J. Pharm. Pharmacol. 1994, 46: 508–510
Received June 14, 1993
Accepted November 29, 1993

Pertechnetate release from a water/oil microemulsion and an aqueous solution after subcutaneous injection in rabbits

M. BELLÒ, D. COLANGELO*, M. R. GASCO†, F. MARANETTO, S. MOREL†, V. PODIO, G. L. TURCO, I. VIANO‡, *Cattedra di Medicina Nucleare, Facoltà di Medicina, Torino, *Istituto di Farmacologia e Terapia Sperimentale, Facoltà di Medicina, Torino, †Dipartimento di Scienza e Tecnologia del Farmaco, Facoltà di Farmacia, Torino, ‡Dipartimento di Scienze Mediche, Facoltà di Medicina, Università di Torino sede di Novara, Italy*

Abstract—A water-oil microemulsion and an aqueous solution, both carrying pertechnetate, were injected subcutaneously in rabbits; release was observed by imaging the administration sites with a gamma-camera. Disappearance from the injection site of pertechnetate in aqueous solution was about ten times faster than that of pertechnetate in a microemulsion.

Many systems have been studied with the aim of obtaining prolonged release of peptides and small proteins by parenteral administration. Besides aqueous viscous solutions, other systems have been proposed, such as liposomes (Weingarten et al 1985), biodegradable microspheres (Sanders et al 1985), polymer conjugates (Nandini 1993) and polymeric controlled-release systems (Boer & Krruisbrink 1987).

In previous studies, LHRH-trp6 was incorporated in a water-oil microemulsion and administered in a single injection in rats; a reduction in plasma testosterone levels was maintained over three weeks (Gasco et al 1990). Insulin was also incorporated in a water-oil microemulsion and injected in rabbits; the results showed a significant increase of t_{max} and $t_{\frac{1}{2}}$, in comparison with the corresponding data using an insulin aqueous solution (Gasco et al 1992).

To investigate the behaviour of a microemulsion at the injection site, the release of pertechnetate from a water-oil microemulsion and an aqueous solution, after subcutaneous administration in rabbits, was observed in this study, by imaging the injection sites with a gamma-camera.

Materials and methods

Materials. Ethyl oleate and egg lecithin were from Merck (Darmstadt); hexanoic acid was from Fluka (Buchs, Switzerland); capric-caprylic triglyceride (CCT Myritol 318) was from Henkel (Dusseldorf, Germany); pertechnetate was from a standard $^{99}Mo/^{99m}Tc$ generator, Sorin Biomedica (Saluggia, Italy). Egg lecithin was purified as described by Hanahan et al (1951).

Composition of the microemulsion for injection. The components of the microemulsion were ethyl oleate and Myritol 318 (1:1) as oils (59%), purified egg lecithin as surfactant (21%), hexanoic acid as cosurfactant (11%) and 0·225% NaCl solution of pertechnetate as aqueous phase (9%). Pertechnetate aqueous solution was obtained from a standard $^{99}Mo/^{99m}Tc$ generator, using NaCl 0·225% aqueous solution as mobile phase, obtaining a concentration of about 6 GBq mL^{-1}. The choice of components and of their amounts was dictated by the need to obtain a microemulsion in the presence of a high-salt aqueous phase; high salinity is necessary to achieve elution of pertechnetate from the standard generator.

Preparation of the microemulsion. Lecithin and hexanoic acid were dissolved in the oil mixture; the aqueous phase was added just before use. The final radioactive concentrations of the various batches of the microemulsion prepared for the various experiments were in the region of 500 MBq mL^{-1}.

Correspondence: M. R. Gasco, Dipartimento di Scienza e Tecnologia del Farmaco, via P. Giuria 9, 10125 Torino, Italy.

Characterization of the microemulsion. The dynamic quasi-elastic light scattering technique, QELS, (PC100 Malvern, Malvern, UK), was used to determine the diffusion coefficient of the microemulsion droplets. The samples were observed at 37°C, in red light at a wavelength of 632·8 nm and at an angle of 90°. Conductivity was measured at 37°C (Orion Research, Boston, MA, USA). Viscosity was measured at 37°C (Ubbelhode Schott-Garäte, Hofheim, Germany).

Injection. Animals used were seven young adult New Zealand White rabbits (Conelli, Novara, Italy). At the start of the study, the rabbits weighed between 4·5 and 5·2 kg; they were acclimatized to the laboratory environment for at least 15 days, examined for signs of ill health shortly before the study started, and allowed free access to tap water and food throughout.

Potassium iodide was injected subcutaneously into the hind paw of each rabbit (7 mg day^{-1} for 15 days before the experiment) to avoid artifacts due to thyroid uptake.

One hour before the beginning of the study, 5 mg diazepam was administered intramuscularly, to tranquillize the animal during imaging.

The radioactivity of the microemulsion was comparable with that of the aqueous solution at injection: 0·7–0·8 mL microemulsion or aqueous solution was injected subcutaneously into the back of each rabbit, after it had been held firmly but gently until calm. Each animal received two injections, one of microemulsion and one of aqueous solution, at an interval of at least one week. The order of the two injections was randomized.

Imaging. Imaging was with an analogue gamma-camera equipped with a low-energy, high-resolution, parallel-hole collimator (S.E.LO. Unicamera, Sesto, Italy). Static images were stored in a commercial nuclear medicine computer in a 128 × 128 pixel matrix (General Electric Star II, Milwaukee, WI); each image was acquired over 10 or 40 s, according to its count rate. Each rabbit was imaged together with a reference source containing 40 MBq of pertechnetate in 15 mL water.

Images were obtained at about 2, 10, 20, 40, 60, 80, 100 and 120 min after injection in all rabbits, and at 3, 4, 5, 6, 7, 9, 11, 13 and 15 h after injection in the rabbits injected with microemulsion.

For each rabbit, the background-corrected count rate obtained from the injection site was divided by the count rate originating from the reference source, in order to compute a radioactivity ratio. In this way, the radioactivity ratio obtained was independent of both the physical decay and the dead-time count-losses of the gamma-camera.

Mathematical and statistical methods. Curves were analysed and fitted with the most appropriate monoexponential or biexponential curve, and the correlation coefficient was computed. Statistical analysis was performed using Student's *t*-test for paired groups.

All radioactivity data were plotted against the time of imaging and for each time-activity curve the best exponential fit was obtained: the slope of the exponential curve was computed.

Results and discussion

Microemulsions are clear systems with indefinite stability, they are reproducible and easily prepared. They can be sterilized by filtration, their droplets always being smaller than 100 nm; their usual components are oil, water, surfactant and cosurfactant. The internal phase of a water-oil microemulsion can behave as a reservoir of hydrophilic molecules dissolved in water.

The components of the microemulsion tested were physiologically compatible (Bremer & Osmundsen 1984; Cotter & Tucker 1991). The conductivity was very low, less than 1·0 μmho as expected for water-oil microemulsions. The microemulsion had a viscosity of 18·16 centistokes at 37°C. The QELS analysis at 37°C confirmed that the diameter of the droplets was 18 nm, the accepted value for microemulsions (Pursey & Tough 1985).

Fig. 1 shows the activity for pertechnetate injected as a microemulsion and as an aqueous solution. The kinetic release of the radionuclide from the injection site after administration of the microemulsion was much slower than from aqueous solution. Fig. 2 reports the sequences of images for the reference source of activity, a rabbit injected with microemulsion and a rabbit injected with aqueous solution. The drop-off of activity at the injection site is much more marked for the aqueous solution than for the microemulsion.

Microemulsion activities decayed monoexponentially ($r \geqslant 0{\cdot}98$) in four rabbits, while in three rabbits the decay was better described biexponentially ($r \geqslant 0{\cdot}99$), $t_{\frac{1}{2}} = 40$ and 296 min, respectively.

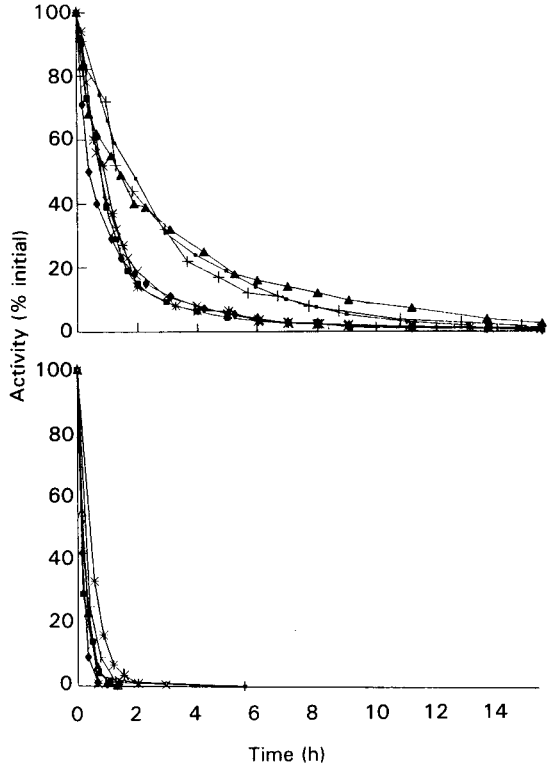

FIG. 1. Individual curves of activity in the injection site after microemulsion administration (upper) and aqueous solution administration (lower).

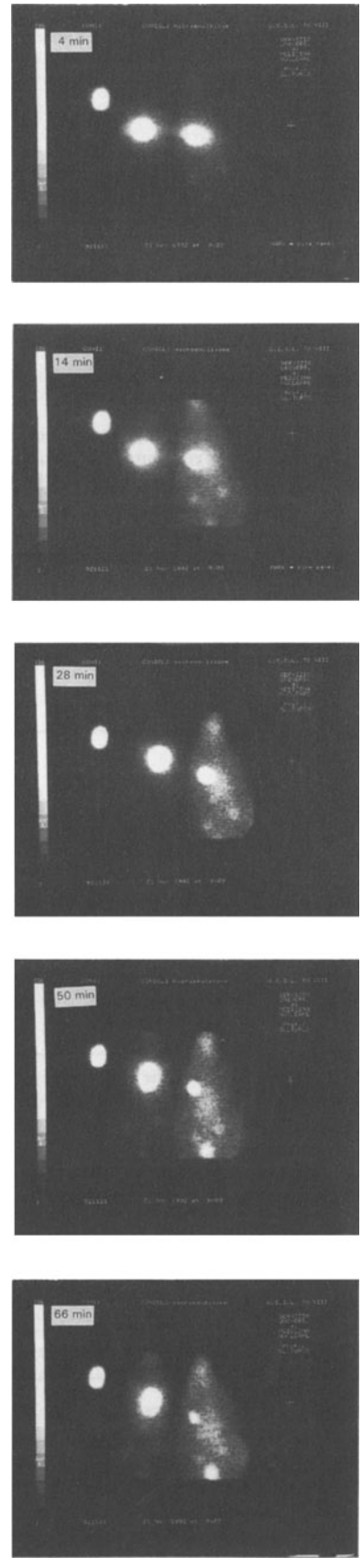

FIG. 2. Temporal sequences of images; time: 4, 14, 28, 50 and 66 min after injection; left to right: reference source of radioactivity, rabbit injected with microemulsion, rabbit injected with aqueous solution.

Table 1. Half-life values of technetium activity in the rabbits injected with technetium in microemulsion or aqueous solution.

Rabbit	Microemulsion	Aqueous solution
1	132	10·6
2	151	12·5
3	57*	17·9
4	46*	12·1
5	69*	9·8
6	122	8·2
7	189	9·2

Mean ± s.d. * These animals showed a biexponential decay: half-lives reported are those of exponential equations with the same intercept and the same AUC as the (biexponential) fitting obtained.

The biexponential curve was simplified to permit statistical analysis and the half-life was computed for a monoexponential curve with the same intercept and the same area under the curve (AUC) as the original biexponential curve.

Table 1 gives half-life data for each animal.

The experimental data show that pertechnetate carried by water-oil microemulsions is released from the site of administration at a slower rate than from pertechnetate administered as an aqueous solution.

References

Boer, G. J., Krruisbrink, J. (1987) A polymeric controlled drug delivery device for peptides based on a surface desorption/diffusion mechanism. Biomaterials 8: 265–274

Bremer, J., Osmundsen, H. (1984) Fatty acid oxidation and its regulation. In: Numa, S. (ed.) Fatty Acid Metabolism and Its Regulation. Elsevier Science Publishers B. V., Amsterdam, pp. 113–154

Cotter, R., Tucker, H. (1991) Parenteral preparations containing short chain fatty acid containing lipids to maintain gastrointestinal integrity and function in patients. Eur. Pat. Appl. EP 451,750

Gasco, M. R., Pattarino, F., Lattanzi, F. (1990) Long-acting delivery systems for peptides: reduced plasma testosterone levels in male rats after a single injection. Int. J. Pharm. 62: 119–123

Gasco, M. R., Morel, S., Tonso, E., Viano, I. (1992) In vivo and in vitro release of insulin from microemulsion. Proc. Int. Sym. Contr. Rel. Bioact. Mat. (19th), p. 502

Hanahan, D. J., Turner, N. B., Deyko, M. E. (1951) Isolation of egg phosphatidylcholine. J. Biol. Chem. 191: 623–629

Nandini, V. K. (1993) The conjugation of proteins with polyethylene glycol and other polymers. Adv. Drug Del. Rev. 10: 91–114

Pursey, P. N., Tough, R. J. A. (1985) Particle interactions. In: Pecora, R. (ed.) Dynamic Light Scattering. Plenum Press, New York, London, pp 154–158

Sanders, L. M., McRae, G. I., Vitale, K. M., Kell, B. A. (1985) Controlled delivery of an LHRH analogue from biodegradable injectable microspheres. J. Contr. Rel. 2: 187–195

Weingarten, C., Moufti, A., Delattre, J., Puisieux, F., Couvreur, P. (1985) Protection of insulin from enzymatic degradation by its association to liposomes. Int. J. Pharm. 26: 251–257

J. Pharm. Pharmacol. 1994, 46: 510–512
Received September 14, 1993
Accepted December 15, 1993

Diuretic effect of N^G-nitro-L-arginine methyl ester in the rat

Q. LI, C. J. BOWMER, M. S. YATES, *Department of Pharmacology, Worsley Medical and Dental Building, The University of Leeds, Leeds LS2 9JT, UK*

Abstract—Intravenous infusion of the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester, L-NAME (10 $\mu g\ kg^{-1}\ min^{-1}$), to anaesthetized rats produced a diuresis and natriuresis. By contrast, infusion of the same dose of N^G-nitro-D-arginine methyl ester had no effect on either urine output or sodium excretion. The effects of L-NAME were first evident 120 min after the start of infusion and by 170 min a fivefold increase in urine volume and sodium excretion was recorded. L-NAME also produced a transient fall in inulin clearance and a persistent decline in renal blood flow. These renal effects of L-NAME were associated with a gradual elevation of mean arterial blood pressure, although this only attained statistical significance, in comparison with saline-infused animals, 170 min after the start of infusion. The findings indicate the diuresis and natriuresis evoked by L-NAME in the rat is a result of a direct tubular action together with a pressure diuresis.

A study in the rat using the NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) revealed the renal cortical vasculature has a relatively high basal release of NO which substantially contributes to the control and maintenance of renal cortical blood flow (Walder et al 1991). Infusion of another NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) in rats has been shown to evoke falls in renal blood flow (RBF), glomerular filtration rate, urine flow and sodium excretion (Lahera et al 1993). An antidiuretic effect of L-NAME has also been demonstrated in the dog (Salom et al 1992). In a recent study, however, using rats treated with cisplatin, we noted that infusion of L-NAME potentiated the diuretic and natriuretic effects of glycine (Li et al 1994). This observation led us to investigate the effects of L-NAME infusion on renal haemodynamics and excretory function in the anaesthetized rat. Some experiments were also conducted in which renal function was monitored during infusion of N^G-nitro-D-arginine methyl ester (D-NAME), the enantiomer of L-NAME, which is inactive against NO synthase (Graves & Poston 1993). These experiments were performed to differentiate the effect on renal function of NO synthase inhibition produced by L-NAME from any effects which amino acid infusion itself might have on renal function (Cernadas et al 1992).

Correspondence: M. S. Yates, Department of Pharmacology, Worsley Medical and Dental Building, The University of Leeds, Leeds LS2 9JT, UK.

Materials and methods

Materials. L-and D-NAME, and inulin were purchased from Sigma Chemical Co., UK. [^{3}H(G)]Inulin (201 mCi g^{-1}) was obtained from DuPont NEN Research Products, UK, and its stated radiochemical purity was greater than 98%.