO, dimethyl sulfoxide.

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Table 1

Comparison of 2 vehicles at different dose levels of DMBA

DMBA was injected i.v. either in Schurr's emulsion (5 mg/ml) or dissolved in DMSO (40 mg/ml). Three days later, the animals were killed and the presence of adrenal necrosis was verified.

Dose of DMBA (mg)	Vehicle	Adrenal necrosis	
		Incidence[a]	Scale, 0–3
2	Schurr's emulsion	0	0
	DMSO	2	0.3
4	Schurr's emulsion	10	3.0
	DMSO	10	3.0
6	Schurr's emulsion	10	3.0
	DMSO	10	3.0

[a] 10 rats/group.

Table 2

Chronological comparison of adrenal necrosis induced by DMBA given in emulsion or solution

Animals received i.v. (at 0 hr) 5 mg of DMBA either in Schurr's emulsion (5 mg/ml) or dissolved in DMSO (40 mg/ml). Between the 24th and 60th hr, at intervals of 6 hr, 5 to 15 rats from each series were killed and the adrenals were examined for the presence of necrosis.

Time of appraisal (hr)	Vehicle	Adrenal necrosis	
		Incidence[a]	Scale, 0–3
24	Schurr's	0/5	0
30	emulsion	0/10	0
36		1/10	0.1
42		4/10	1.2
48		9/15	1.6
54		8/10	2.4
60		10/10	3.0
24	DMSO	0/5	0
30		0/10	0
36		2/10	0.5
42		9/10	2.0
48		13/15	2.5
54		10/10	3.0
60		10/10	3.0

[a] No. of rats showing adrenal necrosis/total no. of rats in the group.

At 54 hr, 8 out of 10 animals receiving DMBA in emulsion showed adrenal necrosis, whereas the DMSO solution elicited in every animal maximal adrenocorticolysis. At 60 hr, all rats of either group exhibited severe bilateral adrenal necrosis.

Induction of Mammary Tumors by DMBA Dissolved in DMSO. For this purpose the schedule of Huggins *et al.* (5) was applied. Twenty animals received 2 mg of DMBA dissolved in 0.05 ml of DMSO i.v. on the 1st, 4th, and 7th day. The results of this experiment are summarized in Table 3. During the whole observation period of 12 weeks, 4 animals died of intercurrent infection; these were excluded from evaluation of tumor development. The 1st palpable tumors appeared 4 weeks after the 3rd DMBA injection. The last animal developed mammary tumors 8 weeks later. Fifty % of the animals had mammary tumors 5.5 weeks after DMBA administration; at the end of the experiment on the 12th week, the average number of tumors per rat was 1.68. Histologically, the tumors were adenocarcinomas without the tendency to develop metastases. In the same strain of rats, both the incidence and the morphological characteristics of tumors were similar when under otherwise identical conditions DMBA was administered in Schurr's emulsion (8).

Table 3

Tumor development following the administration of DMBA dissolved in DMSO

Two mg of DMBA dissolved in 0.05 ml of DMSO was given i.v. on the 1st, 4th, and 7th day. The experiment was terminated 12 weeks after the last DMBA injection.

No. of rats	20
Mortality	4
No. of rats with tumor	16
Appearance of tumors (wk)	
Range	4–12
50%	5.5
Mean	6.75 ± 0.61
No. of tumors/rat (mean)	1.68 ± 0.16

Stability of DMBA Dissolved in DMSO. For verification of its stability, the same solution was used to give injections at weekly intervals to groups of 5 rats each with 4 mg of DMBA in 0.1 ml of DMSO. During the entire observation period of 3 months, all animals given DMBA developed severe bilateral adrenal necrosis. In other words, no decline in the activity of the solution kept under circumstances described in "Materials and Methods" could be observed during 3 months.

The amount (generally 4 mg) of DMBA resulting regularly in bilateral adrenocorticolysis is dissolved in 0.1 ml of DMSO. Using a syringe with a volume of 1 ml, the injection of 10 animals lasts for several minutes. Hence, the question arose whether the light of an operating-room lamp used during the i.v. injections would influence the stability of the solution. For this purpose, 1 ml of the standard solution was exposed to direct illumination for 30 min in a 1-ml syringe kept at a distance of 1 m from an operating-room lamp. Subsequently, a group of 10 rats received this DMBA solution. All animals showed severe bilateral adrenocorticolysis at autopsy.

The Effect of the Amount of DMSO on the Adrenocorticolytic Action of DMBA. It has been postulated that, in order to be converted into its adrenocorticolytic derivative, DMBA has to be metabolized by the liver (15). Thus, it seemed reasonable to investigate whether this process would be influenced, in either way, by DMSO. To clarify this point, we injected 2 mg and 4 mg of DMBA, both dissolved in 1 ml of DMSO, into 2 groups of 10 animals each. Every rat receiving 4 mg of DMBA in this relatively high amount of DMSO developed bilateral adrenocorticolysis, whereas those which received injections of 2 mg of the hydrocarbon showed no adrenal lesions, with one exception. The livers of animals receiving DMBA dissolved in both the usual and the higher (up to 1 ml) amount of DMSO were examined histologically. At

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most, slight vacuolization in a few hepatic cells could be detected only following the larger doses of this solvent.

DISCUSSION

Stable fatty emulsion of DMBA for i.v. injection has been prepared by Schurr (12) and has been proven to be of excellent quality in several laboratories, including ours. However, it is not possible to produce an emulsion of similar quality under ordinary laboratory conditions because it requires a costly special homogenizer and a complicated time-consuming procedure. On the other hand, as revealed by the present experiments, an i.v. injectable preparation which is just as effective as the emulsion can be simply and economically obtained by dissolving DMBA in DMSO. This solvent has already been used for some time in various fields of experimental and clinical medicine (9). Moreover, amounts of DMSO necessary to dissolve both adrenocorticolytic and carcinogenic doses of this hydrocarbon are well tolerated by various species, including the rat (1, 13, 16).

It is known that DMSO influences enzymatic processes in various ways (3, 11). Therefore, the question arose whether the amount of DMSO injected i.v. as the solvent of DMBA could affect reactions known to depend upon enzymes. In additional experiments (not reported here in detail), we tried to inhibit the DMBA-produced adrenocorticolytic changes with enzyme inducer steroids; we obtained similar results by administering DMBA i.v. in Schurr's emulsion, in DMSO solution, or given p.o. in corn oil. As revealed by the present investigation, the amount of 4 mg of DMBA was equally active whether dissolved in 0.1 ml (1st experiment) or in 1 ml (5th experiment) of DMSO. Accordingly, a 2-mg dose of DMBA was practically ineffective in causing adrenal damage when given either in 0.05 ml or 1 ml of the solvent. Therefore, we can conclude that DMSO itself neither inhibits nor aggravates the DMBA-induced adrenal lesions. Furthermore, it had been found earlier that DMSO given in drinking water starting before or after a single gavage of DMBA had no influence on the number and development of tumors or on the mortality rate during an observation period of 18 months (2).

While this work was in progress, Huggins *et al.* (7) described a method for the preparation of fatty emulsions of polycyclic hydrocarbons which are poorly soluble in oil. They dissolved these aromatic compounds in DMSO in order to bring this solution into a 15% fatty emulsion prepared by the method of Schurr. Possibly, these aromatics could also be injected like DMBA in the form of DMSO solutions without introducing into the blood stream other foreign substances (*e.g.*, oil, lecithin, and nonionic detergent) as the constituents of an emulsion.

The melting point of DMSO is 18.45°; hence, the solution should not be kept at low temperatures. However, refrigeration, and even sterilization during the processing of the solution, are superfluous, since it has been shown that DMSO itself is a powerful bactericidal agent (10). Accordingly, we never observed opacity or any other sign of microbial growth in the solution. A bottle was kept for 5 months in the laboratory and was used many times without particular sterility precautions, and, as revealed by subsequent microbiological control, the solution remained sterile.

On the basis of present results, we recommend DMSO as a convenient solvent of DMBA for i.v. injections.

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