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Intramuscular rate of disappearance of oily vehicles in rabbits investigated by gamma-scintigraphy

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Abstract

The fate of oily vehicles administered intramuscularly was followed with whole body gamma-scintigraphy. Groups of six rabbits received injections administered into the upper hind leg. No differences were observed in disappearance rates of various volumes (50–400 μl) of either fractionated coconut oil or sesame oil. Addition of two different concentrations of a drug substance, zuclopenthixol decanoate, to fractionated coconut oil did not influence the disappearance rate of the vehicle. Half-lives of the two oils at the injection site were in the order of 1 week for fractionated coconut oil and 1 month for sesame oil. Both oils spread approximately 25% along the muscle fibres during the first 24 h after administration. Radioactivity was mainly excreted with the urine. Insignificant amounts of radioactivity were found in blood, liver and carcass after 10 days.

Keywords: Oily vehicles; IM administration; Gamma-scintigraphy; Disappearance rates; Spreading; Rabbit

1. Introduction

Parenteral depot preparations intended for intramuscular administration are often formulated as solutions of a drug or a prodrug in an oily vehicle. The drug release rate is suggested to be controlled by the partition coefficient of the drug between the oily vehicle and the tissue fluid (Chien, 1981; Hirano et al., 1981; Davis et al., 1994). Due to the very strong affinity for the oil phase, highly lipophilic drugs may, however, be released concurrently with the disappearance of the oily vehicle from the injection site. The oily vehicle may disappear via blood or lymph or become metabolised directly at the site of injection. Thus, information on the fate of the vehicle at the injection site is relevant for selection of the most suitable oily vehicle during formulation development.

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Traditionally, disappearance of drug and vehicle from an intramuscular injection site has been investigated by excision and analysis of muscle tissue at various time points after administration to experimental animals (the local clearance method). Recent data (Larsen et al., 1998) have revealed that results obtained from whole body gamma-scintigraphy correlated well with observations obtained by the local clearance method. The non-invasive gamma-scintigraphy technique is considered preferable because (i) each animal can be followed over an extended period of time and accordingly function as its own control, (ii) fewer animals are necessary for the study, and (iii) smaller variations in results will occur.

The primary aim of the present study was to measure rate of disappearance of two oily vehicles, administered into the upper hind leg of rabbits from the intramuscular injection site, by means of whole body gamma-scintigraphy. Specifically, the effect of variations in dose volumes and addition of a drug substance on the disappearance rate was investigated. Zuclopenthixol decanoate was the chosen drug, as this is administered via the intramuscular route. In addition, the study aimed at obtaining information on distribution of the vehicle at the injection site.

2. Materials and methods

2.1. Materials

Fractionated coconut oil (Viscoleo) was supplied by H. Lundbeck A/S. Sesame oil and triolein were purchased from Sigma Chemical Co. Clopixol® and Clopixol Conc®. containing, respectively, 200 and 500 mg/ml of zuclopenthixol decanoate (MW 554.5) in fractionated coconut oil, were purchased from PIF Medical Supplies Ltd. Ultra-pure water was used throughout and all reagents were of analytical grade. Materials for radioiodinations were purchased directly by the Medical Physics Department, University Hospital, Nottingham.

2.2. Labelling procedure

Sesame oil was radioiodinated directly by a process involving the addition of ^{131}I to a double bond in the fatty acid chain of the triglycerides. Fractionated coconut oil, consisting of saturated triglycerides only, could not be iodinated directly. Therefore radioiodinated triolein was mixed with either the pure fractionated coconut oil or with the commercial preparations Clopixol® and Clopixol Conc®. The added amount of triolein constituted 2.5–5% of the total dose volume. The radioactivity of the doses was in the order of 1–25 MBq per dose. The labelling procedures for both sesame oil and triolein were performed at the Medical Physics Department, University Hospital, Nottingham using the method of Lubran and Pearson (1958).

2.3. Experimental animals

Groups of six adult female New Zealand White rabbits weighing approximately 3 kg were used throughout. The animals were housed in individual cages for the duration of the study.

2.4. Administration of formulations

Each formulation was administered into the upper hind limb (vastus lateralis) of each of the six rabbits in the group using a 0.6×30-mm needle and a 1-ml syringe. The needle was inserted to a depth of 10–15 mm.

2.5. Gamma image collection

Using a Searle gamma camera and a General Electric gamma camera (Nuclear Portacamera IIc) lateral and ventral whole body images of the rabbits were collected immediately after administration of the formulations and at the specified sampling points. The imaging time was 1 or 2 min throughout.

2.6. Determination of spreading of the oily vehicle at the injection site

Spreading of the dosed vehicle at the injection site was investigated from the gamma camera

images. The unit area at the injection site region, containing the maximum radioactivity, was identified. A line was drawn through this unit area across the widest distribution of radioactivity (Fig. 1A). A curve was generated to represent the distribution of radioactivity along this chosen datum line (Fig. 1B). At a level of 10% of the maximum counts obtained, taken as a background, a line was drawn on the curve. The length of this line was measured and was taken to represent the spread of radioactivity at the injection site (Fig. 1B).

2.7. Radioactivity in blood, urine, faeces, liver and carcass

Two groups of six rabbits received injections of either 400 μl of sesame oil or fractionated coconut oil, respectively, both labelled with ^{131}I as described above. Radioactivity in samples of blood, and in urine and faeces collected for 10 days following administration, was determined by liquid scintillation counting. After 10 days the rabbits were killed, and radioactivity present in liver and carcass was determined by means of liquid scintillation counting and whole body gamma-scintigraphy, respectively.

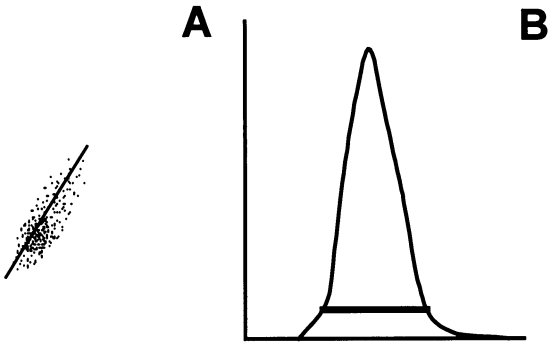

Fig. 1. Method for determination of extent of spreading. (A) Insertion of line across the widest distribution of radioactivity on the gamma camera image. (B) Curve representing concentration of radioactivity along the line in (A) Spreading taken as length of line at 10% of the maximum radioactivity.

Table 1
Radioactivity accumulated in excretions and present in blood, liver, carcass and at injection site 10 days after administration of 400 μl of oily vehicle to rabbits

Compartment	Sesame oil	Fractionated coconut oil
Urine	22	66
Faeces	2	6
Blood	<1	<1
Liver	<1	<1
Carcass[a]	79	40
Injection site	73	29

Values are expressed as percentages of the total amount of radioactivity administered.
[a] Injection site included.

3. Results and discussion

Visual inspection of the gamma images revealed no difference between data collected from ventral or lateral views. Therefore calculations are only based on data obtained from lateral views. Radioactivity found in the area corresponding to the muscle used for injection, was corrected for background counts and decay of radioactivity, and expressed as percentage of the radioactivity determined in the muscle at time zero. Disappearance profiles were plotted from the mean values. In most cases, standard deviations of the six individual determinations in the range 5–15% were observed.

The mass balance data given in Table 1 indicate that the radioactivity was either contained at the injection site or excreted with the kidneys constituting the dominant excretion pathway. There was no evidence of significant distribution elsewhere and measured low amounts of radioactivity in the blood indicated that the material was efficiently cleared from the body concurrent with release of radioactivity from the injection site. Free fatty acids obtained from lipase-mediated hydrolysis of triglycerides are incorporated in fatty tissues or are metabolised to CO_2 with simultaneous liberation of energy (Lehninger, 1982). The high urinary excretion of radioactivity observed in this study suggests that attachment of iodine impaired the normal catabolism of triglycerides most likely due to a steric hindrance of the

β-oxidation pathway of the free iodinated fatty acids. Whereas ^{131}I-labelled triglycerides adequately describe the intramuscular disappearance rate of oily vehicles (Larsen et al., 1998), iodinated triglycerides might not completely mimic the metabolic fate of parent triglycerides.

The relative spread of radioactivity after administration is shown in Table 2. Spreading occurred predominantly during the first day after administration and appeared to be along the line of the muscle fibres. This is in accordance with previous data showing that only little spreading occurred following intramuscular injections of iodinated oils, and that the spreading took place during the first few minutes after administration (Shaffer, 1929).

Disappearance-time profiles obtained after administration of four different dose volumes, 50, 100, 200 and 400 μl, of fractionated coconut oil and sesame oil are shown in Fig. 2A and Fig. 2B, respectively. To enable comparison of the individual rate profiles the data were treated according to first-order kinetics, thus allowing each curve to be expressed by a single parameter. This approximation appears reasonable as apparent from the calculated correlation coefficients close to unity (Table 3). An analysis of residuals reveals that eight of 10 curves can be regarded as linear. No significant difference ($p = 0.95$, t-test) between the relative disappearance rates were observed after administration of oil volumes in the range 50–400 μl. Similarly, equal disappearance rates for various volumes (50–300 μl) of ethyl oleate administered to rabbits have been reported (Howard and Hadgraft, 1983). In contrast, Hashida et al. (1977), found an increase in disappearance rate from rat muscles with increasing volumes (10–50 μl) of sesame oil. These conflicting results may reflect that different animal models were used.

Table 2
Relative spreading of radioactivity from the position at time zero after im administration of 400 μl of oily vehicle to rabbits

Time (h)	Sesame oil	Fractionated coconut oil
0	100	100
24	125 ± 22	131 ± 32
48	123 ± 17	126 ± 30

Mean values and standard deviations are shown.

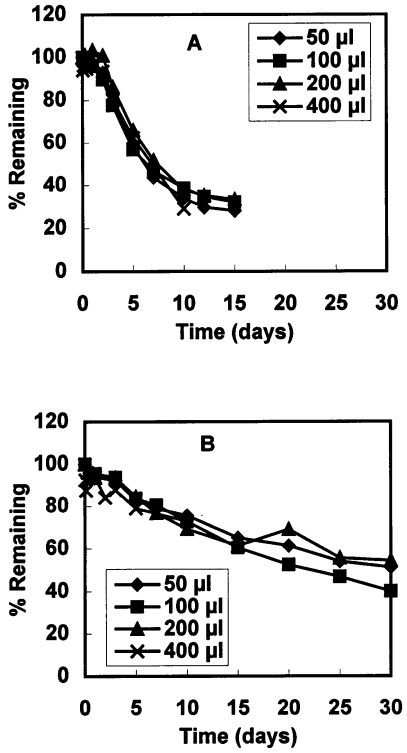

Fig. 2. Disappearance of oily vehicle from the site of injection after administration of various dose volumes into the upper hind limb of rabbits. Mean values of six animals. (A) Fractionated coconut oil. (B) Sesame oil.

Rates of disappearance of the oily vehicles, expressed as half-lives, from muscle tissue are in the order of 1 week for fractionated coconut oil and approximately 1 month for sesame oil. It must be noted that the half-lives are derived from disappearance profiles followed for only 70% (fractionated coconut oil) and 50% (sesame oil) of disappearance of the two oily vehicles. A $t_{1/2}$ of ethyl oleate of 10 days at the injection site has been reported (Howard and Hadgraft, 1983). In addition, the latter authors estimated that the disappearance of 300 μl of arachidis oil from the muscle tissue had a half-life of 23 days. Castor oil, being very viscous, is reported to remain in the tissues almost indefinitely after intramuscular injection (Madan, 1985). Viscosities and half-lives of the various oil vehicles are listed in Table 4.

Table 3
First-order rate constants and half-lives derived from the disappearance profiles obtained after im adminstration of various dose volumes of sesame oil and fractionated coconut oil, respectively

Dose (μl)	Sesame oil			Fractionated coconut oil		
	$k \times 10^2$ (S.D.) (days^{-1})	$t_{1/2}$ (days)	Corr. coeff.	$k \times 10^2$ (S.D.) (days^{-1})	$t_{1/2}$ (days)	Corr. coeff.
50	2.4 (45%)	29	−0.99	10.3 (30%)	7	−0.98
100	3.2 (29%)	22	−1.00	8.7 (23%)	8	−0.98
200	2.1 (46%)	33	−0.94	9.1 (27%)	8	−0.98
400	2.8 (9%)	25	−0.90	12.1 (25%)	6	−0.98
200[a]	—	—	—	8.0 (29%)	9	−1.00
200[b]	—	—	—	6.5 (42%)	11	−1.00

Mean values and standard deviations of the rate constant obtained from data of each animal are given together with half-lives and correlation coefficients corresponding to the mean values.
[a] Clopixol (zuclopenthixol decanoate, 190 mg/ml).
[b] Clopixol Conc. (zuclopenthixol decanoate, 475 mg/ml).

Enhanced disappearance rates with decreasing viscosity of the triglycerides are observed. A priori, it was expected that a low viscosity would facilitate spreading of the oily vehicle, thereby accelerating the disappearance. However, fractionated coconut oil and sesame oil exhibiting different viscosities and intramuscular half-lives, spread to the same extent (Table 2). Likewise, the intramuscular half-life of ethyl oleate exceeds that of the more viscous fractionated coconut oil. Zuclopenthixol possessing a certain degree of surface activity was incorporated into fractionated coconut oil in high concentrations. However, as seen from Table 3 addition of the latter prodrug derivative did not significantly influence the disappearance rate of the oil from the muscle tissue after injection of 200 μl of the formulation. At present no single factor governing the intramuscular half-life of oily vehicles has been identified. Since chemical hydrolysis of the glyceride ester bond is expected to proceed very slowly at physiological pH, an oily vehicle has to be cleared from the muscle in a solubilised state or by enzymatic degradation at the injection site. The latter mechanisms might be influenced by the composition of the oils.

The rationale for investigating the rate of disappearance of oily vehicles from the injection site is to determine the influence of the vehicle on the duration of action of parenteral depot formulations. Probably, the partition coefficient of the drug compound between the oily vehicle and the tissue fluid determines the release mechanism. Extremely lipophilic drugs (for instance decanoate esters) possessing very high affinities for the oily phase, are probably released concurrently with disappearance of the vehicle. Less lipophilic drug compounds may, by simple partition, be released from the oil phase to the tissue fluid prior to disappearance of the oil. Following the intramuscular administration to humans of perphenazine decanoate dissolved in sesame oil, longer t_{max} and lower C_{max} of perphenazine plasma concentration were found, compared with those parameters obtained after administration of a solution in fractionated coconut oil (Knudsen et al., 1985). This

Table 4
Viscosities and muscular disappearance rates of various oily vehicles

Oily vehicle	Viscosity, 37°C (cP)	$t_{1/2}$
Ethyl oleate	3.9[a]	10 days[a]
Fractionated coconut oil	15[b]	1 week[c]
Sesame oil	35[b]	1 month[c]
Arachidis oil	35.2[a]	23 days[a]
Castor oil	286[b]	'Indefinitely'[d]

[a] Howard and Hadgraft (1983).
[b] Hirano et al. (1981).
[c] Present study.
[d] Madan (1985).

is in accordance with the different disappearance rates found here for the two oils, assuming that the very lipophilic drug substance was released simultaneously with the oil vehicle. Two esters of zuclopenthixol, an acetate and a decanoate, dissolved in fractionated coconut oil, gave rise to durations of pharmacological actions of 2–3 days and 2 weeks, respectively, after intramuscular administration to humans (Aaes-Jørgensen, 1989). Assuming that the hydrolysis rates of the two esters in vivo are of the same order of magnitude, these two drugs with different lipophilicities may probably follow two different disappearance mechanisms. Hirano et al. (1981) investigated the release rate of *p*-aminoazobenzene from various vehicles after im administration to rats. The drug disappeared at a faster rate from sesame oil ($t_{1/2} = 2.1$ h) than from fractionated coconut oil ($t_{1/2} = 4.6$ h). In spite of relatively high affinity for the two oily vehicles (log *P* of 3.5 for fractionated coconut oil and 3.1 for sesame oil, respectively), *p*-aminoazobenzene is released according to partition rather than being released to the tissue fluid concurrently with disappearance of the oily vehicle. Based on these observations, it is likely that derivatisation to a more lipophilic compound may be an option, if the drug possesses an insufficient lipophilicity, and in vivo results reveal too rapid release from the depot. Subsequently, the disappearance rate of the oily vehicle alone may determine the duration of action of the depot formulation.

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References

Aaes-Jørgensen, T., 1989. Pharmacokinetics of three different injectable zuclopenthixol preparations. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 13, 77–85.

Chien, Y.W., 1981. Long-acting parenteral drug formulations. J. Parent. Sci. Technol. 35, 106–139.

Davis, J.M., Metalon, L., Watanabe, M.D., Blake, L., 1994. Depot antipsychotic drugs. Place in therapy. Drugs 47, 741–773.

Hashida, M., Egawa, M., Muranishi, S., Sezati, H., 1977. Role of intramuscular administration of water in oil emulsions as a method for increasing the delivery of anti-cancer agents to regional lymphatics. J. Pharm. Biopharm. 5, 225–239.

Hirano, K., Ichihashi, T., Yamada, H., 1981. Studies on the absorption of practically water-insoluble drugs following injection. I. Intramuscular absorption from water-immiscible oil solutions in rats. Chem. Pharm. Bull. 29, 519–531.

Howard, J.R., Hadgraft, J., 1983. The clearance of oily vehicles following intramuscular and subcutaneous injections in rabbits. Int. J. Pharm. 16, 31–39.

Knudsen, P., Bolvig Hansen, L., Larsen, N.-E., 1985. Pharmacokinetic implications of different oil vehicles used in depot neuroleptic treatment. Acta Psychiatr. Scand. 72 (Suppl. 322), 7–10.

Larsen, C., Schultz, K., Fisher, A., Illum, L., 1998. Intramuscular fate of ^{14}C- and ^{131}I-labelled triglycerides. Int. J. Pharm. (in press).

Lehninger, A.L., 1982. Principles of Biochemistry. Worth, New York.

Lubran, M., Pearson, J.D., 1958. A screening test for steatorrhoea using ^{131}I-labelled triolein. J. Clin. Pathol. 11, 165–169.

Madan, P.L., 1985. Sustained-release drug delivery systems: Part V. Parenteral products. Pharm. Manufact. June, 51–57.

Shaffer, L.W., 1929. The fate of intragluteal injections. Arch. Dermatol. Syphilol. 19, 347–364.