International Journal of Pharmaceutics, 16 (1983) 31–39
Elsevier

The clearance of oily vehicles following intramuscular and subcutaneous injections in rabbits

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(Received November 22nd, 1982)
(Accepted January 14th, 1983)

Summary

[^{131}I]-Iodinated oils were administered to rabbits by intramuscular and subcutaneous injection. Clearance of [^{131}I]-iodinated oils from the site of injection was monitored by gamma-scintigraphy. Clearance rates were found to be independent of the site of injection. The volume of injection had no effect on the clearance rate of [^{131}I]-iodinated ethyl oleate when given intramuscularly. Lymphatic absorption did not appear to make a major contribution to the clearance of [^{131}I]-iodinated oils from either site of injection.

Introduction

Oils have been used as vehicles for parenteral administration of drugs for many years. The sustained release effect achieved with certain drug/oil combinations has led to the long-term use of oil-based preparations, notably in psychiatric medicine (Ayd, 1975). While the absorption characteristics of drugs from oily injections given intramuscularly (i.m.) and subcutaneously (s.c.) are well documented, less attention has been directed towards evaluating the rate and mechanisms of absorption of the oily vehicles used.

The present investigation was undertaken to determine the absorption characteristics of arachis oil (AO) and ethyl oleate (EO) following i.m. and s.c. injection in rabbits, using the technique of gamma-scintigraphy.

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0378-5173/83/$03.00

Materials and Methods

Materials

The AO was BP grade (Evans) and the EO was SLR grade (Fisons). The potassium iodide, potassium iodate, sulphuric acid and sodium thiosulphate were all analytical grade (BDH). The solvent ether was obtained from MacFarlane Smith. ^{131}I was obtained as $Na^{131}I$ in aqueous solution from Amersham International.

Labelling procedure

Oils were labelled with ^{131}I using the iodine-monochloride technique described by Lubran and Pearson (1958).

Preparation of iodine-monochloride. To 1 ml of an aqueous solution containing approximately 37 MBq of ^{131}I as $Na^{131}I$ in a glass-stoppered test tube, 1 ml of an aqueous solution of potassium iodide (1 mg/ml) was added. 3 ml of solvent ether was layered on the aqueous phase, 0.5 ml of an aqueous solution of potassium iodate (2 mg/ml) and 0.2 ml of 2 N sulphuric acid were added, liberating iodine in the aqueous phase. The mixture was shaken until the iodine had partitioned into the organic phase. The aqueous phase was then removed and discarded. A freshly prepared solution of chlorine in ether was added dropwise to the iodine solution until the ether layer became colourless.

Iodination of oil. 1 ml of oil was dissolved in 3 ml of solvent ether in a glass-stoppered test tube. The iodine-monochloride solution was added dropwise, and the preparation allowed to stand at room temperature for 2 h.

Purification of iodinated oil. To the iodinated oil ethereal solution an equal volume of an iodide/thiosulphate solution (5 g KI, 5 g $Na_2S_2O_3 \cdot 5H_2O$/100 ml water) was added, mixed and centrifuged and the aqueous phase discarded. This procedure was repeated once with the iodide/thiosulphate solution and twice with distilled water.

The ethereal solution of iodinated oil was then transferred to a clean test tube and placed in a heated block at 60°C to evaporate the ether. The pure iodinated oil was then transferred to a nitrogen-filled vial for storage.

Absorption studies in animals

Female New Zealand White rabbits (3 kg) were used in all experiments. Injections were administered using a 26-gauge 3/8 in. needle (Yale Microlance) connected to a 1 ml syringe (Gillette Surgical). The area around the injection site was shaved prior to injection.

I.m. injection. Injections were made into the vastus lateralis muscle located in the posterior limb above the knee. The needle was plunged into the muscle at an angle of 90° for the full length of the needle giving a uniform injection depth.

S.c. injection. Skin on the mid-thigh was pinched between forefinger and thumb and the injection made at an angle of about 45° for the full length of the needle. Oil did not appear to leak back along the track of the needle. The total dose of radioactivity varied between 5 and 20 MBq depending on the dose of oil administered. Each experiment was performed in triplicate. Animals had free access to food and water and were free to exercise at will.

Data collection

A General Electric MaxiCamera II fitted with a high energy standard parallel collimator was used to image the rabbits. Peak energy of activity was set at 364 keV with an energy window of ±20%. Images of the whole animal were taken.

Data analysis

A standard computer program was employed which enabled regions of interest (ROI) to be drawn around the site of injection, an ROI around the contralateral site was taken as a background reading. Total counts were obtained in the ROIs allowing a correction for background and decay to be made for the total counts at the site of injection.

Absolute viscosity measurements

Kinematic viscosities of the oils were determined with a U-tube viscometer. The densities were measured by using a calibrated 50 ml density bottle. The determinations were performed at 37°C, and the absolute viscosity was calculated from the kinematic viscosity and density.

Surface and interfacial tension measurements

The Wilhelmy Plate method was used to determine the surface tension and oil/water interfacial tension of the oils. Force measurements were made using a CI microforce balance. End plate correction was determined using carbon tetrachloride as a standard. Measurements were conducted at 25°C.

Results

The injection techniques discussed were verified by injection of a dye in oil solution, followed by dissection of the relevant tissues.

If free ^{131}I was present in the injections, it would be expected to concentrate in the thyroid gland following absorption. Therefore the thyroid region was monitored; in no experiment did the levels of activity in the thyroid region exceed the levels of the background count, indicating that the ^{131}I present was totally bound to the oil.

Clearance studies

Fig. 1 shows the clearance of oils from the vastus lateralis muscle, following 300 μl injections. The results are expressed as the amount of oil remaining at the site of injection as a percentage of total dose, and as a function of time post-injection. Fig. 2 shows the corresponding plot following s.c. injection of 300 μl of the oils. Half standard error bars are shown on both plots. It appears that iodinated arachis oil (AOI) is cleared more quickly than iodinated ethyl oleate (EOI) following i.m. and s.c. injections.

Using standard first-order rate of reaction theory, it is possible to derive an elimination rate constant (k_{el}) and half-life ($t_{1/2}$) for the oils at both sites in vivo.

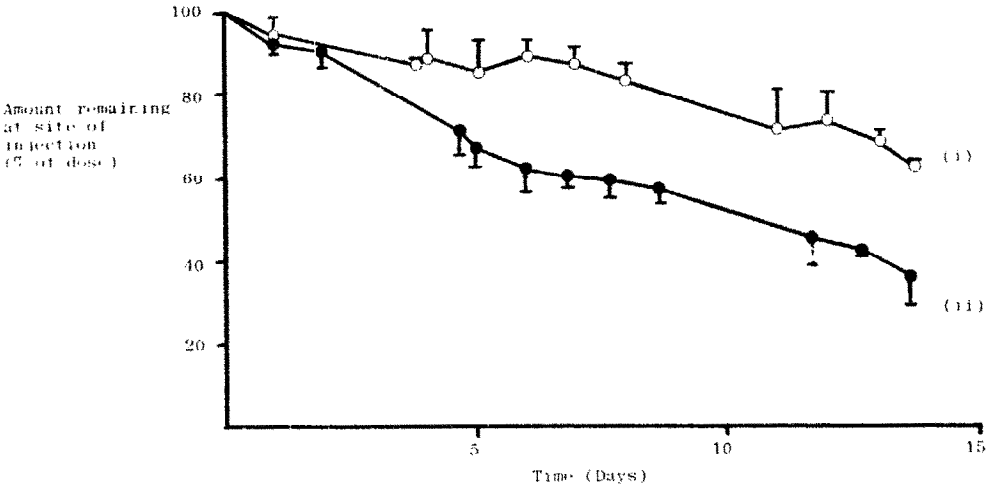

Fig. 1. Clearance of oils following 300 μl i.m. injection. (i) Arachis oil (iodinated); (ii) ethyl oleate (iodinated).

This type of analysis was used on clearance data following 300 μl injections of AOI and EOI by the i.m. and s.c. routes, and on 200 μl and 50 μl injections of EOI by the i.m. route.

Table 1 shows the complete list of results. The results of t-tests, measured at the 99% confidence interval, indicate that the clearance rate of AOI is significantly slower than the clearance rate of EOI when 300 μl is administered i.m. and s.c. Also the results show that the clearance rate of EOI from i.m. and s.c. sites when given in a dose of 300 μl are not significantly different ($P < 0.01$). A similar result is recorded for AOI when comparing the clearance rate following i.m. and s.c. administration. Furthermore, the volume of injection had no significant effect on the

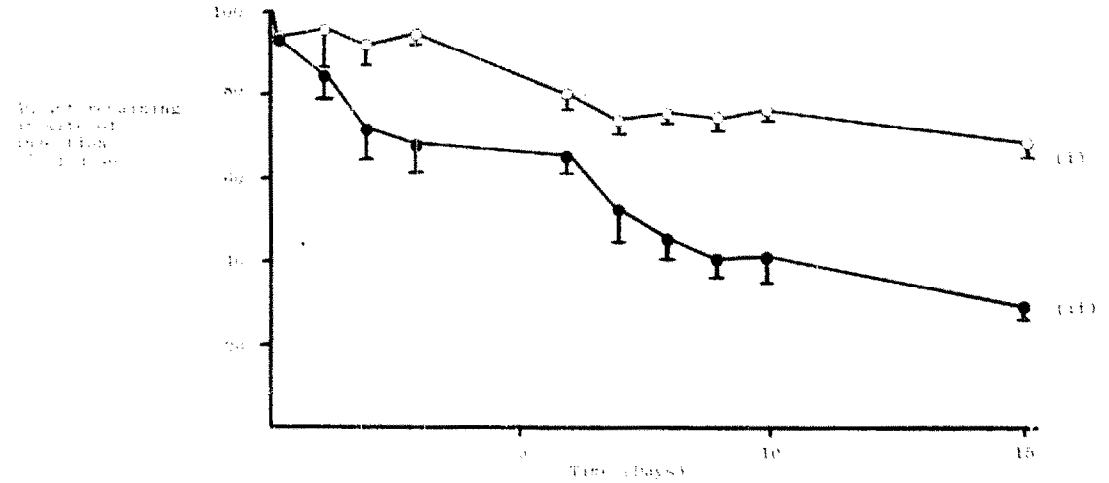

Fig. 2. Clearance of oils following 300 μl s.c. injection. (i) Arachis oil (iodinated); (ii) ethyl oleate (iodinated).

TABLE 1

Results of k_{el} and $t_{1/2}$ calculations for clearance of oils following s.c. and i.m. administration

Oil	Site of injection	Volume of injection (μl)	k_{el} (days)	($\pm$S.E.M.)	$t_{1/2}$ (days)
AO	i.m.	300	0.02971	(0.00322)	23.3
AO	s.c.	300	0.02556	(0.00348)	27.1
EO	i.m.	300	0.06904	(0.00337)	10.0
EO	i.m.	200	0.07568	(0.00554)	9.2
EO	i.m.	50	0.07020	(0.00437)	9.9
EO	s.c.	300	0.07823	(0.00386)	8.9

TABLE 2

Results of viscosity, surface tension and interfacial tension measurements

Oil	Surface tension (mNm^{-1})	Interfacial tension (mNm^{-1})	Kinematic viscosity (centistokes)	Absolute viscosity (centipoise)
AO	37.5	19.9	39.0	35.2
EO	32.3	17.4	4.6	3.9

clearance rate of EOI from the i.m. site ($P < 0.01$).

The low resolution of the system and the fact that data are collected in two-dimensional form (when spreading is clearly a three-dimensional process) preclude useful observations on the spreading characteristics of the oils in vivo.

Table 2 shows that the surface and interfacial tension of EO and AO are similar; however, EO is much less viscous than AO.

Discussion

From the results presented it appears that the clearance rate of the oil is independent of the injection site. This is in agreement with reports that drugs are absorbed at the same rate when given i.m. and s.c. and that the sites are bioequivalent (Hirano et al., 1982; Pfeffer et al., 1980).

Absorption from i.m. and s.c. sites can occur in several ways: by absorption through capillary blood vessels into the general circulation, by lymphatic absorption, by phagocytosis and by metabolism in situ followed by absorption.

An indication of the mechanism of absorption can be obtained by consideration of the anatomy of the circulatory systems at the i.m. and s.c. sites. It is known that muscle is well supplied by capillary blood vessels. However, it is generally recognized that few, if any, lymph vessels exist in muscle (Rusynyak et al., 1967; Pearson, 1962). It is known that lymph vessels exist where the fascial planes enter the muscle and

that lymph fluid flows along the fascial planes between the muscle fibres. Subcutaneous tissue is known to be well supplied with capillary blood vessels and lymphatic vessels (Ballard, 1968). Absorption by capillary blood vessels was advanced as the major factor for clearance of [^{14}C]methyl oleate following i.m. injection; absorption by the lymphatic system was thought to be less important (Tanaka et al., 1974). Absorption of oil by the lymphatic system, following i.m. injection, has been demonstrated in the laboratory. Svendsen and Aaes-Jørgensen (1979) studied the fate of sesame and vegetable oil following i.m. injection. Both oils were absorbed by the lymphatic system, to a maximum of about 5% of the total dose at any particular time. However, very high doses of oil were used (up to 10 times the dose per kg used in this study) over a long period of time. An appreciation of the anatomy of the lymphatic system would seem to indicate that when using chronic high doses of oil, some lymphatic absorption of oil would be inevitable, especially as oil has been shown to spread along the fascial planes of muscle (as does lymph fluid) following i.m. injection (Brown et al., 1944; Shaffer, 1929). Bollinger (1970) found that mineral oil was absorbed by the lymphatic system following s.c. and i.m. injection, as an oil in water emulsion. However, it has been shown that delivery of oil as an emulsion promotes absorption by the lymphatic system (Nakamoto et al., 1975).

Clinically, few reports exist which suggest that oil is absorbed by the lymphatics following i.m. injection. Ahmed and Greenwood (1973) described a case of lymphadenopathy following repeated injections of oil. Unfortunately, the authors do not give any indication of dose or site of injection, but it would appear that lymphatic absorption of oil following chronic parenteral therapy had indeed occurred. Furthermore, Svendsen et al. (1980) reported evidence of lymphogenic absorption of oil in humans receiving depot-neuroleptics i.m. As discussed, in the present study no differences between clearance rates of oils from i.m. and s.c. sites was observed. Considering then the difference in anatomy of the lymphatic system between muscle and s.c. tissue, it is unlikely that the lymphatic system plays a major role in the clearance of oils, in this study.

Several workers have observed that the volume of injection administered i.m. or s.c. can affect the absorption rates of drugs from oily and aqueous vehicles. Most workers report a reduced absorption rate with increase in injection volume (Pfeffer and Van Harken, 1981; Hirano et al., 1981, 1982). Other workers have found this effect to be less predictable (Honrath et al., 1963). Assuming that the major route of absorption of drugs given s.c. and i.m. is by the capillary blood vessels, then the effect of reduced absorption rate with increase in injection volume can be explained by a reduced surface area to volume ratio, with increased injection volume (Pfeffer and Van Harken, 1981). A similar relationship might be expected to exist if the major route of absorption of oily vehicles was by capillary blood vessels. Results presented in this paper show that the clearance rate of EOI following i.m. injection is volume-independent. Therefore, it would appear that absorption of the oil by blood capillaries is not a major factor, in the clearance from the site of injection, or that the surface area-to-volume ratio effect is overcome, possibly by the spreading characteristics of the oil in situ.

Earlier it was stated that Tanaka et al. (1974) found that [^{14}C]methyl oleate was

absorbed mainly by capillary blood vessels. This conclusion was arrived at using one injection volume and the observation that little radioactivity appeared in the lymphatic system. The spreading characteristics of the oil are likely to be a function of the viscosity and interfacial tension of the oil. The interfacial tension of EOI and AOI are similar, but the viscosities differ markedly. Since AO is more viscous than EO it is more resistant to spreading (flow) in this muscle; this might account for the slower clearance rate of AOI from s.c. and i.m. injection sites. Further investigation of a possible link between viscosity (spreading) and clearance rate of oils is merited.

It is possible that over the relatively long period of this study that 'infinite' distribution of the EOI at the various injection volumes has occurred around the site of injection, overcoming the surface area-to-volume ratio effect. If indeed this is the case, then absorption by capillary blood vessels could be important. Certainly the shape of the depot and the distribution of oil varies with time. Most studies reporting a decrease in absorption rate with increase in injection volume have been performed over hours rather than days (Tanaka et al., 1974; Hirano et al., 1981, 1982; Pfeffer and Van Harken, 1981). The shorter the time period the greater the effect of injection volume on the distribution and shape of the injected material is likely to be.

Phagocytosis has been suggested as a significant factor in the clearance of fats and oils following i.m. and s.c. injections (Rees et al., 1967; Bisgard and Baker, 1940). If phagocytosis does in fact contribute to the clearance of fats and oils following injection, it is likely to be related to the inflammatory response of the tissues to the vehicle (or drug) injected (Ballard, 1968).

AO is known to cause an inflammatory response in muscle tissue (Brown et al., 1944). A slight inflammatory response of muscle tissue to EO has been observed by Hem et al. (1974/75). It is possible that some degree of inflammatory response will occur following s.c. and i.m. injection as a result of physical trauma alone (Sidell et al., 1974). So it is feasible that phagocytosis has been responsible, at least in part, for the clearance of AOI and EOI from the injection sites in this study.

Metabolic degradation of the oils at the site of injection is a further possible explanation of the results presented. Svendsen and Aaes-Jørgensen (1979) state that "oil is thought to disappear by local metabolic degradation, absorption in the blood and phagocytosis". The results of this study support that statement, with lymphatic absorption of oils apparently not a major factor. It is probable that all of the absorption routes play some role under different conditions in vivo. Clearly the absorption characteristics of oily vehicles from s.c. and i.m. sites have yet to be fully elucidated, especially the role of metabolism and phagocytosis in situ, in the absorption process.

Several factors affect the choice of vehicle for sustained release formulations (Ballard, 1968; Chien, 1981). Even without knowing the exact mode or modes of the absorption processes of an oily vehicle, a knowledge of the $t_{1/2}$ of oily vehicles could be another useful factor in the choice of an oily vehicle for sustained release formulations.

The $t_{1/2}$ values of oils administered parenterally depend on the animal model (Svendsen and Aaes-Jørgensen, 1979). However, long $t_{1/2}$ values of oily vehicles

following chronic dosing could lead to the presence of multiple-depot sites. This could account in part for the observations of Wistedt et al. (1981), where following long-term use of depot fluphenazines, slow decline of blood drug levels were recorded after the cessation of therapy.

Acknowledgements

We wish to thank Dr. C.G. Wilson, Dr. J.G. Hardy, Dr. M. Frier, Mr. D. Whalley and Mr. A. Perkins for helpful discussions on the techniques of gamma-scintigraphy. We are grateful to the SERC for funding support for J.R.H.

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