Congenital malformations induced by dimethyl sulphoxide in the golden hamster

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Dimethyl sulphoxide (DMSO) is a relatively simple organic compound with a molecular weight of 78. It has been reported to be exceptionally non-toxic and has been used extensively in biological systems as a cell preservative, including freeze storage (Porterfield & Ashwood-Smith, 1962; Dougherty, 1962), and as a radioprotective agent against X-irradiation in mice (Ashwood-Smith, 1961). Recent clinical investigations in man have suggested that DMSO has a remarkable array of effects, including anti-inflammatory activity, bacteriostatic effects, and analgesic activity (Jacob, Bischel & Herschler, 1964). It has been used extensively as a vehicle for the topical application of various drugs because of its dramatic effect on enhancement of penetration of various drugs through the skin (Kligman, 1965).

Toxicologic studies in laboratory animals including dogs, mice and rats have revealed that anemia and peritoneal inflammation are the only detectable results of repeated intraperitoneal injections of this compound. Repeated intravenous injection of DMSO resulted in perivascular inflammation and localized thrombosis in mice and rats and a reversible hemolytic anemia in dogs (Willson, Brown & Timmens, 1965). 90% DMSO caused no systemic symptoms when applied topically to the trunks of healthy human subjects daily over a 6 months period (Kligman, 1965).

Preliminary studies (Ferm, 1966) have shown that DMSO exerts a profound effect on embryonic development in the golden hamster. These initial experiments revealed that a single intraperitoneal injection of 0.5 ml of 100% DMSO into a pregnant female hamster on the eighth day of gestation provoked a significant teratogenic response. Frequent and specific malformations consisting primarily of exencephaly were induced in a large number of embryos. The present study reports further investigations on the teratogenic effect of DMSO and its particular effect on the developing nervous system.

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MATERIAL AND METHODS

Times of matings of female hamsters were obtained, and the morning after the night of mating was considered to be the first day of gestation. On the 8th day of gestation the animals were anesthetized with Nembutal and injected with various dilutions of DMSO ranging from 50 to 8250 mg/kg in saline (Table 1), made up so that the volume injected was 0.5 ml/100 g body weight. Most of the animals were injected intravenously with the dilutions of DMSO. However, the maternal animals receiving levels of 5500 mg/kg of DMSO (undiluted) did not tolerate this level and died within a few minutes, showing signs of muscle spasm and hyper-extension of the extremities. Another series of animals received 5500 mg/kg as a single intraperitoneal injection into unanesthetized animals. However, for some reason, when this same dose-level was diluted to 1.0 ml with saline, the maternal animals survived the intravenous injection. The DMSO was obtained from Nutritional Biochemicals Co., Cleveland, Ohio. The dose-level was calculated on the basis of the density of DMSO (1 ml = 1.1 g). All animals tolerated the injections well except that those receiving 2500, 3800 and 5500 mg/kg (diluted to 1.0 ml) showed signs of generalized muscular tremors for about 1 min following injection and those receiving 5500 and 8250 mg/kg intraperitoneally had some abdominal muscle rigidity for the same post-injection period.

Three days later (day 11 of gestation) the maternal animals were sacrificed and the embryos recovered. The effect of the treatment on maternal weight changes, and the number of embryonic resorption sites were recorded. The embryos were examined for gross external malformations under a dissecting microscope, and examples of certain developmental malformations were photographed. A few other animals, not included in Table 1, were sacrificed later in gestation in order to record specifically the progress of the exencephalic lesion.

RESULTS

Table 1 summarizes the data obtained from these experiments. Low levels of intravenously injected DMSO produce no significant embryocidal or teratogenic response, no different than several hundred saline-injected control animals used in this laboratory over the past several months. Not until levels approaching 2500 mg/kg are reached is there any significant effect on embryonic development. The developmental malformations noted in these 11-day-old embryos were strikingly specific. A lesion of the cranial end of the embryo was noted as early as the ninth day of gestation (Plate 1, fig. A). This lesion consisted of non-closure of the neural tube as compared to normal development for this day (Ferm, 1965). On the tenth day of gestation the consistency of this defect persisted and could be detected in surviving embryos from then until near term (Plate 1, fig. B; Plate 2, figs. C, D, E). Other malformations were also observed.
Fig. A. Well-preserved hamster litter-mates recovered on the 11th day of gestation from a mother who had received 8250 mg/kg DMSO intraperitoneally on day 8 of pregnancy. These embryos demonstrate a severe arrest of development and are similar to 9-day-old embryos. The anterior neural tube in all of them has failed to close and appears to be quite expanded. ×6.

Fig. B. 11-day-old hamster litter-mates from a mother who had received 5500 mg/kg of 100 % DMSO on day 8 of pregnancy. The embryo at the lower left is grossly normal, but the rest of the litter demonstrates a remarkably consistent degree of exencephaly. ×2.5.

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Fig. C. 11-day-old hamster embryos from a mother who had received 5500 mg/kg of 100% DMSO intraperitoneally on day 8 of pregnancy. Four other litter-mates had the same defect. Compare with Fig. B. ×8.

Figs. D and E. Although not included in the data of Table 1, these figures are included to show the progression of the exencephaly in older fetuses. Fig. D is the sole surviving 12-day-old embryo of a litter from a mother receiving 8250 mg/kg on day 8 of pregnancy: note marked exencephaly and bilateral cleft lip (×6.5). Fig. E is a 13-day-old hamster from a mother treated with 5500 mg/kg of DSMO intraperitoneally 5 days previously: compare the degree and extent of exencephaly with the previous figures. ×4.

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These consisted of cleft lip and palate (Plate 2, fig. D), limb abnormalities, rib abnormalities and a most unusual case of conjoined twins. The incidence of exencephaly was by far the most consistent finding and on three occasions entire litters were affected to the same degree (Plate 2, fig. B).

Except for the immediate post-injection reaction described, the maternal animals suffered no apparent ill-effects from this treatment. Their weight gain was normal and histologic examinations of the placentas revealed no obvious lesions.

Table 1. Teratogenic and embryocidal effect of dimethyl sulphoxide on hamster embryos when injected into pregnant hamsters on the eighth day of gestation

<table>
<thead>
<tr>
<th>Dose-level (mg/kg)</th>
<th>Route of administration</th>
<th>No. of mothers treated</th>
<th>Av. wt. gain following treatments (g)</th>
<th>Total no. of embryos treated</th>
<th>Total no. of dead embryos</th>
<th>Total no. of abnormal embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>I.V.</td>
<td>5</td>
<td>13</td>
<td>66</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>250</td>
<td>I.V.</td>
<td>5</td>
<td>14</td>
<td>57</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>500</td>
<td>I.V.</td>
<td>5</td>
<td>18</td>
<td>77</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>1000</td>
<td>I.V.</td>
<td>5</td>
<td>12</td>
<td>66</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>2500</td>
<td>I.V.</td>
<td>5</td>
<td>14</td>
<td>67</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>5500</td>
<td>I.V.*</td>
<td>6</td>
<td>9</td>
<td>77</td>
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<tr>
<td>5500</td>
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<td>5</td>
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<td>67</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>8250</td>
<td>I.P.</td>
<td>5</td>
<td>8</td>
<td>64</td>
<td>10</td>
<td>18</td>
</tr>
</tbody>
</table>

* Diluted to 1·0 ml per animal.

DISCUSSION

As a teratogenic agent, DMSO provoked malformations not unlike those induced by vitamin A (Marin-Padilla & Ferm, 1965). The induction of exencephaly in the golden hamster embryo following vitamin A treatment has been attributed to a direct toxic effect of the vitamin on head mesenchyme inducing necrotic changes in the head mesenchyme and cephalic somites (Marin-Padilla, 1966). DMSO has been reported (Weissmann, 1965) to affect the solubility of steroids and thus to increase their effect on the stability of lysosomes, a phenomenon which suggests a similarity of action to the effect of vitamin A on the lysosomes of certain tissues. It is of interest that for some unknown reason no cases of spina bifida have been yet observed in any of the DMSO-treated animals. In contrast to the findings with vitamin A, the incidence of other malformations, such as rib fusions, microphthalmia, limb abnormalities, and cleft lips, have been unusually low.

The remarkable frequency and similarity of the developing exencephaly in the treated embryos points to a rather specific teratogen–organ response, which may well be time-related. Examples of this teratogen–organ response may be
found in the well-known effect of thalidomide on the developing human limbs (Taussig, 1962), and the effect of cortisone on raising the incidence of cleft palates in an inbred strain of mice (Fraser, Kalter, Walker & Fainstat, 1954). While this teratogen-organ effect may be time-related, further studies on the effect of DMSO in relation to earlier and later stages or pregnancy seem warranted, but it is important to note that the hamster embryo differentiates from a pre-somite stage to a limb-bud embryo between the eighth and ninth days of gestation (Ferm, 1965).

The transient muscular tremors following intravenous injection of DMSO under anesthesia and the abdominal muscular rigidity following intraperitoneal injection have been noted in non-pregnant rats and mice (Willson et al. 1965). These responses and reactions may be of significance in the present experiments but their transient nature and the almost immediate recovery of the maternal animals makes this explanation for the teratogenicity of DMSO unlikely. Direct observations of older hamster fetuses in utero during maternal DMSO administration may be useful in evaluating the uterine smooth muscle response and the possibility of placental transmission of DMSO with a similar direct stimulatory effect on the fetus.

The relationship of the amounts of DMSO used in these experiments to therapeutic and possible teratogenic levels in the human are difficult to determine. In most clinical uses of DMSO the drug has been applied directly to the skin (Kligman, 1965) from where it disseminates to systemic tissues. It has also been used as a vehicle for the percutaneous administration of other drugs. Thus, both the rate and total amount of DMSO absorbed in these clinical situations of acute and chronic administration of DMSO is not known. Moreover, the differential susceptibility of various mammalian species to the same teratogen is most striking. Thalidomide, for example, has a potent teratogenic effect on the human embryo (Taussig, 1962) but a significantly less striking teratogenic effect on the hamster embryo (Homburger, Chaube, Eppenberger & Nixon, 1965).

The reported effects of DMSO on cell permeability and skin penetration raise some interesting questions in relation to the problem of placental transfer. While the small molecular size of this compound makes it almost certain that it itself crosses the placental membrane with ease, its important solvent properties for a wide range of compounds provides the basis for further investigations, including experiments combining this drug with a variety of other teratogenic, subteratogenic or suspected teratogenic agents.

**SUMMARY**

1. Pregnant golden hamsters were injected with dilutions of dimethyl sulphoxide ranging from 50 to 8250 mg/kg on the eighth day of gestation. Examination of the embryos 3 days later revealed that no embryocidal or teratogenic effect was noted until levels of 2500 mg/kg were reached. At higher levels, malfor-
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1. On a injecté à des femelles gestantes de hamster doré des dilutions de diméthylsulfoxide allant de 50 à 8250 mg/kg, le 8ème jour de la gestation. Les embryons recueillis trois jours plus tard ont montré qu'il n'y avait pas eu d'effet embryocide ou tératogène jusqu'à ce qu'on ait atteint des doses de 2500 mg/kg. Pour des doses plus élevées, on a trouvé des malformations telles que exencephalie, fusion des côtes, microphthalmie, anomalies des membres et bec-de-lièvre.

2. L'exencephalie a été la malformation la plus communément notée et trois portées complètes étaient atteintes de cette malformation, presque au même degré.

3. Il n'y a pas eu d'effet appréciable du DMSO sur le gain de poids ou la santé maternels au cours de la période expérimentale.

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REFERENCES


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