EFFECT OF INTRA-UTERINE ADMINISTRATION OF DIMETHYLSULFOXIDE ON FERTILITY OF RATS

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Summary : A single intra-uterine injection of 60 μl of dimethylsulfoxide prevented implantation when administered before mating and induced resorption of the conceptus in rats when given during early pregnancy.

Key words : dimethylsulfoxide, intra-uterine, implantation, resorption, litters

INTRODUCTION

Dimethylsulfoixide (DMSO) had no effect on implantation or conceptus maintenance when administered orally or intraperitoneally (9). However, a broad spectrum of pharmacological properties and therapeutic applications were reported for DMSO (7, 10, 12). Microquantities of DMSO was reportedly used as solvent in the chemical contraception for the males (8) and as a chemical irritant, its effect on the vas deferens luminal epithelium was extensively studied (11). Accordingly, the effect of a single intrauterine injection of microquantities of DMSO on implantation and conceptus maintenance was investigated in rats.

MATERIAL AND METHODS

Adult male and cycling female rats of Holtzman strain weighing between 200 to 250 *g* were used in this study. Normal saline (60 μl/horn) was injected into both uterine horns of one group of female rats. DMSO (60 μl/horn; E. Merck, 99% pure) was injected into one uterine horn of one group and both horns of another group of rats. Care was taken so as to prevent any accidental puncture of blood vessels while injecting. These rats were cohabitated with adult male rats of proven fertility during proestrus after about 7 days of injection. The mated females were sacrificed on the 10th day of pregnancy. The number of corpora lutea and the number of normal and resorbed implantation sites were counted.

In another set of experiment, the female rats were mated with males and after confirmation of mating normal saline (60 μl/horn) and DMSO (60 μl/horn) were administered in both uterine horns of different groups of animals after 3, 6, 7 and 10 days of mating. Following laparotomy the number of corpora lutea were counted after 3 and 6 day of mating and the number of implantation sites were counted after 7 and 10 days of mating at the time of injection. The animals were kept under observation till parturition and the litter size was noted at delivery. The percentage of survival of fetuses are calculated (4) using the formula:

$$\frac{\text{Number of litters delivered}}{\text{Number of corpora lutea/number of implantation sites}} \times 100$$

The ovary, oviduct, uterus and cervix of animals treated with saline or DMSO before mating and only the uterus of animals treated after mating were dissected and fixed in Bouin's fluid for histological studies.

RESULTS

The treatment with normal saline had no effect on implantation (Table I). Unilateral administration of DMSO prevented implantation not only in ipsilateral treated but

TABLE I : Anti-implantation effect of intrauterine administration of dimethylsulfoxide (7 days before mating).

Treatment (60 µl)	*Uni-/Bilateral*	*No. of normal implantation sites (Mean±SE)*	*No. of resorbed implantation sites Left/Right*	*No. of rats implantation*		*No. of Corpora lutea (Mean±SE)*
				With	*Without*	
Normal Saline (6)*	Bilateral	10.15±1.08	Nil	6	0	12.5±2.58
DMSO (9)	Unilateral (left)	Nil	2/5(1) 0/4(1)	2	7	10.9±0.69
DMSO (12)	Bilateral	Nil	5/5(1) 4/4(1) 5/0(1)	3	9	11.8±3.24

*Figure in parenthesis indicates number of animals.

also in the contralateral uterine horn except in two rats where resorbed implantation sites were observed. One rat had 7 resorbed implantation sites, 2 in the treated and 5 in the control horn whereas the other rat had 4 only in the control horn but none in the treated horn. Bilateral administration of DMSO prevented implantation in 9 out of 12 rats and 3 rats had 10, 8 and 5 resorbed implantation sites respectively. The treatment had no significant effect on the number of corpora lutea and histological appearances of the genital organs.

The maintenance of conceptus was not affected by treatment with normal saline except on day 10 postmating where the percentage of survival of foetuses was reduced to 89.2 (Table II). The percentage of survival was nil when DMSO was administered on day 3 postmating and 2.7 when it was injected on day 6. The survival rate was increased to 25.5 and 73.9 when DMSO was administered on day 7 and day 10 postmating.

TABLE II : Effect of intrauterine administration of dimethylsulfoxide at different days mating.

Treatment (60 µl)	*Injection at days of pregnancy*	*No. of rats pregnant/ tested*	*%Survial* of the fetuses*
Normal Saline	3	12/12	94.3
	6	12/12	91.7
	7	11/12	93.5
	10	12/12	89.2
DMSO	3	0/18	0
	6	1/12	2.7
	7	4/13	25.5
	10	9/12	73.9

*Percent of survival = $\frac{\text{No. of litters delivered x 100}}{\text{No. of corpora lutea/No. of implantation sites.}}$

DISCUSSION

Intra-uterine administration of DMSO was effective in reducing the percentage of survival of foetus only when it was administered during the early phases of embryonic development. Subsequently, the effect was gradually reduced. The embryo-toxic effect was noted only on day 7 when the implants were only one day old and not on day 10 when the embryos were firmly established. It has been suggested that closure of the uterus (clasping) on the blastocyst is a prerequisite for the interaction of the blastocyst with the uterus (6). Accordingly, when the embryos are firmly established DMSO administered probably could not reach all over the implantation sites and therefore was not effective or the effect of DMSO was not sufficient to cause drastic changes of the uterine stroma for embryotoxic action. The appearance of resorbed implantation sites (Table I) in the control horn could possibly due to spilling of some DMSO to that side of the horn during the injection.

DMSO could also affect locally the milleau of the uterine fluid and endometrium so that substances required for the implantation process or subsequent development of the embryo, including hormones (2, 5) hormonally induced proteins (13), ions, immunoglobulins (11) and neutrophils were altered.

Contrary to the observation of Dubin *et al.* (4) intrauterine administration of normal saline did not prevent implantation. However, the anti-implantation or embroyotoxic effect of normal saline depend on the volume as well as the day of pregnancy when it is administered. Further studies on ultrastructure and biochemistry of uterine epithelium are necessary to elucidate the anti-implantation action of DMSO.

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