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Absorption and lymphatic uptake of 5-fluorouracil in the rat following oral administration of w/o/w multiple emulsions

J.A. Omotosho [2], A.T. Florence [1] and T.L. Whateley [1]

[1] *Department of Pharmacy, School of Pharmacy and Pharmacology, University of Strathclyde, Glasgow G1 1XW (U.K.)* and [2] *Department of Pharmaceutics, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife (Nigeria)*

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Summary

The influence of the nature of the oil phase of w/o/w emulsions on the oral absorption of 5-fluoro[^{3}H]uracil in the rat is described. Liver levels and lymphatic accumulation of the drug following oral administration of w/o/w emulsions were studied. The absorption of 5-fluoro[^{3}H]uracil orally administered in a water/isopropyl myristate/water emulsion was enhanced compared to an aqueous solution while a w/o/w emulsion prepared with octane as the oil phase reduced intestinal uptake. The suggested mechanism underlying the enhancement noted with isopropyl myristate emulsions may be attributed to the inhibitory effect of the oil on the gastric emptying processes. Both emulsion systems showed potential as lymphotropic carriers of water-soluble drugs to the mesenteric lymph nodes following oral administration.

Introduction

Multiple emulsions are emulsions in which globules of the disperse oil phase encapsulate smaller aqueous droplets. A typical system used in this study is illustrated in Fig. 1. In multiple w/o/w emulsions, the internal and the external aqueous phases are separated by an oil layer and require for their formation and stability at least two stabilizing surfactants, one having a low HLB to form the primary water-in-oil emulsion and the other of higher HLB to achieve secondary emulsification. Such emulsion systems have the advantage of being less viscous and therefore more convenient to inject than the primary w/o emulsion.

The potential of multiple emulsions as parenteral prolonged drug delivery systems has been discussed previously (Davis, 1976; Omotosho, 1987). Comparative in vivo studies of an aqueous solution, a w/o/w and a water-in-oil emulsion showed that administering 5-fluorouracil (5-FU) in either emulsion forms significantly prolonged the in vivo release of 5-FU from an intramuscular injection site in the rat. A more sustained blood concentration with a delayed blood peak level was observed following intramuscular injection of w/o/w emulsions as compared with aqueous drug vehicle (Omotosho, 1987).Multiple w/o/w emul-

Correspondence: T.L. Whateley, Department of Pharmacy, School of Pharmacy and Pharmacology, University of Strathclyde, Glasgow G1 1XW, U.K.

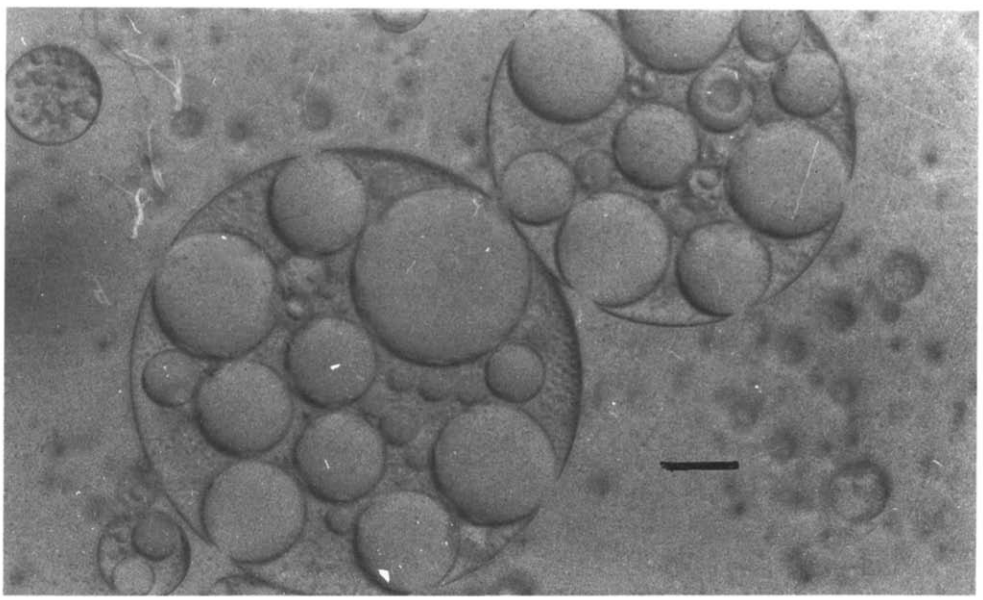

Fig. 1. Photomicrograph of w/o/w emulsion droplets prepared with 2.5% Span 80 and 0.2% BSA as primary emulsifiers, 1% Tween 80 served as the secondary emulsifier with octane as the oil phase (Bar scale = 10 μm).

sions have also been studied for potential application to facilitate gastrointestinal absorption of non-absorbed peptides and other biopolymers such as insulin (Engel et al., 1968; Shichiri et al., 1974, 1975). Intraduodenal injection of a w/o/w emulsion containing insulin in the internal phase resulted in a significant hypoglycaemic activity. However, the multiple w/o/w emulsions used for these studies tended to be unstable and the emulsions had to be prepared immediately before they were used, as described by Davis (1976). The w/o/w emulsions prepared in the present study are stabilized by interfacial complexation in which the formation of an interfacial film gives rise to enhanced stability and satisfactory in vitro drug release characteristics (Omotosho et al., 1986). The main objective of this study was to investigate whether formulating a water-soluble drug in a w/o/w emulsion would enhance intestinal uptake, thus allowing some reduction in the dose and consequently lowering the incidence and intensity of side-effects of cytotoxic agents. The potential of these systems to promote lymphatic accumulation of orally administered 5-fluoro[^{3}H]uracil in rats has also been investigated.

Materials and Methods

Materials

Span 80 (Sorbitan monooleate) was obtained from Koch-Light Labs. Tween 80 (polyoxyethylene sorbitan monooleate), isopropyl myristate (purity 98%), octane (purity 99%), crystallized and lyophilised bovine serum albumin (BSA), essentially globulin-free, and 5-fluorouracil (5-FU) were obtained from Sigma, UK.

5-Fluoro[^{3}H]uracil (specific activity 40.7 GBq/mmol) was obtained from Amersham International, U.K. Soluene-100 and emulsifier scintillators (ES 299) were obtained from United Technologies Packard. Water was freshly double distilled.

Methods

Preparation of w/o/w emulsions. The emulsions were prepared by a two-stage emulsification procedure. The aqueous phase containing 0.2% BSA, unlabelled 5-FU (1 mg/ml) and 5-fluoro [^{3}H]uracil in normal saline was added to an equal weight of either isopropyl myristate or octane containing 2.5% Span 80 in a 25 ml glass vial. Emulsification was carried out using a small vortex mixer (Whirlmixer, Fisons Scientific, Loughborough) to produce a water-in-oil emulsion. The w/o/w emulsion was prepared by re-emulsification of the water-in-oil emulsion with an equal volume of water containing Tween 80 (1% w/v). A 1 ml portion of the final w/o/w emulsion contained 5 μCi of 5-fluoro[^{3}H]uracil. Prior to oral administration of the emulsion to rats, four 5-μl aliquots were removed for scintillation counting.

Absorption studies in rats. Male rats (Sprague-Dawley strain), weighing 300–350 g were fasted overnight before the experiments. An adapted needle with a bulb end was used to dose the rats orally with 5-fluoro[^{3}H]uracil contained in either 1 ml of normal saline or in 1 ml of a multiple emulsion. Each rat was given 5 μCi/300 g body weight. After administration of the formulations the rats were returned to restraining cages. At specified time intervals the rats were killed and blood samples (0.2 ml) were collected from the heart using a hypodermic needle from each experimental rat. The mesenteric lymph nodes and the liver were removed, weighed, frozen at $-20\,^\circ$C and prepared for scintillation counting. The procedure for the preparation of samples for liquid scintillation counting have been reported previously (Omotosho, 1987).

All measurements were performed in a Tri-carb 4000 liquid Scintillation spectrometer (Packard Instruments) equipped with automatic external standardisation. Quenching curves were prepared by the addition of known amounts of tritium to portions of bleached and solubilised blood samples of size and type similar to the unknown but taken from non-radioactive animals.

Results and Discussion

Fig. 1 shows a typical photomicrograph of w/o/w emulsion droplets prepared by interfacial complexation between Span 80 and BSA with octane as the oil phase. The long-term stability of these emulsion systems has been described previously (Omotosho et al., 1986). There was no significant change in the number of multiple drops and the mean droplet diameter on storage in emulsions prepared with hydrocarbons, indicating good stability in these systems. Not only is the choice of oil and concentration of stabilising emulsifiers important in optimizing the stability of the systems, but the presence of additives such as electrolytes is also critical (Omotosho, 1987). In vitro studies (Fig. 2) from w/o/w emulsions showed that the release rate of 5-FU was faster for those systems with smaller internal droplets, due

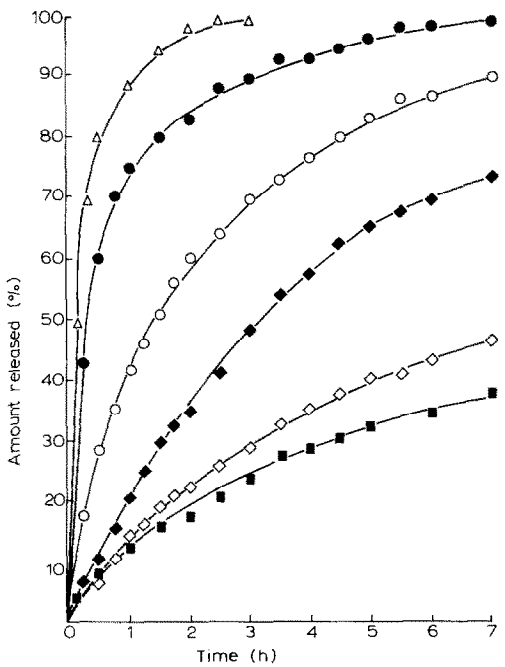

Fig. 2. Release of 5-fluorouracil from w/o/w emulsions prepared as indicated in Fig. 1 with the following oil phases: (■) octane, (◇) cyclohexane, (◆) dodecane, (○) hexadecane, (●) toluene, (△) isopropyl myristate.

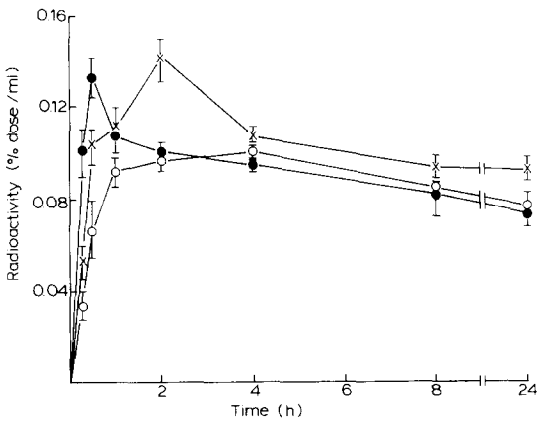

Fig. 3. Blood levels of 5-fluoro[^{3}H]uracil following oral administration of various formulations. Each value is the mean ± SE: (●) aqueous solution, w/o/w emulsions prepared with (×) isopropyl myristate and (○) octane.

to the increased interfacial area available for drug transport across the oil phase barrier. The rank order of release rates was isopropyl myristate > hexadecane > dodecane > octane emulsions (Omotosho et al., 1986).

The whole blood radioactivity levels following oral administration of 5-fluoro[^{3}H]uracil in w/o/w emulsions and in solution to rats are shown in Fig. 3. Compared with aqueous vehicles, multiple w/o/w emulsions prepared with isopropyl myristate enhanced the oral absorption of 5-fluoro[^{3}H]uracil while octane emulsions actually reduced the intestinal uptake of the drug. Peak blood radioactivity levels were observed after 30 min when 5-fluoro[^{3}H]uracil was administered in solution, compared with about 2 and 4 h for emulsions prepared with isopropyl myristate and octane, respectively. The delay in the time of occurrence of the blood peak concentration of 5-fluoro[^{3}H]uracil following oral administration in the emulsion may be attributed to slow release of 5-fluoro[^{3}H]uracil from the encapsulated internal phase.

The increased gastro-intestinal absorption of 5-fluoro[^{3}H]uracil following oral administration of isopropyl myristate emulsions may be attributed to slow release of 5-fluoro[^{3}H]uracil from the emulsions and/or increased gastric retention time producing a prolonged absorption phase. A number of physiological processes have been identified as affecting the mechanisms of intestinal uptake of drugs from lipid systems. These include reduced gastric retention time which has been reported to vary with the chemical structure of the oil. With the series of straight chain saturated fatty acids, shorter chain length fatty acids (C_2–C_8) have little effect on the gastric retention time while longer chain fatty acids are more effective, the most potent being myristic acid (C_{14}) (Palin, 1985). It is therefore possible that a relatively large fraction of 5-fluoro[^{3}H]uracil may be retained within the emulsions prepared with octane and excreted in the faeces because of its rapid transit through the gastrointestinal tract.

The enhancement noted with isopropyl myristate emulsion may, on the other hand, be attributed to the inhibitory effect of the oil and/or metabolite (myristic acid) on the gastric emptying processes. The rapidity and efficiency of the digestion process of oils to fatty acids that precedes the inhibitory effect have been shown to increase through emulsification which increases the effective surface area exposed to the hydrolytic action of pancreatic lipase (Bates and Sequeira, 1975). Whitehill (1981) has suggested that the presence of a hydrophilic surfactant emulsifier at the external phase of w/o/w emulsions may be responsible for the increase in the oral absorption of methotrexate. However, the differences in the absorption characteristics of 5-fluoro[^{3}H]uracil observed in the present study cannot be attributed to this effect, since the two w/o/w emulsion systems investigated contain at the external phase similar amounts of the non-ionic surfactant Tween 80.

Oil-in-water emulsions have been studied by several authors for the purpose of facilitating the absorption of water-soluble drugs (Carrigan and Bates, 1973; Chakrabarti and Belpaire, 1978). Since the drug has to be placed in the aqueous phase of the emulsion where it is more soluble, formulating the drug in a w/o/w emulsion could offer the additional advantage of protecting the drug molecule from the external environment and of reducing the toxic effects of irritant drugs.

Fig. 4 shows radioactivity levels in the liver following oral administration of the various formulations. Both the peak liver radioactivity levels and the time to the peak liver levels were different

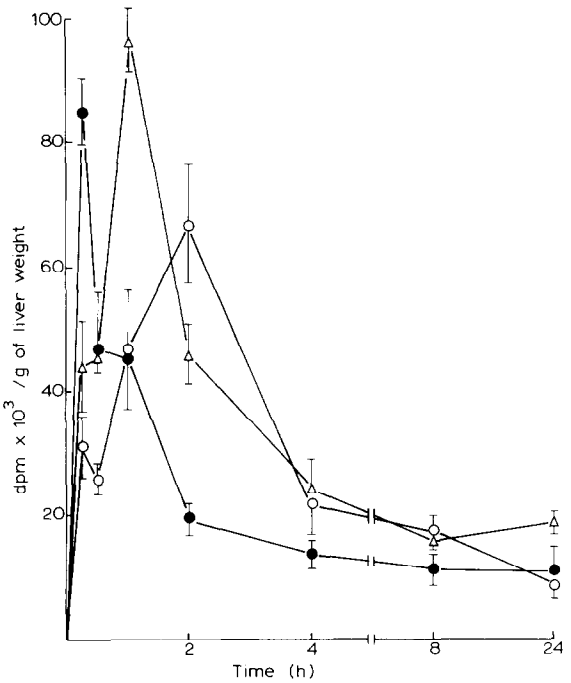

Fig. 4. Radioactivity levels of 5-fluoro[^{3}H]uracil in the liver: (●) aqueous solution, w/o/w emulsions prepared with (△) isopropyl myristate and (○) octane. Each value is the mean ± SE.

for each formulation. These effects again are indicative of the amount of 5-fluoro[^{3}H]uracil released into the systemic circulation. Higher liver radioactivity levels were obtained with the emulsions prepared with isopropyl myristate compared with the oral administration of 5-fluoro[^{3}H]uracil in solutions.

The potential of w/o/w emulsions as lymphotropic carriers of water-soluble drugs to the mesenteric lymph nodes has been investigated. Fig. 5 shows the radioactivity levels in the mesenteric lymph nodes following oral administration of 5-fluoro[^{3}H]uracil in w/o/w emulsions and in solution. No detectable differences in radioactivity levels of 5-fluoro[^{3}H]uracil between the blood (see Fig. 3) and the mesenteric lymph nodes (Fig. 5) can be demonstrated following oral administration of 5-fluoro[^{3}H]uracil in solution. This will be expected for a small molecule such as 5-FU which would be absorbed primarily via the blood supply rather than through the lymphatics and could explain the lack of accumulation of the drug in the mesenteric lymph nodes. On the other hand, oral administration of 5-fluoro[^{3}H]uracil in w/o/w emulsions gave higher concentrations and a prolonged slow release appearance of 5-fluoro[^{3}H]uracil in the mesenteric lymph nodes. The actual mechanism of transport to the mesenteric lymph nodes remains to be elucidated, however, one might suggest that both the emulsion droplets and 5-fluoro[^{3}H]uracil were transported there together. The encapsulation of the drug by the oil phase would place it in a more lipophilic environment and would allow it, therefore, to be selectively absorbed into the lymphatic vessels rather than into the portal circulation. The differences in the levels of 5-fluoro[^{3}H]uracil in the lymph nodes following oral administration of the two emulsion systems suggest that the droplet size of the emulsion globules may be an important factor affecting the lymphatic uptake.

Of considerable clinical importance is the fact that for effective cancer chemotherapy a high concentration of the drug has to be accumulated at target sites, which may include not only the main lesion but also the regional lymph nodes. Selective absorbtion of drug into the lymphatic system will be useful for maintaining drug concentrations within the therapeutic range in the lymph vessels.

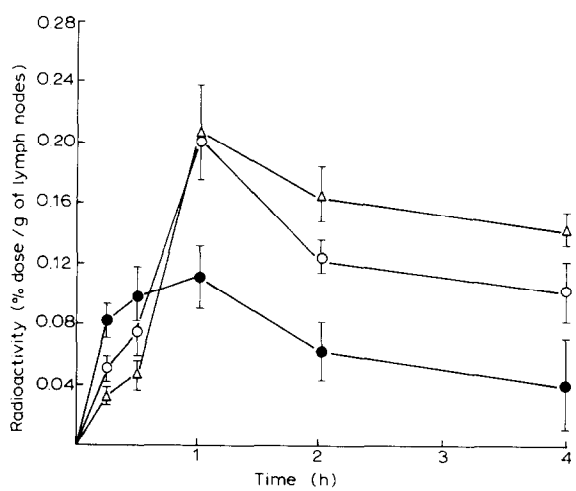

Fig. 5. Radioactivity levels of 5-fluoro[^{3}H]uracil in the mesenteric lymph nodes following oral administration of (●) aqueous solution, w/o/w emulsions prepared with (○) octane and (△) isopropyl myristate. Each values is the mean ± SE.

In addition w/o/w emulsions might act as carriers for the delivery of polypeptide/protein drugs, which require both protection from the gastric fluids and delivery via the lymph nodes.

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