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The effect of oils on the lymphatic absorption of DDT

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Plasma concentrations of DDT were measured in conscious rats following oral administration in arachis oil, Miglyol 812 (fractionated coconut oil), liquid paraffin or as a fine suspension in water. The total absorption of DDT, calculated as the area under the plasma concentration time curve, was significantly greater for the arachis oil formulation compared to the other vehicles. In anaesthetized rats it was shown that DDT absorption was almost totally via the lymphatic system, and that lymph levels of DDT were highest following administration in arachis oil. Measurement of lymph flow showed that this enhanced absorption of DDT in the presence of arachis oil was not due to an increased flow as both arachis oil and liquid paraffin stimulated lymph production to the same extent.

The oral absorption of a variety of drugs has been shown to be altered in the presence of oil and emulsion vehicles (Chakrabarti & Belpaire 1978; Fischler et al 1973). Various physiological mechanisms have been proposed to explain this effect including altered gastrointestinal motility (Bates & Sequeira 1975), increased bile flow leading to drug solubilization (Bates & Sequeira 1975), increased mucosal permeability (Muranushi et al 1980) and enhanced mesenteric lymph flow (DeMarco & Levine 1969).

The lymphatic absorption of drugs from oily vehicles following oral administration is of interest for two reasons. First, metabolism of the drug in the liver (first pass effect) may be reduced by absorption of the drug directly into the mesenteric lymph. Second, it may be possible to target cytotoxic drugs directly into the lymphatic system.

In an attempt to determine whether lymphatic absorption may be enhanced by co-administration of lipids, the oral absorption of DDT from different oils has been investigated in the rat. DDT was selected as a model compound as it is highly lipid soluble and preferentially absorbed via the lymphatic route (Sieber et al 1974).

MATERIALS AND METHODS

Male Wistar rats, weight range 190–210 g, were used in all the experiments described.

2,2,bis(p-chlorophenyl)1,1,1-trichloroethane (*p,p*-DDT) was obtained from Aldrich Chemicals Co. Ltd (Gillingham, Dorset). Heptachlorepoxide was a generous gift of Vesicol Chemicals Corporation (Chicago, U.S.A.). The oils investigated were Arachis oil B.P. (Evans Ltd, Liverpool), Miglyol 812 (a fractionated coconut oil) (Dynamit Nobel, Slough, Berks) and liquid paraffin B.P. (Shell Ltd, London). All other reagents were 'Analar' grade and were used without further purification. Dulbecco's solution (A + B) was obtained from Oxoid Ltd, Basingstoke, Herts.

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Animal procedure

An adapted 19 gauge needle with a bulb end was used to orally dose rats (4 per group) with DDT (100 mg kg^{-1}) in 1 ml volumes of solution in the three oils and as fine suspension in water containing 6% Tween 80. Plasma was collected following tail tip blood sampling at intervals over 24 h, and stored at −20 °C before analysis.

In another series of experiments each animal was anaesthetized 1 h after dosing using sodium pentobarbitone (*i.p.* 90 mg kg^{-1}). Dulbecco's solution (A + B) containing heparin 1 unit ml^{-1} was infused at 2 ml h^{-1} through a cannula placed in the left jugular vein. In addition, the abdominal thoracic duct was cannulated immediately above the cisternae chyli (Ford & Hunt 1973) and the lymph collected over 4 h. Plasma samples were collected as before at half-hour intervals. A 'sham' operation procedure was carried out on a further group of rats to account for the effect of anaesthesia and surgery on DDT absorption. This was performed in the same manner previously described but without insertion of the cannulae, plasma samples being taken as before.

Analysis of DDT

An adaptation of the method described by Dale et al (1970) was used for the determination of the DDT concentration in the plasma and lymph samples. Samples (30–50 μl) were extracted with equal vol-

umes of n-hexane after pre-treatment with one volume of formic acid. Heptachlorepoxide was used as the internal standard. Determination of the p,p-DDT concentration in the hexane phase was made with a Perkin-Elmer Model F33 Gas Chromatograph fitted with an electron capture detector, using a 2 metre × 0·4 cm i.d. column of OV225 (3% w/w) on an acid washed Chromosorb W(HP) support (80–100 mesh, PhaseSep Ltd, Queensferry). The flow rate of the carrier gas (oxygen free nitrogen) was 90 ml min^{-1} at an oven temperature of 225 °C and a detector/injector temperature of 275 °C.

RESULTS

The plasma concentration versus time curves following administration of DDT in different vehicles to conscious animals, exhibit significant differences between the formulations (see Fig. 1). For each curve the maximum plasma concentration (Cp_{max}), the time to the Cp_{max} (T_{max}) and the total drug absorption, calculated as the area under the curve between 0 and 24 h (AUC_{0-24h}) using the trapezoidal method, were determined (see Table 1). Whilst the arachis oil formulation produced a greater absorption of DDT compared with the water formulation ($P < 0·05$ for Cp_{max} and AUC_{0-24h}, unpaired Student's *t*-test), liquid paraffin reduced the AUC_{0-24h} ($P < 0·05$). There was no difference in the AUC_{0-24h} value between the Miglyol 812 and the water formulations, although Miglyol 812 gave rise to a higher peak plasma concentration ($P < 0·05$).

Table 1. Absorption data following oral administration of DDT (100 mg kg^{-1}) in different vehicles (1 ml volumes) to unanaesthetized rats. (Data are given as means (with s.d.) of 4 values). C_{pmax} = maximum plasma concentration. T_{max} = time to maximum plasma concentration. AUC_{0-24} = area under plasma versus time profile between 0 and 24 h.

	C_{pmax} (μg ml^{-1})	T_{max} (h)	AUC_{0-24h} (mg ml^{-1} h^{-1})
Arachis oil	11·08 (1·96)	7·0 (2·0)	118·3 (6·6)
Miglyol 812	4·67 (0·74)	6·0 (0·0)	57·9 (10·2)
Liquid paraffin	1·72 (0·05)	4·0 (0·0)	23·9 (4·2)
Water containing 6% Tween 80	2·88 (0·37)	10·0 (2·0)	44·6 (5·3)

Following 'sham' operation procedures, the plasma levels of DDT after dosing with Miglyol 812 and arachis oil were reduced compared with the levels in conscious animals ($P < 0·05$) (see Figs 1 & 2), whereas the plasma levels of DDT following administration of the liquid paraffin formulation were similar in both groups of animals. However DDT absorption was still greatest from the arachis oil formulation as in the experiment with the conscious animals.

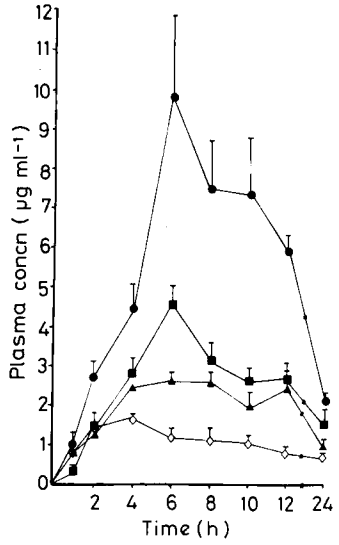

FIG. 1. The effect of different vehicles (1 ml volumes) on the absorption of orally administered DDT (100 mg kg^{-1}) in unanaesthetized rats. ● = Arachis oil ■ = Miglyol 812 ◇ = Liquid paraffin ▲ = Water containing 6% Tween 80. (Data are given as means ± s.e.m. of 4 values).

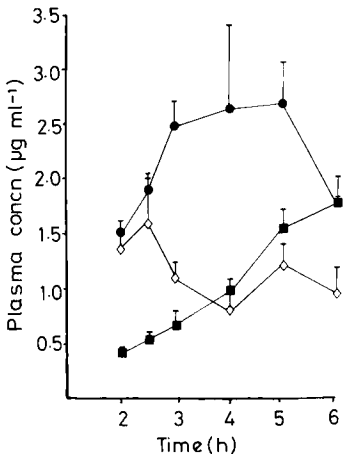

FIG. 2. The effect of different oils (1 ml volumes) on the absorption of orally administered DDT (100 mg kg^{-1}) in anaesthetized rats. ● = Arachis Oil ■ = Miglyol 812 ◇ = Liquid paraffin. (Data are given as means ± s.e.m. of 4 values).

Whilst arachis oil and liquid paraffin stimulated lymph flow compared with the basal lymph flow in anaesthetized rats, Miglyol 812 inhibited the flow (see Fig. 3). This inhibition was gradually overcome with time and was reflected by an increase in the plasma DDT concentration with time.

Thoracic duct cannulation virtually abolished the appearance of DDT in the plasma (see Fig. 4), the lymph concentration of DDT being higher than that in the plasma with all the formulations studied. Peak lymph concentrations were higher ($P < 0{\cdot}05$) following administration in arachis oil than in either Miglyol 812 or liquid paraffin. However the time to the peak lymph concentration was shorter ($P < 0{\cdot}05$) following administration in liquid paraffin than in either Miglyol 812 or arachis oil.

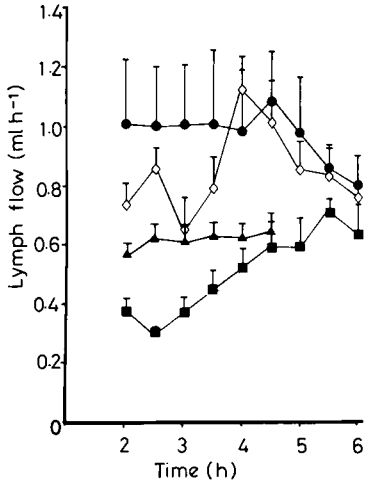

FIG. 3. Thoracic duct lymph flow in anaesthetized rats following oral administration of different oils (1 ml volumes) containing DDT (100 mg kg^{-1}). ● = Arachis Oil ■ = Miglyol 812 ◇ = Liquid paraffin ▲ = Basal lymph flow. (Data are given as means + s.e.m. of 4 values).

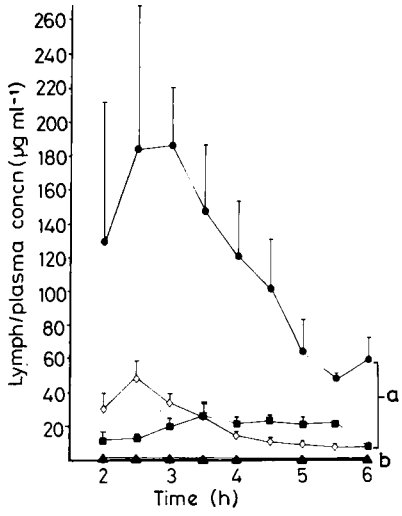

FIG. 4. The effect of different oils (1 ml volumes) on the absorption of orally administered DDT (100 mg kg^{-1}) into (a) lymph ● = Arachis Oil ■ = Miglyol 812 ◇ = Liquid paraffin and (b) plamsa (▲). (Data are given as means ± s.e.m. of 4 values).

DISCUSSION

The initial study in conscious animals clearly demonstrated that the extent of oral absorption of DDT was dependent on the nature of the oily vehicle. Cannulation of the thoracic duct showed that in the presence of all three oils the major, if not the only route for DDT absorption was via the lymphatic system. This is in agreement with the findings of Rothe et al (1957), Pocock & Vost (1974) and Sieber et al (1974). In addition, it has been demonstrated that the nature of the oil vehicle influences the concentration of DDT in the thoracic duct lymph.

The higher lymph levels of DDT following administration in arachis oil compared with other formulations suggest that some facet of arachis oil absorption promotes lymphatic uptake. Digestion of arachis oil liberates long chain unsaturated and saturated fatty acids which are incorporated into mixed bile salt micelles. This increases the capacity of the micelle to solubilize lipophilic molecules such as cholesterol or DDT (Hofman & Small 1967; Treadwell & Vahouny 1968). It has been suggested that mixed bile salt micelles containing unsaturated fatty acids increase the permeability of the mucosal membrane (Muranushi et al 1980) which in turn might further increase DDT absorption. The presence of a long chain unsaturated fatty acids within the enterocyte stimulate the synthesis of chylomicra (Caselli et al 1979) in which DDT is transported in the lymph (Pocock & Vost 1974). Thus arachis oil may promote the lymphatic uptake of DDT by a series of physiological mechanisms.

The fatty acids liberated by the hydrolysis of Miglyol 812 are sufficiently water-soluble to pass through the intestinal aqueous phase and be absorbed into the portal vein without solubilization by the bile salt micelles. Fatty acids that are absorbed into the lymph stimulate the formation of very low density lipoprotein particles rather than chylomicra as they are saturated in structure (Caselli et al 1979). Therefore neither Miglyol 812 or its digestion products would be expected to promote DDT absorption.

As liquid paraffin is a non digestible oil, absorption of DDT is dependent upon absorption of the oil or on partitioning of the molecule from the oil phase into the micellar phase. However as only a small proportion of liquid paraffin is absorbed (Albro & Fishein 1970), it is likely that a relatively large fraction of DDT is retained within the intestinal oil phase and excreted in the faeces. Similar findings have been reported for cholesterol and DDT in the presence of sucrose polyester, a non-absorbable lipid (Mattson et al 1976; Volpenhein et al 1980).

Comparison of the plasma DDT levels in conscious and anaesthetized rats showed that anaesthesia reduced the plasma concentration of DDT following dosing in arachis oil and Miglyol 812 but that there was no difference in the levels following administration in liquid paraffin. Pentobarbitone anaesthesia is known to reduce splanchnic blood flow and gastrointestinal motility (Lee 1965; Green 1979). Lower drug absorption from the intestinal tract would therefore be expected during pentobarbitone anaesthesia. Previous studies (Palin et al 1980) have suggested that DDT absorption from liquid paraffin is limited by the rapid transit of this oil through the gastrointestinal tract. It is possible that during pentobarbitone anaesthesia the transit of this oil was slowed allowing more time for DDT absorption, thereby compensating for reduced absorption resulting from other physiological changes.

A marked depression of lymph flow and plasma DDT levels was observed during the initial experimental period with anaesthetized animals dosed with DDT in Miglyol 812. The only indication of such an inhibitory action in conscious animals is at the 1 h data point when the plasma concentration of DDT was lower following administration in Miglyol 812 than with any other formulation ($P < 0{\cdot}05$). It is suggested that by some unknown mechanism Miglyol 812 initially inhibits lymph flow and drug absorption. However in conscious animals this effect is only short-term whereas in anaesthetized animals, because of the depression of blood flow in the intestinal region and the reduction in gut motility, recovery is not as rapid, resulting in the gradual increase in lymph flow and plasma DDT levels observed during the experimental period.

These experiments have demonstrated that it is possible to alter the oral absorption of DDT by changing the oily vehicle in which it is administered. The extent of absorption would appear to be dependent on the nature of the oil, DDT absorption being greatest following administration in triglycerides containing long chain unsaturated fatty acids which provide the most favourable conditions for lymphatic uptake and transport. It may therefore be possible to enhance the lymphatic absorption of a lipophilic drug by careful selection of a suitable oily vehicle and so increase the total concentration of drug absorbed into the systemic circulation.

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