SOLVENT ARTIFACTS LIKELY TO BE INDUCED BY DIMETHYLFORMAMIDE*

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Summary : Dimethylformamide (DMF) is widely used as a drug solvent. We found DMF to have wide-spread pharmacological effects including depressant effect on CNS evidenced by a decrease in locomotor activity, body and limb tone and rectal temperature, and potentiation of pentobarbitone sleep. A dose-dependent hypotensive effect was seen in cats and rats. In rats, it was partially blocked by atropine and was associated with bradycardia. DMF antagonised the contractions of smooth muscle induced by many agonists. An atropine sensitive spasmogenic effect was observed on rabbit ileum at 20 *ml/l* and a direct relaxant effect at 50 *ml/l*. A positive inotropic effect on guinea pig atria was observed with 5 *ml/l*. The results indicate DMF concentrations that may not perhaps produce 'solvent artifacts' when used as a solvent.

Key words : DMF solvents pharmacology

INTRODUCTION

Dimethylformamide (N, N-dimethyformamide, DMF), the "Universal Solvent" has been used as a solvent in pharmacological screening programmes (1,8,11). However, DMF has its own pharmacodynamic effects (5,11,13,16). DMF results in hepatotoxicity (9), alters cell culture characteristics and causes a loss of tumorigenicity in cultured human carcinoma cells (6), exhibits cytotoxic effects (8) and inhibits lymphocyte mitogenesis (10).

In view of this, attempt was made to define solvent concentrations which could be safety employed in pharmacological studies.

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MATERIAL AND METHODS

Albino mice (18-22 *g*), albino rats (250-300 *g*), guinea pigs (350-450 *g*) and cats (2.5-3.5 *kg*) of either sex, and virgin rats (150-180 *g*) were used. All experiments were conducted at an ambient temperature of 24±0.5° C.

The following drugs were used : Dimethylformamide (analytical grade, 99.5%), acetylcholine chloride, adrenaline bitartrate, barium chloride, carbachol chloride, histamine dihydrogen phosphate, 5-hydroxy tryptamine creatinine sulphate, isoprenaline hydrochloride, noradrenaline bitartrate, atropine sulphate, chlorpheniramine maleate, pentobarbitone sodium, phentolamine methanesulphonate and propranolol hydrochloride. DMF was diluted with distilled water.

Intact animal experiments :

Acute toxicity and CNS effects : DMF was diluted in a manner so that each animal received a fixed volume (10 *ml/kg*. Control animals received equivalent volume of normal saline. Maximum tolerated dose (ip, po) was found in mice after administering graded doses of DMF. Experiments on general behaviour, locomotor activity, forced locomotor activity, inclined plane, hypnotic potentiation and effect on rectal temperature were conducted by a blind method to avoid subjective error, following the methods described earlier (14). Graded doses of DMF were administered to groups of mice, 5 in each group unless otherwise mentioned.

Blood pressure, heart rate, electrocardiogram and respiration : Cats were anaesthetised with pentobarbitone sodium (*mg/kg*, iv). Blood pressure was recorded from the right carotid artery by a mercury manometer and respiration was recorded with Marey's tambour. Femoral vein was cannulated for the administration of drugs. Standard lead II electrocardiogram was recorded on a polygraph (Grass, Model-7D) and heart rate was determined from the ECG signal. Responses to carotid occlusion (20 sec), vagal stimulation (2v, 10 Hz, for 15 sec.) and to adrenaline, noradrenaline, acetylcholine, isoprenaline and histamine (in doses of 0.5-1.0 $\mu g/kg$) were recorded before and after iv administration of DMF (0.025 to 0.5 *ml/kg*, volume 0 5 *ml/kg*).

Similar experiments were conducted in rats anaesthetized with urethane (1.5 *g/kg*. ip), blood pressure being recorded from right carotid artery with pressure transducer connected to a recorder (Ugo Basile). Drugs were given via femoral vein (volume, 0.5 *ml/kg*).

Isolated tissues :

Rabbit ileum, guinea pig ileum and vas deferens (emptied of their contents) were

set up in bath containing oxygenated Tyrode solution at 37±0.5°C : resting tension was 1.2 *g*, 1 *g* and 0.5-0.8 *g*, respectively. Rat uterus was set up using standard technique (7) at room temperature. Tone was recorded with isotonic levers (magnification, X 5-7). Tissues were rested for 30 min before experiments. Agonist (a single, submaximal dose, see Table II) was added till tissue responses were reproducible. The responses were elicited again after exposing the tissue to various concentrations of DMF (contact time, 2-10 min; enough for maximal inhibition) and % inhibition of agonist responses noted. EC_5 and EC_{50} of DMF for producing inhibition of agonistic effect was determined from plots (% inhibition vs concentration, log probability paper).

Guinea pig paired atria were mounted in oxygenated Ringer Locke solution at 37±0.5°C. The force and rate of contractions were recorded by a transducer (Type DYO, Ugo Basile) and a recording microdynamometer. DMF was added in cumulative manner at 2 min intervals.

RESULTS

Intact animal experiments :

Acute toxicity and CNS effects : The maximum tolerated dose of DMF in mice (ip and po) was 2.5 and 10 *ml/kg*, respectively. DMF (ip) had pronounced neurological effects as compared to po administration which had milder effects. There was marked decrease in respiration, body and limb tone, pinnal reflex and body temperature accompanied by piloerection, crouching, ptosis and ataxia on ip administration.

There was decrease in the locomotor activity in mice. The effect started 30 min after administration of DMF (2.5 *ml/kg*, ip) and the peak effect (89.82%) was observed at the 60th min. However, on po administration (5.0 *ml/kg*) the peak effect (80.22%) was at the 90th min. DMF (2.5 *ml/kg*, ip) resulted in development of neurological deficit in mice and 40% of animals fell from rota-rod. On po administration (5.0 *ml/kg*), only 20% of the animals showed this effect. DMF (2.5 *ml/kg*, ip or po) significantly ($P<0.01$, t-test) prolonged the pentobarbitone-induced sleeping time in mice (control sleep, min 85±7.08 : after ip DMF, 151.4±15.80; after po DMF, 148.4±14.99), though lower concentrations had no significant effect.

Effect on blood pressure, heart rate, electrocardiogram and respiration : An appreciable decrease in blood pressure was observed from 0.1 to 0.5 *ml/kg* doses in cats and rats (Table I). There was little or no effect on the heart rate, ECG and respiration of cats. Bradycardia was found in rats with high doses 0 2 *ml/kg* (Table I). The ECG and respiration were unaffected, however, transient apnoea was observed in a few animals.

TABLE I : Effect of dimethylformamide (DMF) on blood pressure of cat and on blood pressure and heart rate of rat.

DMF Concentration (ml/kg)	Cat*	Rat**	
	% decrease in blood pressure mm Hg. (Mean±S.E.M)	% decrease in blood pressure mm Hg. (Mean±S.E.M.)	% decrease in heart rate (Mean±S.E.M.)
0.1	9.57±3.40	10.10±6.58	—
0.2	20.15±3.19	17.85±2.35	8.93±1.09
0.4	40.50±5.75	29.34±1.24	64.37±2.92
0.5	53.35±3.32	31.38±2.07	65.71±7.73

*Number of experiments = 5-6

**Number of experiments = 6-8

In order to find out the mechanism of depressor response, various antagonists were tried and it is concluded that in cat it was a direct effect as the same was not blocked by atropine, propranolol or mepyramine treatments. In rats however, the depressor response was partially blocked by atropine pretreatment but was unaffected by pretreatment with propranolol or mepyramine. In bilateral vagotomised or atropinised rats and also in pithed rats, the depressor response was almost 50% less as compared to control; however, the effect on heart rate was completely antagonised.

Isolated tissues :

DMF *per se* had no major effect on tone of guinea pig ileum, guinea pig vas deferens and on rat uterus. However, responses to various agonists were inhibited when concentrations reached a threshold. Table II shows the potency of DMF in terms of threshold ihhibiting concentration (EC_5) and EC_{50}. Apart from this, safe limits of DMF concentrations were found to be as below (1) guinea pig ileum :- 5 *ml/l* for acetylcholine and histamine, 15 *ml/l* for barium; (2) guinea pig vas deferens :- 5 *ml/l* for acetylcholine and 10 *ml/l* for noradrenaline; (3) rat uterus :- 2.5 *ml/l* for 5-HT and 1.5 *ml/l* for carbachol. Responses of rabbit ileum to acetylcholine and noradrenaline were unaltered by DMF (2.5 *ml/l*). Concentrations more than 2.5 *ml/l* produced some spasmogenic effect which were not dose-dependent; however, 20 *ml/l* produced a marked spasmogenic effect which resisted chlorpheniramine maleate (upto 2.56×10^{-6}M, n=6), but was blocked by atropine (5.76×10^{-8}M, n=5) 50 *ml/l* of DMF had a relaxant effect which was not altered by propranolol (6.8×10^{-6}M, n=6) or phentolamine (2.6×10^{-5}M, n=6) or their combi-

nation (n=7). Effect of DMF on agonistic responses was reversible on washing (recovery time, 5-40 min).

TABLE II : Concentration of DMF (*ml/l*) producing (a) perceptible inhibition (EC_5) and (b) definite inhibition (EC_{50}) of responses of smooth muscle to various agonists.

Agonist	*Dose used (m)*	*Test model*		
		Guinea pig ileum	*Guinea pig vas deferens*	*Rat uterus*
Acetylcholine	1.1×10^{-7}	a) 2.62	3.00	—
	4.4×10^{-5}	b) 11.25	11.75	
Histamine	6.5×10^{-6}	a) 8.50	—	—
		b) 15.25		
Barium	7.4×10^{-4}	a) 10.50	—	—
		b) 22.50		
Noradrenaline	4.1×10^{-5}	a) —	9.00	—
		b)	20.75	
5-Hydroxytryptamine	9.9×10^{-6}	a) —	—	2.50
		b)		6.12
Carbachol	1.6×10^{-6}	a) —	—	0.95
		b)		2.65

Values are mean from 4-6 experiments
A single dose of agonists causing submaximal effect was used.

DMF (upto 2.5 *ml/l*) had no direct effect on either force or rate of contraction, however, concentrations of 5 *ml/l* to 25 *ml/l* produced positive inotropic effect (25% above the control or around, n=6). The concentration of 50 *ml/l* produced positive inotropic (34.02%±3.39, n=5) and a negative chronotropic (25.57%±3.23, n=4) effect.

DISCUSSION

That solvents used to dissolve a drug may interfere, through their own actions, the interpretation of drug action is well known. Actions of many solvents have been documented (2,3,4,12,14,15). DMF is known to exert CNS effects (5,13,16) as well as cardiovascular effects (11) Depressant effect on CNS function was confirmed in mice in this work, alongwith the reported potentiation of pentobarbitone sleep (16). Likewise, depressor effect reported in cat (11) was confirmed. Our experiments with antagonists suggest

that the effect may be direct in cats, while it was partly mediated through cholinergic mechanism in rats, suggesting species variability in DMF effects. Isolated rabbit jejunum responded to DMF with atropine sensitive spasm (moderate doses) or a direct relaxation (higher doses). Again, other smooth muscles did not exhibit a spasm, though responses to various agonists could be adversely affected (Table II). Our results clearly indicate that DMF concentration employed to dissolve a drug may become important deterrant factor in drug-effect analysis.

We tried to define which doses/concentrations of DMF could be used without producing solvent artifacts (as such or by altering agonist responses). Dose less than 1 *ml/kg* (po, ip), 0 1 *ml/kg* (iv) in intact animals and a concentration upto 2.5 *ml/l* in isolated tissue experiments may appear safe enough, though it should be less than 1.5 *ml/l* in rat uterus experiments. It may be, however, appreciated that even smaller amounts of DMF may lead to artifacts depending upon alteration in experimental conditions (viz., species/strain of animals, composition and temperature of bath fluids etc). Our work serves to warn against indiscriminate use of DMF as a 'safe solvent', and effect of solvent must always be provided as control in pharmacological analysis.

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