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Effects of Polysorbate 80 on the Absorption and Distribution of Oral Methotrexate (MTX) in Mice

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Summary. *This study is part of a programme of work aimed at improving the bioavailability of oral methotrexate (MTX). In preliminary experiments no significant effect of non-ionic surfactant polysorbate 80 (Tween 80) on absorption of 0.5 mg MTX · kg^{-1} in NMRI mice was observed except when the drug was given together with 6% polysorbate 80 in solution. Absorption from a higher dose of 3 mg MTX · kg^{-1} was increased when the drug was administered with 2% or 6% polysorbate 80. Plasma MTX measurements confirmed the significantly higher levels of MTX with 6% polysorbate 80 PO. In subsequent experiments, when Porton mice were used and 4 mg MTX · kg^{-1} was administered PO, higher plasma and brain levels of MTX were measured in animals given the drug with 6% polysorbate 80, suggesting the enhancement of MTX uptake by this non-ionic surfactant. Although the amount of MTX in the liver and kidney of mice given MTX with polysorbate 80 were not significantly different from the amounts in mice given MTX alone, the lower observed levels suggested that polysorbate 80 perhaps facilitates the elimination of the drug from these organs. The amount of plasma MTX in mice measured 1 h after oral administration of various MTX doses in the presence of 6% polysorbate 80 were significantly higher than the levels in mice given the drug without surfactant, but the significantly higher amounts of MTX in the brain were only observed following the doses of 2 and 6 mg MTX · kg^{-1}.*

Introduction

Although the maximum tolerated dose of systemic MTX in man ranges from 80 mg to 900 mg · m^{-2} without administration of citrovorum factor and was from 3,000 mg to 30,000 mg · m^{-2} when accompanied by citrovorum rescue [3], the use of MTX PO is only suitable for low-dosage regimens [19], due to the erratic and incomplete absorption of MTX from doses beyond 30 mg · m^{-2} [11, 21]. Only about 15%–50% of the drug is absorbed from a 50 mg · m^{-2} oral dose [18]. Bischoff et al. [2] have shown that the saturation point of the active transport mechanism lies between 0.1 mg and 1 mg · kg^{-1}, thus, at dosages exceeding this limit, the absorption is incomplete because the uptake in this region depends on slow passive diffusion of the drug [17]. An increase in the bioavailability of high-dose MTX from oral preparations might be achieved by giving the drug in divided doses [4, 16]. Christophidis et al. [5] have reported a bioavailability of over 80% when 50 mg MTX was given hourly to five outpatients with normal creatinine clearences.

Methotrexate has two carboxyl groups at the side chain, exerting a net negative charge. Since the drug is lipophobic, it requires a large activation energy to cross the negatively charged membrane barrier. The limited entry into avascular tumour masses [24] and poor penetration through the blood-brain barrier limit the use of MTX in treatment of solid tumours and brain cancers; if the drug is indicated in such cases a very high dose has to be given IV to achieve the required therapeutic levels followed by folinic acid rescue.

Surfactants, ionic or non-ionic, have been shown to be capable of increasing or decreasing the absorption of many drugs [6, 7]. Some of the factors determining the effects observed include the polarity and size of drug molecule, the micellar concentration of surfactants, drug-surfactant interaction, and the nature of the surfactant molecule [7, 8, 20]. Our unpublished data indicate the ability of polysorbate 80 to solubilise MTX. Polysorbate 80 has been administered PO at dosages up to 15 g daily for several months, with neglibible toxicity [14].

Polysorbate 80 was choosen as the model surfactant in our studies because of its low toxicity, allowing its sudy in patients. We have explored the possible usefulness of polysorbate 80 in increasing the absorption of MTX from the gastro-intestinal tract and its effect on the distribution of MTX in mice following the oral administration. Our studies have suggested that polysorbate 80 increases the absorption of MTX from the gastro-intestinal tract and enhances the uptake of the drug into the brain.

Materials and Methods

Materials. Methotrexate (MTX) powder obtained from Lederle Corporation was used without further purification. The purity of this drug assayed by HPLC was found to be 95%. Polyoxyethylene sorbitan mono-oleate (Polysorbate 80) manufactured by Honeywill Atlas was obtained commercially from Sigma Chemical Company, St. Louis, USA. The MTX analog, 4-[4[(2,4 diamino-6-quinazolinyl) methylamino] bonzoyl]aspartic acid, kindly supplied by Dr Leonard H. Kedda, Drug Synthesis and Chemical Branch, National Cancer Institute, Bethesda, IND., USA, was used as internal standard during the analysis of MTX with high-pressure liquid chromatography (HPLC). All other chemicals and the solvent were of analytical reagent grade.

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Analysis of MTX in Plasma or Serum by Enzyme Multiple Immunoassay Technique (EMIT®). The EMIT kit for MTX assay was obtained from Syva Ltd and reconstituted as prescribed. Plasma or serum to be assayed was separated from blood cellular components by centrifugation of heparinised or clotted blood samples. The samples and reagents were mixed according to Syva protocols, using pipette diluter (Syva), samples volume 50 µl and buffer volume 250 µl. The amount of MTX was measured by micro-sample spectrophotometer (Gilford Stasar II) and calculated by EMIT Clinical Processor (Syva CP-500).

Analysis of MTX in Tissue by HPLC. The method used by Watson et al. [23] for measuring MTX in plasma was adapted for measuring MTX in tissue samples. The HPLC instrument used was the ALTEX twin pump (Model 100A) with solvent flow controlled by the ALTEX programmer unit (Model 420). Detection was carried out at 254 nm by a variable wavelength LC-UV detector (PYE-UNICAM). The pre-packed stainless steel column used (0.46 cm internal diameter, 25 cm length), containing a strong anion exchange resin with average particle diameter of 10 µm (PARTISIL-10-SAX), was connected to an ALTEX 210 valve injector (100 p volume 20 µl). Tissue samples were homogenised by a CITENCO variable-speed homogeniser in the presence of 2 ml 2 *N* perchloric acid. The supernatants were separated after centrifugation by a MINSTREL centrifuge (2,000 rpm, 20 min) and were then transferred to tubes containing about 5 g ammonium sulphate. MTX was extracted with 2 ml 10 : 1 ethylacetate : isopropanol. The resultant organic layers were separated and evaporated in a stream of nitrogen. The residues were dissolved in 75 µl water. Samples (25 µl) were applied and eluted at a constant flow rate (1.5 ml · min^{-1}) with 0.025 *M* sodium phosphate buffer solution, pH 7. Standard curves of MTX, using area under the peak ratio of the drug versus internal standard, were generated by adding appropriate amounts of MTX to the blank tissue samples.

Experiments. Forty male and female NMRI mice weighing 25–35 g were divided into eight groups. Each of the first four groups was given 0.5 mg MTX · kg^{-1} body weight PO as solution containing zero, 0.1%, 2% or 6% polysorbate 80, while the other four groups received 3 mg MTX · kg^{-1} with the same order of polysorbate concentrations. The volume of each preparation administered was equivalent to 4 ml · kg^{-1} body weight. At intervals one mouse from each group was sacrificed at intervals, the blood withdrawn from the heart, transferred into tubes, and centrifuged, and the serum assayed for MTX by EMIT.

In another experiment, two groups of five NMRI mice each were given 3 mg MTX · kg^{-1} PO. One group received the drug as solution (4 ml · kg^{-1}) with 6% polysorbate 80 and the other group was given the drug without the surfactant. The samples were collected from the tail vein at intervals into heparinised capillary tubes. The samples were spun in a microhematocrit centrifuge and the plasma MTX was measured by EMIT.

Male Porton mice were used in the subsequent experiments. Two groups of 25 mice weighing 25–40 g were given 4 mg MTX · kg^{-1} PO or 4 ml · kg^{-1} as solution, formulated with or without 6% polysorbate 80. At intervals blood samples were collected from five mice in each group. The animals were killed and the brain, kidney, and liver were collected, and washed twice with ice-cold phosphate buffer saline. After blotting of the excess fluid, the organs were weighed for the wet weight and stored at −20° C until assayed for MTX. Plasma samples were assayed by EMIT and the tissues by HPLC.

In the last experiment, each of eight groups of mice received MTX PO as shown in Table 2. At 1 h post administration the animals were sacrificed, the blood samples were collected from the heart, and the brain was dissected and treated as before. Both blood and brain samples were assayed by HPLC.

The statistical analysis was performed using Student's *t*-test.

Results

The doses of MTX used in this study, 0.5 mg and 3 mg · kg^{-1}, represent the low- and higher-dose regimens, respectively. One lies below the saturation point of active transport as reported by Bischoff et al. [2] while the other is very much higher than the saturation limit. At 0.5 mg · kg^{-1}, no significant effect of surfactant on MTX levels in serum was observed except when the drug was given together with 6% polysorbate 80, where the maximum level was slightly higher than in mice given MTX without the surface-active agent. The higher maximum levels of MTX in mice given 3 mg MTX · kg^{-1} with 2% and 6% polysorbate (0.19 and 0.24 µg MTX · ml^{-1} serum, respectively), compared with 0.15 µg · ml^{-1} in mice given the same dose without the surfactant, suggest the enhancement of MTX absorption from the gastro-intestinal tract.

As shown in Fig. 1, there were significantly higher plasma levels of MTX in NMRI mice given 3 mg MTX · kg^{-1} with 6% polysorbate 80 at 30, 45, and 60 min post administration than in mice given the drug without the surfactant ($P = 0.025$, 0.05, and 0.05, respectively). In this experiment it was not possible to obtain plasma sample volumes sufficient for assay by HPLC and hence the EMIT system was used. Although the EMIT assay for plasma levels will probably cross-react with some MTX metabolites, the results here unequivocally show that polysorbate 80 caused an increase in overall absorption of the higher-dose MTX from the gastro-intestinal tract. In view of cross reactivity of MTX with the metabolites in the EMIT assay this second experiment was repeated. The HPLC method described was employed to analyse both serum and tissue

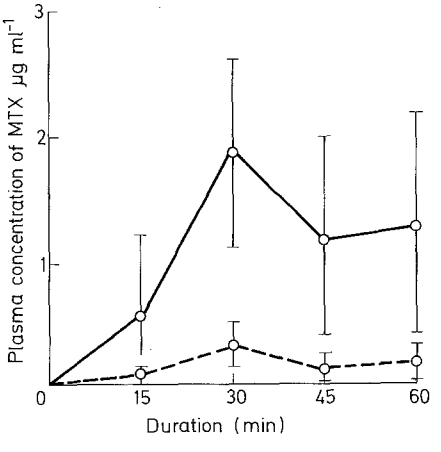

Fig. 1. Absorption of 3 mg MTX · kg^{-1} from the gastro-intestinal tract in NMRI mice following MTX solution with 6% polysorbate 80 (O———O) or MTX solution without polysorbate 80 (O- - - -O)

Table 1. The absorption and distribution of 4 mg MTX · kg^{-1} from the gastro-intestinal tract in Porton mice given the drug as solution with or without 6% polysorbate 80 (PS 80)

Tissue	MTX preparation given	Duration (h)	Amount of methotrexate (μg · ml^{-1} plasma or μg · g^{-1} tissue)[a]				
			0.5	1	2	4	24
Blood	Without PS 80		0.16 ± 0.09	0.09 ± 0.06	0.12 ± 0.06	0.08 ± 0.06	0.03 ± 0.06
	With PS 80		0.13 ± 0.06	0.29 ± 0.11	0.20 ± 0.06	0.18 ± 0.06	0.03 ± 0.04
Brain	Without PS 80		Not detected				
	With PS 80		–	1.07 ± 0.07	0.32 ± 0.11	0.32 ± 0.11	–
Liver	Without PS 80		2.86 ± 1.88	3.49 ± 1.80	2.85 ± 0.84	1.69 ± 0.43	0.53 ± 0.45
	With PS 80		1.86 ± 1.87	1.36 ± 1.09	1.25 ± 0.89	3.17 ± 0.89	0.67 ± 0.25
Kidney	Without PS 80		23.2 ± 10.9	21.6 ± 12.2	–	9.2 ± 10.3	–
	With PS 80		16.1 ± 11.3	4.9 ± 3.6	–	11.4 ± 10.4	3.6 ± 0.1

[a] The values given are means of 3–5 measurements ± standard deviation

Table 2. The levels of MTX in the blood and brain of Porton mice, 1 h after the administration of various oral MTX doses as solution with or without 6% polysorbate 80 (PS 80)

Tissue	MTX preparation given	Dosage of MTX (mg · kg^{-1})	Amount of methotrexate: (μg · ml^{-1} serum or μg · g^{-1} brain)[a]			
			0.5	2	4	6
Blood	Without PS 80		0.10 ± 0.03	0.11 ± 0.03	0.10 ± 0.02	0.23 ± 0.06
	With PS 80		0.20 ± 0.12	0.27 ± 0.05	0.19 ± 0.05	0.34 ± 0.06
Brain	Without PS 80		Not detected	0.03 ± 0.01	0.16 ± 0.02	0.12 ± 0.02
	With PS 80		Not detected	0.10 ± 0.06	0.25 ± 0.15	0.24 ± 0.06

[a] The values given are means of 3–5 measurements ± standard deviation

samples. The results confirmed the above findings, statistically higher levels of MTX again being achieved in mice given the drug with the surfactant, at 15, 30, and 45 min after the administration of MTX solutions, (P = 0.05, 0.05, and 0.05, respectively).

Plasma MTX levels in Porton mice given 4 mg MTX · kg^{-1} with 6% polysorbate 80 (Table 1), were significantly higher than in mice given the same dose without the surfactant at 1, 2, and 4 h after the drug was administered (P = 0.025, 0.05, and 0.05, respectively). There was no significant difference between the amount of MTX present in the livers and kidneys of the two groups of mice, although the levels measured up to 2 h seem to be lower in the organs of mice given MTX with 6% polysorbate 80. No MTX was detected in the brain of mice given the MTX solution without the surfactant, but polysorbate 80 allows penetration of significant levels of the drug.

Table 2 shows the significantly higher levels of serum MTX in mice given 2 mg, 4 mg, and 6 mg MTX · kg^{-1} with 6% polysorbate 80 than in mice given the drug without the surface-active agent P = 0.01, 0.05, and 0.05, respectively. The amount of MTX taken up into the brain was higher in mice given MTX solutions PO with polysorbate 80, although only significant at the doses of 2 mg and 6 mg · kg^{-1} (P = 0.05 and 0.01).

The difference noted in the amount of blood and brain MTX measured in mice given MTX with 6% polysorbate 80 (experiments 3 and 4) could perhaps be accounted for by the experimental errors in handling the small samples and by the difference in ages of mice used. Comparison of the plasma levels of MTX in NMRI mice and Porton mice given MTX with 6% polysorbate 80 indicates an as yet unexplained difference in the extent of drug absorption.

Discussion

Various sorption promotors have been used to increase the absorption of a wide range of drugs. These sorption promotors include organic solvents, saponins, enzymes, complexing agents, carbohydrates, and surface-active agents [6, 15]. The non-ionic surfactant polysorbate 80 is known to increase the absorption of vaccines, mineral oils, and other fat-soluble substances [14], phenobarbitone [12], and indomethacin [13]. Incorporation of polysorbate 80 into oral emulsions also enhances the absorption of many drugs such as MTX (our unpublished data) and 5-FU [22].

We have observed an increase in the absorption of the higher-dose MTX from the gastro-intestinal tract of mice (Tables 1 and 2; Fig. 1), and there may be enhanced penetration into the brain (Tables 1 and 2) when MTX is given together with polysorbate 80. The latter, if confirmed, is of more importance clinically than the former.

The increase in the absorption from the gastro-intestinal tract could be accounted for by the increased permeability of the barrier membrane [7]. Increased absorption might also result from the increased solubility or decreased precipitations of MTX in presence of polysorbate 80 at the low pH values that would be encountered in the stomach. A recent paper [10], however, has suggested that increased blood levels of adriamycin were the results of an osmotic reduction in plasma volume caused by the high amount of polysorbate 80 injected together with the drug into the peritoneal cavity. This is unlikely to take place in the present studies, as relatively low amounts of polysorbate 80 were administered into the gastro-intestinal tract and were subjected there to dilution and loss by absorption.

Some of any polysorbate 80 given orally is known to be absorbed from the gastro-intestinal tract [14]. Our experiments on mice and rats given I^{125}- and I^{123}-labelled polysorbate 80 have shown that about 20% of the surfactant is absorbed from the alimentary tract. There is sufficient evidence in the literature to suggest that surface-active molecules such as the phenothiazines can increase the permeability of the blood-brain barrier [9]. It is reasonable to expect that the agent used in this work exerts the same action, although it has not yet been confirmed that the observed increase in the brain levels is due to increased permeability of the blood-brain barrier, since it is possible that the enhanced uptake is due to the increased plasma levels. This is being studied further.

Polysorbate 80 appeared to reduce the amount of MTX distributed into the liver and kidney of Porton mice (Table 1). The lower levels noted, although not significant, might indicate facilitated elimination of MTX from the liver, since polysorbate 80 is known to promote bile excretion [1].

The animal experiments have suggested a role of polysorbate 80 in improving the bioavailability of high-dose MTX from the oral formulation. Early trials with patients have confirmed the lack of effect of polysorbate 80 on the absorption of low-dose MTX. Trials with higher doses are planned.

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