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Intramuscular absorption and biodistribution of dexamethasone from non-aqueous emulsions in the rat

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Abstract

Non-aqueous or oil-in-oil emulsions may be used as reservoirs to deliver lipophilic or hydrolytically unstable drugs. Emulsions of castor oil-in-silicone oil (co/so) release drugs slowly *in vitro*. To investigate the potential use of such formulations as depot preparations *in vivo*, drug absorption and distribution from an intramuscular injection site to various organs in the rat was studied. ^{3}H-dexamethasone (0.1 mg/kg) was incorporated into the castor oil (disperse phase) of co/so emulsions and in castor oil-in-water (co/w) emulsions, the latter serving as control. ^{3}H-dexamethasone was absorbed after intramuscular injection of co/w emulsions, reaching a plasma C_{max} of 0.078 μg/ml at 2.0 h (T_{max}). For co/so emulsions, a lower C_{max} (0.048 μg/ml) was observed with a longer T_{max} (4.0 h). No significant difference was found between the two formulations in the area under the plasma concentration–time curve (AUC_{∞}), or in clearance (CL). Administration of ^{3}H-dexamethasone in the co/so emulsion improved the mean residence time (MRT) and the elimination half-life ($t_{1/2}$) in comparison to the co/w emulsion. The clearance of ^{3}H-dexamethasone from the co/so emulsions at the injection site was also slower and at 4.0 h post-injection the amount of drug remaining in the muscle was found to be eight times higher than with the co/w emulsions. For both formulations, a high uptake of ^{3}H-dexamethasone was identified in the liver and kidneys whereas smaller amounts were found in other tissues. Non-aqueous emulsions could be considered as depot formulations for sustained release drug delivery, but further studies on the choice of the continuous phase are necessary to optimize effects.

Keywords: Non-aqueous emulsions; Lipophilic drug; Distribution; Intramuscular absorption; Dexamethasone

1. Introduction

The use of emulsions as depot preparations to be given by intramuscular injection was first discussed in the 1970s. Nakamoto et al. (1975) suggested that the parenteral administration of mitomycin C (MMC) as an emulsion preparation (either water-in-oil, w/o; or oil-in-water, o/w) was more effective for lymphatic transport than administration as an aqueous solution. These authors reported an increase in the lymph:plasma concentration ratio following the intramuscular injection of w/o emulsions of MMC when compared to the o/w emulsions. The absorption of emulsions of ^{131}I-iodohippuric acid (a hydrophilic model compound) in the rat from muscle into lymph nodes was enhanced in the following order: aqueous solution < w/o emulsions < gelatin-containing w/o emulsions (Hashida et al., 1977). More recent studies on w/o emulsions have suggested their possible use as vehicles for the sustained release of hydrophilic drugs from intramuscular injection sites (Bjerregaard et al., 2001). Multiple water-in-oil-in-water (w/o/w) emulsions have also been reported to achieve prolonged release of hydrophilic compounds after intramuscular administration in animals such as the beagle dog (Florence et al., 1976), the rabbit (Davis et al., 1987) and the rat (Omotosho et al., 1989). The use of emulsions may provide some advantages over oily solutions or suspensions, because of the capacity to vary parameters such as droplet size, phase volume, and viscosity (Bjerregaard et al., 1999).

Non-aqueous or oil-in-oil emulsions are composed of two immiscible non-aqueous or oil phases. However, there are relatively few reports which deal with such systems (Hamill et

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al., 1965; Hamill and Petersen, 1966a, 1966b; Cameron and Sherrington, 1996). Imhof and Pine (1997) used non-aqueous emulsions for the preparation of nanoparticles or as templates in the formation of silicate microstructures. We have recently investigated this particular emulsion type because such a formulation may prove useful as a depot, or reservoir vehicle, for poorly water-soluble compounds (Sakthivel et al., 2001). *In vitro* studies have shown that non-aqueous emulsions were able to sustain drug release over a period of 48 h (Jaitely et al., 2004; Suitthimeathegorn et al., 2005). In this paper castor oil-in-silicone oil (co/so) emulsions were investigated in an *in vivo* study in the rat to evaluate the potential of non-aqueous emulsions to act as drug reservoirs.

2. Materials and methods

2.1. Materials

^{3}H-dexamethasone (specific radioactivity 261 mCi/mg) was purchased from Amersham Biosciences (Buckinghamshire, UK), and dexamethasone, castor oil, hydrogen peroxide, and isoamyl alcohol were supplied by Sigma (Gillingham, Dorset, UK). Silicone oil (Dow Corning, DC 200 Fluid, 20 cSt) was supplied by S. Black Ltd. (Hertfordshire, UK) and silicone surfactant (DC3225C; cyclomethicone/PEG/PPG-18/18 dimethicone) was from Dow Corning (Thailand). Biosol and Bioscint were obtained from National Diagnostics (Hessle, Hull, UK). A Polytron PT 3000 homogeniser (Kinematica AG, Switzerland) was used for tissue homogenization.

2.2. Preparation of ^{3}H-dexamethasone emulsions

Dexamethasone 6 mg was dissolved in 2 ml absolute ethanol in a round bottomed flask and ^{3}H-dexamethasone 600 μCi added. The solution was dried in a rotary evaporator. The ^{3}H-dexamethasone was dissolved in the castor oil phase due to the high castor oil-silicone oil partition coefficient ($K = 20{,}417$). Castor oil-in-silicone oil (co/so) and castor oil-in-water (co/w) emulsions were prepared by sonication. The silicone surfactant (DC3225C) and a mixture of polysorbate 20:sorbitan monooleate 80 (75:25) were used as the surfactants to emulsify the co/so and co/w emulsions, respectively. A similar mean particle size for both emulsions was found, namely 1.2 (±0.16) and 1.3 (±0.16) for the co/so and co/w emulsions, respectively (Mastersizer S, Malvern Instruments, Worcestershire, UK). The physicochemical properties of co/so emulsions have previously been discussed (Suitthimeathegorn et al., 2005). All emulsions were freshly prepared and the uniformity of distribution of the tracers was checked prior to administration.

2.3. Animals

Male, Sprague–Dawley rats (Harlan, Blackthorn, Bicester, Oxon, UK), mean body weight 282 g, were caged in groups of three to four and acclimatized for at least 7 days before the start of each experiment; animals were allowed free access to diet (Extruded Global Rodent Diet; Harlan Teklad, Blackthorn, Bicester, Oxon, UK) and water. A temperature of 19–22 °C was maintained, with a relative humidity of 45–65%, and a light:dark cycle of 12:12 h (lights on at 07.00 h). All procedures followed the UK Home Office (1989) "Code of Practice for the Housing and Care of Animals used in Scientific Procedures".

2.4. Intramuscular injection

Two experiments were carried out. In the first experiment, rats ($n = 28$) were lightly anesthetized with isoflurane and injected with a single dose of 100 μl ^{3}H-dexamethasone in a co/so emulsion into the gastrocnemius (calf) muscle of the lower left hind limb. ^{3}H-dexamethasone was given at a dose of 0.1 mg/kg and the specific activity of ^{3}H-dexamethasone was 100 μCi/mg. For the injection, a 25G (5/8) 0.5 × 16 needle (Becton Dickinson, Oxford, UK) was used and care was taken to achieve uniformity of the injection site in all animals. After injection, animals were allowed free access to diet and water. A second (control) experiment was conducted using the same procedure as in the first study but the animals ($n = 28$) were injected with ^{3}H-dexamethasone in co/w emulsions. In each experiment an additional group of four rats served as blank controls, the animals being injected with the corresponding emulsions without drug.

In addition to the two main experiments (above) preliminary studies were performed to validate all procedures (e.g. the presence and location of the emulsion at the injection site and the amount of radioactive drug in different organs).

2.5. Blood and organ sampling

In each of the two experiments, four rats were autopsied at 0 (control), 0.5, 2, 4, 8, 24, and 48 h post-injection following the intraperitoneal (i.p.) injection of pentobarbital sodium (Euthatal, Merial, Harlow, Essex, UK). Blood was withdrawn from the abdominal aorta and collected into 4.5 ml lithium-heparin tubes (Sarstedt, Beaumont Leys, Leicester, UK) which were centrifuged (2500 × *g*, 10 min, room temperature) and the plasma harvested and stored at −80 °C. All muscle tissue at the injection site and surrounding the tibiofibular was removed and weighed. Organs were removed (contralateral muscle, spleen, liver, adrenals, kidneys, heart, lungs, stomach, cervical lymph nodes, thymus and testes), weighed and stored at −80 °C. For paired organs, both organs were taken.

2.6. Preparation of organs for liquid scintillation counting

Tissues were homogenized with an equal amount (w/w) of PBS. An aliquot of 200 mg of tissue homogenate was solubilized in 1.0 ml of tissue solubilizer (Biosol®) and shaken overnight at 55 °C. After incubation, 17 ml of self-acidified (Bioscint®) scintillation liquid was added to each sample and kept in darkness for 72 h before counting in a Beckman LS6500 Multi-Purpose Scintillation Counter (GMI, Ramsey, Minnesota, USA). Coloured samples were decolorized with 200–400 μl of 30% hydrogen peroxide, and isoamyl alcohol was added to reduce foaming.

2.7. Pharmacokinetic analysis

Pharmacokinetic parameters for ^{3}H-dexamethasone were determined according to model-independent analysis. The maximum plasma concentration (C_{max}) and the corresponding time (T_{max}) were obtained directly from the individual plasma concentration–time profile. The elimination rate constant (k_e) was estimated from the slope of a semilogarithmic concentration–time curve in the terminal phase and the elimination half-life ($t_{1/2}$) was then obtained from $\ln 2/k_e$. The area under the plasma concentration–time curve ($AUC_{0\text{-}t}$) and area under the first moment curve (AUMC) were calculated by the trapezoidal rule (Gibaldi and Perrier, 1975). The other parameters such as the area under the plasma concentration–time curve from time zero to infinity (AUC_∞), mean residence time (MRT) and total body clearance (CL) were calculated using the following standard equations (Wagner, 1993):

$$AUC_\infty = AUC_{0-t} + \frac{C_t}{k_e},$$

$$AUMC_\infty = AUMC_{0-t} + \frac{C_t T}{k_e} + \frac{C_t}{k_e^2}, \qquad MRT = \frac{AUMC}{AUC},$$

$$CL = \frac{Dose}{AUC}$$

where C_t is the plasma concentration observed at time t (T).

2.8. Statistical analysis

Mean values of unpaired data were analyzed using Student's t-test using the Microsoft 'Excel' programme. Differences between values were considered statistically significant where P values were less than 0.05.

3. Results

3.1. Dexamethasone plasma concentrations

The dexamethasone plasma concentration profiles in the rat following a single intramuscular injection of the co/so emulsions and the co/w emulsions are shown in Fig. 1. Statistically significant differences ($P < 0.05$) were found between the co/so and the co/w emulsions in the mean levels at each time point, except at 48 h post-dosing. The pharmacokinetic parameters are summarized in Table 1.

Following the intramuscular injection of the co/w emulsion a peak plasma concentration (T_{max}) of the drug appeared at 2.0 h post-dosing (Fig. 1). However, following the administration of the non-aqueous emulsions, T_{max} occurred at 4.0 h post-dosing. A lower maximum plasma concentration (C_{max}) was detected after the administration of the non-aqueous emulsions (0.048 μg/ml) compared to the oil-in-water system (0.078 μg/ml). No significant difference was found between the areas under the plasma concentration–time curve (AUC_∞).

The mean residence time (MRT) value was 1.42 times greater for the co/so emulsion than for the co/w emulsion (Table 1). The prolonged release of drug from the co/so emulsions was also reflected in the elimination half-life ($t_{1/2}$) which was 1.39 times longer.

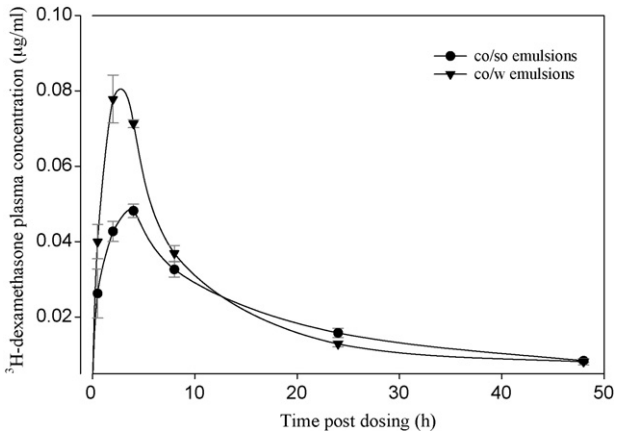

Fig. 1. A plot of ^{3}H-dexamethasone plasma concentrations as a function of time following the intramuscular injection of single dose of (●) castor oil-in-silicone oil emulsions and (▼) castor oil-in-water emulsions; each datum point is the mean value (±S.D.) of four rats; statistically significant differences ($P < 0.05$) were found between the two emulsions at each time point, except at 48 h post-dosing.

3.2. Absorption of ^{3}H-dexamethasone from the injection site

The clearance of ^{3}H-dexamethasone from the site of the intramuscular injection of the two types of emulsions is shown in Fig. 2. The drug was cleared more slowly from the non-aqueous formulation: 49% of the drug remained at the injection site 2.0 h after administration of the co/so formulation compared to 19% for the co/w emulsion. At 4.0 h post-dosing, the percentages were 33% and 4%, and at 8 h, 18% and 1%, respectively.

Assuming that the amount of drug released from the two emulsions was totally absorbed into the blood, the rate of drug release from the emulsions (k_r) is equivalent to the rate of drug absorption from the injection site in the muscle (k_a). A semilog plot of ^{3}H-dexamethasone remaining at the injection site versus time, is non-linear, and appears to comprise at least an initial

Table 1
Plasma pharmacokinetic parameters[a] following the intramuscular injection of rats with ^{3}H-dexamethasone in castor oil-in-silicone oil (co/so) emulsions and castor oil-in-water (co/w) emulsions[b]

Parameter (units)	Co/so emulsions	Co/w emulsions
k_e (h^{-1})	0.033	0.046
T_{max} (h)	4.0	2.0
C_{max} (μg/ml)	0.048	0.078
$t_{1/2}$ (h)	20.87	15.04
AUC_∞ (μg/(h ml))	1.232	1.258
MRT (h)	28.19	19.85
CL (l/(h kg))	0.080	0.077

[a] Abbreviations: k_e, elimination rate constant; T_{max}, time to reach maximum plasma concentration; C_{max}, maximum plasma concentration; $t_{1/2}$, elimination half-life; AUC_∞, plasma concentration–time curve from time zero to infinity; MRT, mean residence time; CL, total body clearance.

[b] Values are means; there were four rats in each emulsion group at each time point: 0 (control), 0.5, 2, 4, 8, 24 and 48 h post-dosing.

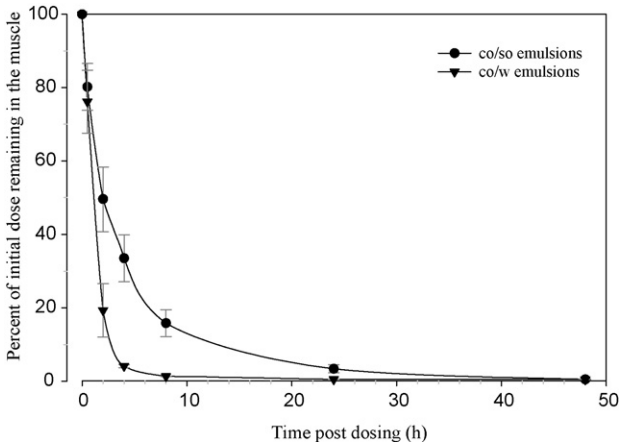

Fig. 2. Clearance of ^{3}H-dexamethasone from the injection site in the gastrocnemius muscle of the rat after the intramuscular injection of (●) castor oil-in-silicone oil emulsions and (▼) castor oil-in-water emulsions. The results are expressed as the percentage of the initial dose remaining in the muscle after injection for up to 48 h post-dosing. Each datum point is the mean value (±S.D.) of four rats at each time point.

phase of rapid absorption followed by a phase of slower absorption (data not shown). The absorption half-life ($t_{1/2}$) of drug in the immediate post-dosing period from the co/so emulsions and from the co/w emulsions was found to be ca. 200 and 50 min, respectively ($t_{1/2} = 0.693/k_a$).

3.3. Tissue distribution of ^{3}H-dexamethasone

The percentages of the administered dose of ^{3}H-dexamethasone (per g of wet tissue) in various organs after the dosing of the co/w emulsions were generally higher than the percentages for the co/so emulsions (Figs. 3 and 4). For both emulsions, the distribution patterns of ^{3}H-dexamethasone from 0.5 h to 48 h in each organ reflected the plasma concentration profiles (Figs. 3 and 4, insets).

The liver contained the highest concentration of the drug at 4.0 h post-dosing, followed by the kidneys (Fig. 3), for both formulations. Little radioactivity (a 10 times lower level than in the liver) was detected in the contralateral gastrocnemius muscle, and in the thymus gland and in the testes. The uptake of ^{3}H-dexamethasone into the tissues at the 4.0 h time point was in the following order: liver > kidneys > spleen > lungs > stomach > adrenals > heart > lymph nodes > thymus > contralateral gastrocnemius muscle > testes.

The results for tissue distribution following the injection of the co/w emulsions were generally similar to those obtained from the co/so emulsion (Fig. 4). The ranked order of ^{3}H-dexamethasone tissue distribution at the 2.0 h time point post-dosing was liver > kidneys > adrenals > lungs > heart > stomach > lymph nodes > spleen > thymus > contralateral gastrocnemius muscle > testes.

The amount of ^{3}H-dexamethasone in the liver and kidneys at 2.0 h after the injection of the oil-in-water emulsions was approximately 1.80 and 1.95 times higher than for the non-aqueous emulsions (Fig. 5), a result reflected in the plasma level at 2.0 h post-dosing (Fig. 1).

4. Discussion

A reduced peak plasma concentration (1.63 times lower) with a longer T_{max} (prolonged 2.0 times) was observed for the non-aqueous emulsion when compared with a co/w emulsion and the MRT and $t_{1/2}$ were also increased. The mean residence time (MRT) (Yamaoka et al., 1978) is a useful parameter for assessing the sustained-release behaviour of a drug after intramuscular administration. Here it shows that the co/so emulsions caused a delay in the absorption of ^{3}H-dexamethasone.

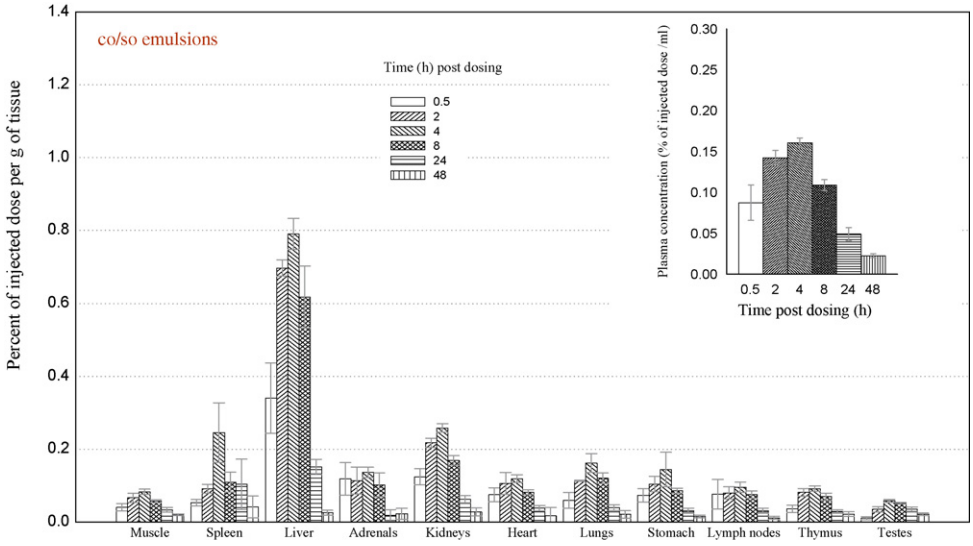

Fig. 3. Tissue distribution of ^{3}H-dexamethasone (0.1 mg/kg) in the rat following intramuscular administration into the gastrocnemius muscle as castor oil-in-silicone oil (co/so) emulsions. The amount of ^{3}H-dexamethasone distributed in each organ is shown at 0.5, 2, 4, 8, 24, and 48 h post-dosing. The results (mean ± S.D.) are expressed as the percentage of the injected dose per g of wet tissue. There were four rats at each time point. The inset shows the ^{3}H-dexamethasone plasma concentration profile after injection of the co/so emulsions. Muscle refers to the contralateral gastrocnemius muscle.

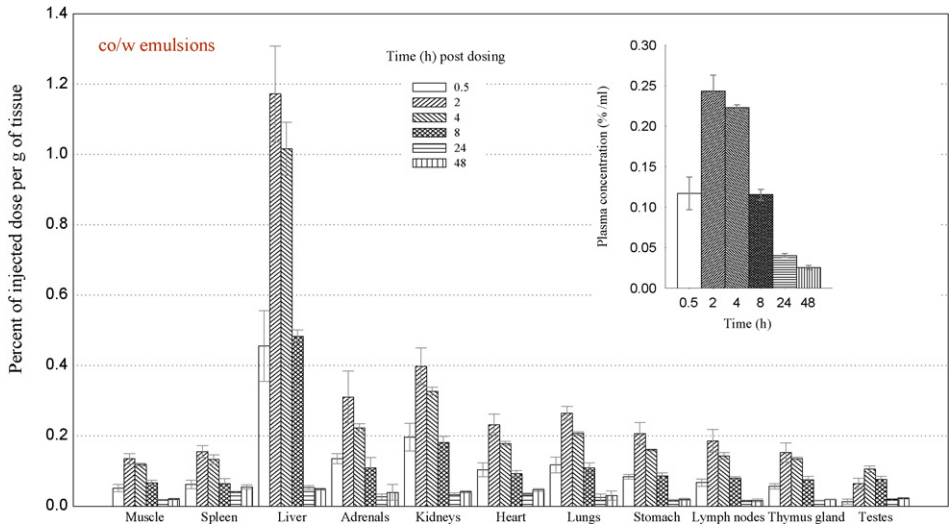

Fig. 4. Tissue distribution of ^{3}H-dexamethasone (0.1 mg/kg) in the rat following intramuscular administration into the gastrocnemius muscle as castor oil-in-water (co/w) emulsions. The amount of ^{3}H-dexamethasone distributed in each organ is shown at 0.5, 2, 4, 8, 24, and 48 h post-dosing. The results (mean ± S.D.) are expressed as the percentage of the injected dose per g of wet tissue. There were four rats at each time point. The inset shows the ^{3}H-dexamethasone plasma concentration profile after injection of the co/w emulsions. Muscle refers to the contralateral gastrocnemius muscle.

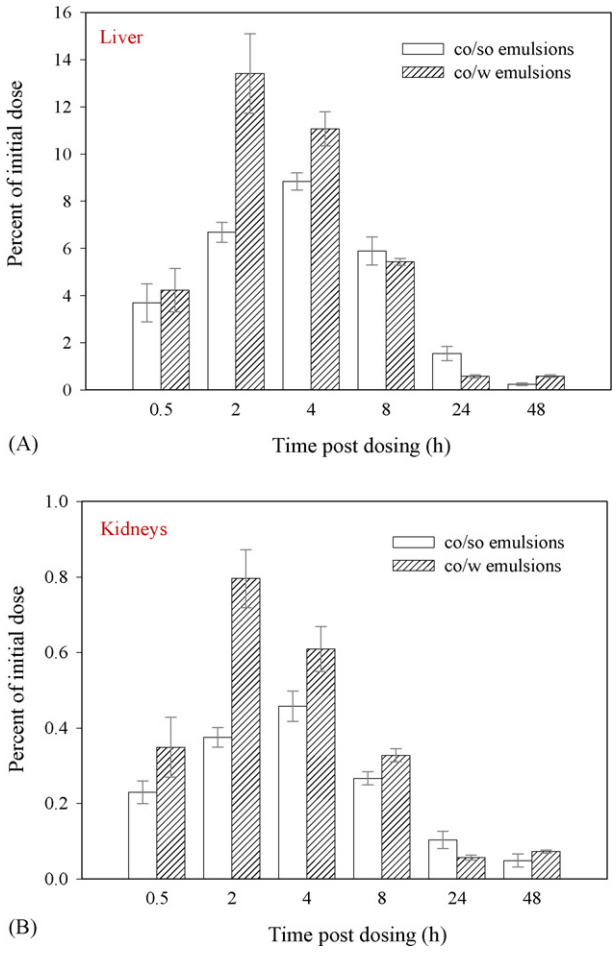

Fig. 5. The percentage of the injected dose of ^{3}H-dexamethasone distributed in A; the liver, and B; the kidneys, at 0.5, 2, 4, 8, 24, and 48 h post-intramuscular injection. The results are for castor oil-in-silicone oil (co/so) emulsions, and castor oil-in-water (co/w) emulsions. Each datum point is the mean value (±S.D.) of four rats.

However, although the co/so emulsions brought about a slower absorption of the drug than was the case for the co/w emulsions, both formulations demonstrated a modestly prolonged drug absorption when compared to reports in the literature with studies on dexamethasone aqueous solutions (Varma and Mulay, 1980; Hansen et al., 1999). These present results also find a parallel with studies from other laboratories. Mager et al. (2003) reported a relatively short MRT (7.80 h) and $t_{1/2}$ (5.55 h) following the intravenous injection of dexamethasone sodium phosphate. Furthermore, Samtani and Jusko (2005) found that dexamethasone was absorbed rapidly, reaching a maximum concentration (T_{max}) within 45 min after the intramuscular administration of dexamethasone sodium phosphate (an aqueous solution), and these workers concluded that the pharmacokinetic profile of the drug given by intramuscular route was not significantly different from dosing via the intravenous route.

Tanaka et al. (1974) and Hirano et al. (1981) studied the mechanism of absorption of drugs from oily solutions after intramuscular injection; it was suggested that there were two different absorption routes to be considered. Firstly, drug molecules were taken up together with the direct absorption of small oil droplets, and secondly, drug was absorbed after being transferred from the oily depot into the aqueous phase which surrounded the injection site. The latter route appeared to be the major one for absorption; therefore, the transport process by which the drug moves from the oil phase into the aqueous phase, might be the rate-limiting step. However, in emulsion delivery systems the presence of two interfaces (i.e. the disperse/continuous phase, as well as the continuous phase/aqueous body fluid phase) has to be considered in estimating the absorption rate of the drug after intramuscular injection. The drug concentration in aqueous body fluids depends on the partition of drug molecules through both interfaces, and here the slower transport process would be considered as the rate limiting step.

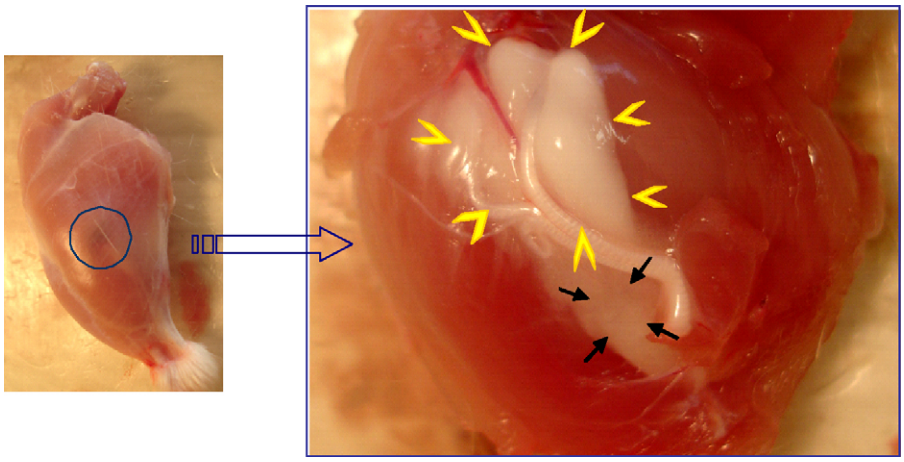

Fig. 6. The lower hind limb of a rat autopsied at 48 h after a single injection of ^{3}H-dexamethasone-co/so emulsions. The superficial muscle has been dissected to reveal the deposit of the emulsions (yellow arrowheads). There is an area of fat below the injected emulsion (black arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

The extent to which emulsions retain their integrity following intramuscular injection is not known. When water is the continuous phase, this must be lost rapidly leaving the castor oil droplets to coalesce or disperse throughout the muscle tissue. However, drug molecules were released slowly from the co/so emulsion droplets due to their need to partition through the castor oil/silicone oil interface, as well as the silicone oil/aqueous body fluid phase. A similar finding was also suggested from the gross pathological findings at the injection site at autopsy. At all time points up to 48 h after intramuscular injection, at autopsy the ^{3}H-dexamethasone-co/so emulsion deposits were still clearly visible between the muscle bundles of the hind leg (Fig. 6).

For both formulations, only relatively minimal amounts of ^{3}H-dexamethasone were identified away from the muscle injection site in other tissues apart from in the liver and kidneys (the metabolism and excretion of dexamethasone mainly take place in the liver and kidney). Farshi et al. (1996) noted that high levels of dexamethasone sodium phosphate were detectable in the kidney, spleen and liver after the intramucosal injection of the dose in solution. It has also been reported that there is a high uptake of dexamethasone by the liver, kidney and adrenal glands; furthermore, dexamethasone metabolism in the liver is slow and excretion is mainly via the urine (Dumasia et al., 1986; Dollery, 1998; Agnew et al., 2003).

Administration of ^{3}H-dexamethasone as a non-aqueous emulsion could be a method of reducing the systemic drug concentration in the tissues; this may be an important factor if the adverse effects of the drug are being considered. Dexamethasone sodium phosphate encapsulated in liposomes has a similar sustaining effect at the site of administration (Farshi et al., 1996).

In the present study it has been demonstrated that the administration of ^{3}H-dexamethasone in a co/so non-aqueous emulsion showed slower absorption following intramuscular injection compared to an o/w formulation but did not bring about very prolonged plasma levels. One advantage, however, may be that the co/so emulsions can be used as anhydrous vehicles where the presence of water is undesirable. Further work on the optimization of formulations is necessary.

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