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The in vivo evaluation of emulsion formulations administered intramuscularly

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Summary

A multiple emulsion system (w/o/w) formulated from arachis oil, thickened with cetostearyl alcohol and aluminium stearate has been administered intramuscularly to rabbits in order to assess its potential as a sustained release vehicle. Iodine-131-labelled iodohippuric acid was contained in the inner aqueous phase and its clearance was followed by gamma scintigraphy. Water in oil (w/o) and simple aqueous solution were used as controls. Both w/o/w and w/o systems demonstrated sustained release capabilities.

Introduction

The intramuscular (i.m.) administration of a drug is a well recognised method of providing a depot for sustained, controlled or delayed release into the systemic circulation. The kind of a depot provided will be markedly dependent upon the nature of the system administered. Aqueous solutions are generally cleared relatively quickly from an i.m. injection site. Consequently oil and polymeric solutions, drug-polymer conjugates and various colloidal systems (e.g. nanoparticles, liposomes, microspheres and emulsions) have all been employed in attempts to produce sustained drug release. Multiple emulsions of the water-in-oil-in-water type ($w_1/o/w_2$ MES) have found success as sustained release delivery vehicles for a variety of drugs and biologically active solutes (including phenothiazines, antigenic materials and antineoplastic agents in various animal species (Florence and Whitehill, 1982; Davis and Walker, in press). However, such systems are generally difficult to formulate as products with acceptable stability (e.g. shelf life) and drug loading characteristics.

Previously we have described studies on the physical properties of $w_1/o/w_2$ MES formulated using vegetable oils. The stability of such systems has been enhanced by the addition of cetostearyl-alcohol (CTA) and aluminium stearate (Davis and Walker, 1983). The present studies on i.m. administration, conducted in the rabbit were designed to evaluate the in vivo release characteristics of this $w_1/o/w_2$ MES formulation using iodohippuric acid (o-IHA) as a model drug. [^{131}I]o-IHA has found wide acceptance as a diagnostic agent for the in vivo evaluation of renal function. In normal individuals it is rapidly and completely cleared

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from the systemic circulation by the kidneys (Tubis et al., 1960). Its disposition in an experimental species can be followed through either non-invasive gamma scintigraphy or periodic serum sampling and analysis. Since [^{131}I]o-IHA is cleared rapidly from an intramuscular injection site it has also found use as a diagnostic agent in assessing muscular blood flow (Chezem, 1973). Thus it can be considered to be an ideal agent for the evaluation of potential sustained release drug delivery systems administered by the i.m. route.

Materials and Methods

Production of [^{131}I]o-iodohippuric acid delivery systems

Preparation of stock solution of [^{131}I]o-iodohippuric acid (I-o-IHA): 1.6 ml of a solution of [^{131}I]o-IHA of nominal activity 493 MBq/ml was used to label 1.3 ml of a standard o-IHA solution (15 mg/ml in pH 7.4 phosphate-buffered saline) to give a stock solution of approximate activity 259 MBq/ml. This solution was then used in the preparation of the one aqueous and two emulsified [^{131}I]o-IHA delivery systems.

Aqueous control solution: 1.0 ml of [^{131}I]o-IHA stock solution was diluted to 10.0 ml with a standard solution of 15 mg/ml o-IHA in pH 7.4 phosphate buffered saline. A 200 μl dose had an approximate activity of 5.18 MBq.

w_1/o control emulsion: 0.2 ml of [^{131}I]o-IHA stock solution and 1.8 ml of oil phase (10% w/w Span 80, 2% w/w Tween 80, 1% w/w cetostearyl alcohol, 3% w/w aluminium stearate, 84% w/w arachis oil) were emulsified in a 30 ml stoppered glass vial for five minutes using a VM20 vortex mixer. A 200 μl dose had an approximate activity 5.18 MBq.

$w_1/o/w_2$ MES test emulsion: a previously described two-stage emulsification procedure was adopted (Davis and Walker, 1983). Specific formulation details are presented in Table 1. The control and test emulsion systems were prepared immediately prior to administration.

Experimental injection procedure

Female New Zealand White rabbits of average weight 2.5 kg were obtained from the University of Nottingham Breeding Unit. 200 μl of [^{131}I]o-IHA delivery system (aqueous solution, w/o and $w_1/o/w_2$ emulsion systems) were injected over a period of thirty seconds into the vastus lateralis muscle on the left thigh using a 25 mm 19-gauge needle. The injection depth was 10 mm, controlled using a needle stop. The relatively wide needle bore was chosen to allow the control w/o emulsion system to be injected without undue shearing and to provide minimal disturbance to the multiple droplets of the test system (previously determined geometric mean multiple droplet diameter = 16.5 μm). The rabbits (3 per group) were

TABLE 1

Formulation of labelled $w_1/o/w_2$ multiple emulsion systems

Internal w_1 aqueous phase	Oil phase composition (% w/w)	External w_1 aqueous phase (% w/w)	Approximate activity of 200 μl dose (MBq)	Other formulation parameters
[^{131}I]o-IHA solution	10% Span 80 2% Tween 80 1% Cetostearyl alcohol 3% Aluminium stearate 84% Arachis oil	2% Tween 80 in pH 7.4 phosphate-buffered saline	2.59	Phase volume w_1/o = 0.1 Phase volume $w_1/o/w_2$ = 0.5 Emulsification time for primary emulsion = 5.0 min Emulsification time for secondary emulsion = 0.25 min Volume of system produced = 10 ml

then examined by whole body gamma scintigraphic imaging, as described elsewhere. Acquisition was undertaken at a single detection channel tuned into the gamma emission energy of ^{131}I, 364 keV.

Immediately after i.m. administration of each delivery system, a 30-min dynamic lateral view of the injection site (consisting of 60 consecutive acquisition frames each of 20 s) was made.

The amount of gamma activity present in the i.m. injection site at a given time was obtained by constructing a region of interest (ROI) on a computer-generated display.

Seven days after the administration the rabbits were sacrificed and each injection site was excised and visually inspected prior to histological examination.

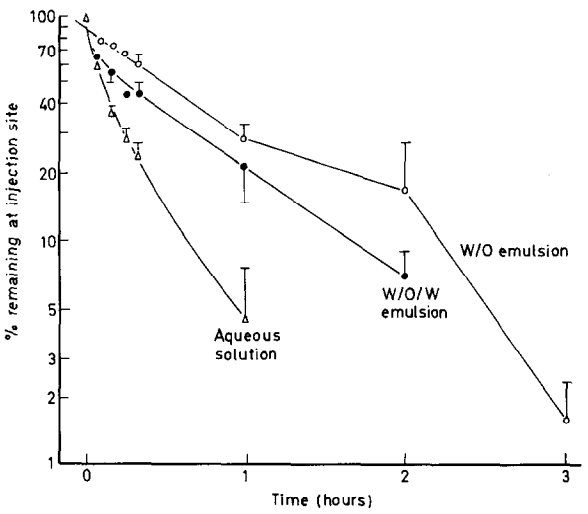

Fig. 1. Release of [^{131}I]o-iodohippuric acid from an intramuscular injection site in rabbits ($n = 3$) (mean ± S.D.). △, aqueous solution; ○, w/o emulsion; ●, w/o/w emulsion.

Results and Discussion

The clearance of [^{131}I]o-IHA from the intramuscular injection site has been expressed in terms of the percentage of ^{131}I activity at $t = 0$ remaining at the injection site at specific time intervals during the experimental procedure (Fig. 1). The results of the triplicate evaluation of each [^{131}I]o-IHA delivery system were highly reproducible.

The intramuscular clearance of drugs formulated in aqueous solutions, oily solutions, w_1/o and $w_1/o/w_2$ emulsions, has been shown previously to follow a monophasic first-order kinetic process (Muranishi et al., 1979). Therefore, the mean data for the clearance of [^{131}I]o-IHA have been subjected to first-order kinetic analysis. However, the clearance of [^{131}I]o-IHA administered in all 3 delivery systems was found to follow a biphasic first-order profile with a short initial clearance phase followed by a longer, slower secondary phase. Characteristic first-order clearance rate constants were derived by regressional analysis of lines of best fit (Table 2). Hashida et al. (1977) also reported deviations from the monoexponential process and noted a biphasic first-order clearance of anticancer agents from an i.m. injection site in the rat following administration of a w_1/o emulsion formulation. However, as in the present case, the initial clearance phase was extremely short compared to the slower secondary phase. Derived times for half-clearance for the longer second phase are given in Table 2. The K_{cl}

TABLE 2

The first order clearance of [^{131}I]o-iodohippuric acid from an i.m. injection site in rabbit

	Aqueous control	w_1/o	$w_1/o/w_2$
Initial clearance phase system			
Time period (min)	0–3.3	0–4.7	0–3.0
First-order rate constant k_{cl} ($min^{-1} \times 10^{-2}$)	8.7	5.3	10.6
Correlation coefficient	0.95	0.98	0.98
Number of data points	10	15	9
$\frac{K_{cl(system)}}{K_{cl(aqueous)}}$	1.00	0.61	1.23
Secondary clearance phase			
Half time for clearance ($t_{1/2}$) (min)	5.8	34.5	13.8
$\frac{t_{1/2(system)}}{t_{1/2(control)}}$	1.00	5.98	2.43

values for the w_1/o and $w_1/o/w_2$ emulsion systems are approximately 6 and 2.5 times greater respectively, than that for the aqueous control system. That is both emulsion systems were effective in retarding the i.m. clearance of [^{131}I]o-IHA, the w_1/o system being more effective than the $w_1/o/w_2$ MES.

Potential modes of i.m. clearance of [^{131}I]o-iodohippuric acid following administration in emulsion systems

The slower clearance of [^{131}I]o-IHA from the injection site when administered in the emulsion systems can be attributed to the retention of solute physically entrapped within the administered dosage form at the injection site.

The clearance of [^{131}I]o-IHA from an i.m. injection site, when formulated in either a w_1/o or $w_1/o/w_2$ emulsion system can be considered to occur in two distinct but potentially concurrent modes, namely; release of [^{131}I]o-IHA from the dosage form followed by transport into the systemic circulation, and transport of [^{131}I]o-IHA-containing emulsion away from the injection site, probably into the lymphatic system.

The more rapid clearance of [^{131}I]o-IHA formulated in the $w_1/o/w_2$ MES as compared to w/o can be ascribed to the presence of a proportion of the solute in the external w_2 aqueous phase, which will be cleared as for the aqueous solution.

The possibility that the w/o/w emulsion underwent *rapid* breakdown at the injection site and thereby released the entrapped material has been considered. This is thought to be unlikely since in vitro studies (Davis and Walker, 1983) have indicated that the system should have acceptable in vivo stability characteristics, certainly for the time period over which the experiments were conducted.

The greater mobility of multiple droplets within the musculature compared to the relatively static depot formed by a w_1/o emulsion will also lead to a faster clearance.

Any [^{131}I]o-IHA entrapped within the aqueous phase of the emulsions at pH 7.4 will be almost totally ionised and thus oil phase impermeable. This would indicate that the release of [^{131}I]o-IHA from these systems is caused by the slow breakdown of the emulsion and that in vivo stability of the emulsions rather than the oil phase permeability determines i.m. clearance profiles after i.m. injection.

Davis and Walker (in press) have reviewed the contributing factors which may affect the release of solutes from a $w_1/o/w_2$ MES and noted that little regard has been paid to the in vivo stability. They considered that the following factors could have various degrees of influence: muscle movement, the presence of endogenous solutes (electrolytes) altering the nature of emulsifier layers, osmolarity of the w_1 aqueous phase, phagocytosis of multiple droplets and metabolism of the oil. For the present work none of the above is considered to be particularly relevant considering the rapid release of the entrapped marker. Calculations show that the internal aqueous phase w_1 is practically isotonic.

Physiological compatibility of the emulsion systems

Macroscopic and histological examination of the excised intramuscular injection sites both indicated that no tissue damage was evident 7 days after the administration of the 3 different systems. In agreement with this finding, the literature indicates that, following the intramuscular administration of aqueous and oily dosage forms, minimal histological changes are seen if no macroscopic changes are present (Rasmussen and Svendsen, 1976). Mineral oils administered i.m. are known to cause intolerable inflammatory and necrotic lesions at the injection site whereas vegetable oils are relatively well tolerated (Brown et al., 1944). Arachis oil/aluminium stearate gels have been developed as physiologically acceptable oily delivery vehicles for the sustained release of i.m. administered antibiotics and antigenic materials. Procaine penicillin G injection in the current United States Pharmacopoeia is formulated using this system, which has also been used for the i.m. administration of vitamin B_{12} and narcotic antagonists (Thompson and Hecht, 1959). More recently, $w_1/o/w_2$ MES adjuvants based upon such gelled oil phases have been shown to be both immunologically effective and physiologically acceptable (Kimura et al., 1978). Thus it is not

surprising that the emulsion systems in the present study based upon modified arachis oil were well tolerated.

Conclusions

The clearance of [^{131}I]o-IHA from an intramuscular injection site in the rabbit has been followed by a non-invasive scintigraphic technique. Formulation of the model drug compound into the internal aqueous phase of both w_1/o and $w_1/o/w_2$ emulsion systems retarded clearance relative to an aqueous control solution; the w_1/o system providing the greater degree of retardation. The first-order [^{131}I]o-IHA clearance profiles of all 3 delivery systems were biphasic but with a very short first phase, and thus could be characterised by the respective first-order clearance rate constants.

Post-mortem macroscopic histological examination of the excised injection sites indicated that all 3 delivery systems were well tolerated. It can be concluded that both the w_1/o and $w_1/o/w_2$ emulsion systems possess potential as sustained release delivery systems for the i.m. administration of water soluble drugs.

Future studies will investigate the potential of such systems to promote the lymphatic accumulation of intramuscularly administered drug, with specific reference to the administration of antineoplastic agents.

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