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PII: S0168-3659(07)00296-9
DOI: doi: 10.1016/j.jconrel.2007.06.013
Reference: COREL 4549

To appear in: *Journal of Controlled Release*

Received date: 6 March 2007
Revised date: 7 June 2007
Accepted date: 14 June 2007

Please cite this article as: Claire M Deadman, Ian W Kellaway, Mohammed Yasin, Paul A Dickinson, Sudaxshina Murdan, An investigation into the influence of drug lipophilicity on the in vivo absorption profiles from subcutaneous microspheres and *in situ*-forming depots, *Journal of Controlled Release* (2007), doi: 10.1016/j.jconrel.2007.06.013

An investigation into the influence of drug lipophilicity on the in vivo absorption profiles from subcutaneous microspheres and *in situ*-forming depots

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Keywords - microspheres, in situ forming implants, lipophilicity, drug absorption, in vivo, subcutaneous, PLGA

Abstract

Drug lipophilicity is known to have a major influence on in vivo drug absorption from intramuscularly and subcutaneously administered solutions. Indeed, chemical modification to increase drug lipophilicity is used to enable sustained drug release from solutions. In contrast to the wealth of knowledge on drug release from simple solutions, the influence of drug lipophilicity on its release from controlled release formulations, such as, microparticles and in situ forming depots, have not been systematically studied. Controlled release vehicles are designed to 'control' drug release, hence, in vitro studies show negligible influence of drug lipophilicity on release. The situation could however be different in vivo, due to interactions between the vehicle and biological tissue. We therefore investigated the influence of drug lipophilicity on its in vivo release in rats from two controlled release formulations, PLGA microparticles and in situ forming depots. Both systems exhibited a burst drug release. Subsequent to the burst release, we found that lipophilicity did not influence the rate or extent of drug absorption from the two formulations over a 10-day study period, which would imply that drug partitioning out of the depots was not the main mechanism of drug release from both formulations. This study must however be repeated with a greater number of animals to increase its power.

Introduction

Sustained release of drugs from depot formulations has a number of advantages, such as, reduction in dosing frequency, increased patient compliance, optimisation of the drug's pharmacokinetic profile, hence increased efficacy with reduced toxicity and cost. Sustained release drug formulations range from simple oil solutions and aqueous suspensions to more complex formulations such as polymeric microspheres and in situ forming implants. The rate of drug release from the more complex vehicles depends on a large number of parameters, and in this paper, the influence of drug lipophilicity from polymeric microspheres and in situ forming implants is reported.

Polymeric microspheres have been extensively investigated as controlled release vehicles for the past decades and a number of preparations have been commercially available for a number of years. The formulations are available as lyophilised microspheres which are reconstituted with a diluent prior to subcutaneous or intramuscular administration. Drug is released from the depot over a period of weeks to months, by drug diffusion out of the polymeric matrix and/or by erosion of the matrix. Thus parameters which influence these 2 processes control the drug release profiles [1]; these include polymer chemistry and erosion mechanism, polymer molecular weight, copolymer composition, crystallinity, polymer-drug interactions, excipients, microsphere size and porosity, drug distribution. [1-2].

More recently, an in situ forming drug depot - Eligard™ - was approved by the FDA. This consists of a solid component (the drug leuprolide acetate and the polymer PLGA) which is dissolved in an organic solvent (N-methyl-2-pyrrolidone, NMP)

immediately prior to injection. Upon administration, the water miscible NMP dissipates into the surrounding tissue, which leads to polymer precipitation into a depot (entrapping the drug) at the site of injection. The obvious advantage of such in situ forming implants over polymeric microspheres is their relative ease of preparation – a water-insoluble polymer (e.g. PLGA) and the drug are dissolved in a water-miscible solvent. The in situ forming implant is also easier to inject – we found injection of microsphere suspensions to be hampered by needle clogging by particulates. The obvious disadvantages are the fact that an organic solvent is administered (which can lead to toxicity) and the variable shape and size of the implant formed in vivo, which leads to variable rates of drug release [3]. Many parameters are expected to influence the drug release profile, and some of these have been investigated in vitro and/or in vivo. For example, the in vitro burst release was related to polymer molecular weight [4], solvent nature [4-6], presence of additives [7] and polymer concentration [4, 6, 8]. The duration of drug release in vivo was related to polymer molecular weight [9], polymer nature [10] and drug loading (3 vs 10% studied; [8]. Some of the parameters were found to have no significant effect on duration of implant efficacy when narrower ranges were investigated (3-6% leuprolide acetate drug loading and 40-50% polymer concentration studied [9]). To our knowledge, the influence of drug lipophilicity has not been studied and this is the first report on the effect of drug lipophilicity on release from in situ forming implants.

In addition, as mentioned above, the influence of drug lipophilicity on the in vivo release from microspheres is addressed in this paper. Despite the extensive literature on microspheres as drug carriers, the influence of drug lipophilicity on in vivo release profile has received scant attention. Thus, in this paper, the 2 depot systems

(microspheres and in situ forming implants) are compared with respect to release rates. Despite the fact that polymeric microspheres and in situ forming implants are two of the most common complex sustained release parenterals, there is currently very little literature where the two systems have been compared. Comparison of the 2 depot systems could only be performed to a certain extent, as the 2 depot systems did not have the same drug:polymer ratio (the animals received 15 mg drug and 75 mg PLGA (in situ forming implant) or 150 mg PLGA (microspheres)). The smaller amount of PLGA used in the in situ forming implant formulation was due to the fact that volume of NMP required to administer the higher amount of PLGA (0.3ml) was found to be irritating to experimental animals.

Rats were used as the experimental animals, and octanoate salts of the beta-blockers, metoprolol and alprenolol, which have similar molecular weights and pKas, but different lipophilicities, were used as model drugs. Log P of metoprolol and alprenolol octanoates were experimentally determined to be 0.6 and 1.25 respectively [11]. The octanoate salts were used as these had a significantly reduced in vitro burst release from PLGA microspheres compared to the tartrate and hydrochloride salts [11], which enabled investigations into sustained drug release. The octanoate salts (hydrophobic ion pairs) were prepared and loaded into microspheres and in situ forming implants. Experimental animals were subcutaneously injected with drug-loaded microspheres or in situ forming implants, then bled at time intervals to determine the plasma drug levels. Subsequently, Wagner-Nelson deconvolution calculations were performed to obtain and compare the absorption profiles from the 2 depot systems. Control animals received subcutaneous injections of aqueous solutions of the octanoate salts to confirm the effect of encapsulation within

microspheres and in situ forming implants. In separate experiments, the drugs were administered intravenously to rats to establish the pharmacokinetic parameters for use in deconvolution calculations.

Materials and Methods

Alprenolol hydrochloride, metoprolol tartrate, propranolol hydrochloride, sodium octanoate, Tween 80, *N*-methyl-2-pyrrolidone (NMP) and formaldehyde 37 - 40% (molecular biology grade) were obtained from Sigma-Aldrich (Poole, UK). The polymer PLGA 5050 DL 2.5A was purchased from Alkermes Inc. (Medisorb® Ohio, USA). Disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate and sodium chloride were all analytical grade and purchased from VWR International Ltd. (Poole, Dorset, UK). Acetonitrile (HPLC grade), methanol (HPLC grade) and formic acid were purchased from Fisher Scientific (Loughborough, UK). Hypnorm™ was supplied by Janssen Pharmaceutical (Oxford, UK). All chemicals and reagents were used as purchased.

Male Wistar rats weighing 162 - 265 g were purchased from Harlan (UK) and allowed to acclimatise for a minimum of 7 days prior to experimentation. Food and water were provided *ad libitum* before and during experimentation. All procedures had been approved by the School's Ethical Review Committee and were conducted in accordance with UK legal and Welfare standards.

Intravenous (IV) administration of drug solutions

Rats (lightly anaesthetised with intraperitoneally administered 0.2mL of Hypnorm™) were intravenously dosed with solutions of the beta-blockers (0.5mL, containing 1.15 or 1.28 mg alprenolol HCl and metoprolol tartrate respectively in phosphate buffered saline, pH 7.4, equivalent to 1 mg of free base) via the tail vein. The rats were then bled from the tail veins at time intervals over a 3 hour period and blood samples (each approx. 150-200 μL) were collected into anticoagulant (EDTA) - coated centrifuge tubes (Microvette CB300, Sarstedt, UK). To separate the plasma, the blood samples were centrifuged for 10 minutes at 3000 rpm (Eppendorf Centrifuge 5415D, Eppendorf AG, Hamburg, Germany), after which the plasma (supernatant) was collected and frozen (*ca* -20 °C) until assayed. The last blood samples were collected by cardiac puncture, after the animals had been euthanised.

Preparation of hydrophobic ion pairs of metoprolol and alprenolol

The hydrophobic ion pairs, metoprolol and alprenolol octanoates, were prepared by the dropwise addition of an aqueous solution of sodium octanoate into an aqueous solution of each beta blocker (alprenolol hydrochloride or metoprolol tartrate; 5ml of 20mg/mL), such that the sodium octanoate:beta blocker molar ratio was 1:1.5. In the case of alprenolol, a white precipitate – alprenolol octanoate, was formed, which was collected by centrifugation with subsequent freeze-drying of the pellet (Drywinner 110, Heto-Holten A/S, Gydevang, Denmark). Metoprolol was collected by lyophilisation. The formation of the hydrophobic ion pairs was confirmed by analysis of the melting points of the original salts and of the hydrophobic ion pairs [11]. The aqueous solubility of alprenolol and metoprolol HIPs in PBS was found to be 5.4 mg/mL and greater than 5 mg/mL respectively.

Subcutaneous administration of aqueous solutions of the hydrophobic ion pairs

Alprenolol octanoate HIP (10.8 mg) and metoprolol octanoate HIP (10.24 mg) were each dissolved in 10 mL PBS to give solutions equivalent to 1 mg (free base)/1.5 mL. The experimental rats were then subcutaneously dosed with 1.5 mL of an HIP solution into the dorsolateral area of the neck. Subsequently, the animals were bled over a 6-hour period (at 5, 15, 30, 60, 90, 120, 240 and 360 minutes post administration) and the plasma was collected, as described above.

Preparation and subcutaneous administration of HIP-loaded PLGA microspheres

HIP-loaded PLGA microspheres were prepared by spray drying using a Büchi B-191 Mini Spray Dryer (Büchi Labratoriums, Flawil, Switzerland) fitted with a 0.5 mm nozzle and a high performance cyclone. The feed solution was prepared by dissolving 150 mg freeze dried HIP (alprenolol or metoprolol octanoate) and 1.5 g PLGA in 50 mL dichloromethane (DCM), giving a nominal loading of 9.1% w/w HIP. This solution was spray dried at an inlet temperature of 45 °C, an outlet temperature of 36 - 40 °C, a solution feed rate of 4%, aspiration ratio of 65% and compressed air flow of 800 L/h. The spray dried microspheres were stored in a glass vial, under desiccation and protected from light until used. Scanning electron microscopy revealed the HIP loaded microspheres to be smooth and spherical. Laser diffraction particle size analysis revealed volume median diameters of 4.8 ± 0.2 μm and 6.1 ± 0.1 μm for alprenolol and metoprolol HIP loaded microspheres respectively (n = 3).

Immediately prior to in vivo experiments, HIP-loaded microspheres (180mg) were reconstituted with 1.8 mL of PBS pH 7.4 with 0.1% w/v Tween 80 in a glass vial which was shaken and briefly bath-sonicated (Grant Ultrasonic Bath XB6, Grant Instruments, Cambridge, England). 1.5 mL of this suspension was then subcutaneously administered into the dorsolateral region of the neck. On occasions, when the needle became blocked partway through dosing, the needle was withdrawn and replaced with a new needle, and injection recommenced. In such cases, the dose was adjusted in subsequent calculations to account for the slight loss of volume. The animals were bled over a 10-day period (at 0.5, 1, 2, 4, 8 and 24 h, then daily for 7 days and a final sample on day 10) and the plasma was collected as described above.

Preparation and subcutaneous administration of *in situ*-forming implants

In situ forming implants were prepared immediately prior to in vivo administration. The plunger from a 1 mL disposable syringe (Syringe A) was removed, 30 mg of a lyophilised HIP (alprenolol octanoate or metoprolol octanoate) was loaded into the barrel, after which the plunger was carefully replaced. Into a second syringe (Syringe B), 0.3 mL of a 50% w/v solution of PLGA in *N*-methyl-2-pyrrolidone was drawn up. The two Syringes A and B were coupled together via a male-male Leuer Lock® connector (kindly donated by Atrix Laboratories) and the contents of the syringes were thoroughly mixed by alternately depressing the plungers for 50 cycles, or until a homogenous solution was formed. The syringes were then uncoupled and excess solution was discarded, leaving 0.15 mL of the polymer/drug solution in NMP in Syringe A. The formulation (0.15 mL, containing 15 mg HIP) was then injected subcutaneously into the dorsolateral region of the neck of rats. The animals were bled at the same time intervals as those receiving microspheres and plasma was separated

as described above. It must be noted that the drug loading of the *in situ*-forming implant was double that of the microspheres (i.e. 20% w/w drug/polymer *cf* 10% w/w); a higher drug loading was used to minimise the volume of NMP administered to rats. Despite having a subcutaneous LD_{50} > 2 g/Kg in rats, an injection volume of 0.3 mL was previously found to cause significant discomfort to the animals. Drug loading was therefore doubled to enable an equivalent drug dose to be given (15 mg HIP) in half the volume of the polymer solution.

Analysis of drug levels in plasma

Alprenolol and metoprolol concentration in rat plasma was measured, following their extraction, by HPLC with electrospray ionisation tandem mass spectrometry (LC-MS/MS) using propranolol as the internal standard. The frozen plasma samples were thawed by standing at room temperature for 1.5 h, after which the analyte and the internal standard were extracted from the plasma using a protein precipitation procedure, as follows. Fifty µL of each plasma sample was transferred to borosilicate glass tubes (Fisher Scientific, UK). Fifty µL of acetonitrile:water (1:1 v/v) was added, followed by the internal standard (425 µL of a 50 ng/mL propranolol solution), followed by 300 µL of acetonitrile as the crash solvent. The mixtures were vortexed for 2 minutes, after which they were centrifuged at 4500 rpm for 10 minutes at 4 °C (Mistral 3000I, MSE, Leicester, UK). The supernatants were transferred into 100 µL glass vial inserts, which were placed in 2 mL amberglass auto-injector vials. The latter were capped. Calibration standards were prepared by adding 50 µL of an analyte solution of appropriate concentration to 50 µL of naïve rat plasma, after which the analyte and the internal standard were extracted as described above.

An HPLC system (Agilent Series 1100, Agilent Technologies, Germany), equipped with a HTC PAL autoinjector (Leap Technologies, France), was employed. A 3 μm C_{18} ACE column (50 x 4.6 mm i.d., HiChrom Ltd, UK) was used in conjunction with a Phenomenex MAX RP guard column (4 x 3 mm) at a temperature of 35 °C. The mobile phase comprised of water (solvent A), acetonitrile (solvent B) and a 2.5% v/v aqueous formic acid solution (solvent C). The composition of the mobile phase (with respect to percentage of solvents A, B and C) was controlled using a quaternary pump over the course of each run. Solvent C was maintained at 2% throughout. At 0 - 0.5 min, isocratic conditions were run at 10% B. Solvent B was increased from 10% to 90% in the time range from 0.5 - 2.5 min, and from 90% to 95% from 2.5 - 3 min (linear gradient). Re-equilibration was performed from 3.5 to 4.5 min at 10% B. A sample volume of 10 μL and a mobile phase flow rate of 1.0 mL/min with a total run time of 4.5 minutes were used.

Data were collected using a PE SCIEX API 4000 triple quadrupole mass spectrometer (ABI-SCIEX, Toronto, Canada) operated in Multiple Reaction monitoring (MRM) mode. Atmospheric pressure chemical ionisation was performed, using a Turbo Spray ion source, in the positive ion mode. The vaporiser temperature was set to 700 °C, and the ionisation voltage 3500 V. Nitrogen was used for curtain gas (setting 10 psi), nebuliser gas (50 psi) and heater gas (55 psi). The CAD gas setting was 5 psi and the declustering potential was 81 V. The ions monitored were: alprenolol m/z 250.3→ 116.2, metoprolol m/z 268.3 → 116.3 and propranolol m/z 260.38 → 116.3. Fragmentation of these ions using collision activated dissociation (CAD) and a collision energy of *ca* 27 eV in the Q2 region of the mass spectrometer resulted in

strong product ions for the analytes and the internal standard. The predominant ion observed from the fragmentation was at m/z 116 for all three compounds.

To validate the assay, the linearity of the standard curve was determined by plotting the area ratios of analyte to internal standard against the actual concentration using 1/x weighted least squares regression over a calibration range 0.2 - 250 ng/mL. Six quality control (QC) standards (2 each of low, medium and high QC concentrations) were analysed before, during and after each batch of test samples. The acceptability of each batch of test samples depended upon the data from the calibration standards and the QC samples, fulfilling the following requirements: a) a minimum of four out of six QC's being within ± 15% of their respective calculated concentrations and no more than one QC be of greater than ± 15% at any one concentration and b) at least 5 calibration samples being within ± 15% of their respective target concentrations or ± 20% at the lower limit of quantification. In order to minimise the possibility of carry over effects, mobile phase injections were run between samples when it was necessary to follow a high concentration sample with a low concentration sample. The liquid chromatography-tandem mass spectrometry method developed gave good levels of specificity and sensitivity for the quantitative determination of analytes and internal standard in 50 μL of rat plasma. Retention times for alprenolol and metoprolol were *ca* 2.7 and 2.5 minutes respectively and no interference from the rat plasma was observed. The assay showed excellent linearity and the calibration curves had regression coefficients greater than 0.999. The lower limit of quantification (LLOQ) was 0.2 ng/mL, while the lower limit of detection (LLOD) was in the region of 0.05 ng/mL.

Pharmacokinetic analysis

Firstly, all plasma drug concentration values were normalised to a rat weight of 250 g to correct for differences in rat body weight. When the drug plasma concentration was below the lower limit of quantification, it was considered to be 0.05 ng/mL (LLOD) when the plasma volume of 50 μL was available. When a 50μL plasma volume was not available, the data point was removed. The plasma concentration data obtained following intravenous drug administration were fitted to an unweighted non-compartmental model using WinNonlin® and the area under the plasma drug concentration-time curve (AUC) from time t = 0 to infinity ($AUC_{(0-\infty)}$), the apparent volume of distribution at steady state (V_{ss}), the elimination half life ($t_{1/2}$) and the rate of clearance of drug from the plasma (CL) were obtained.

Plasma drug concentration data from the subcutaneous administration of aqueous solutions, microspheres and in situ forming implant were fitted to an unweighted non-compartmental model for a single extravascular input using WinNonlin®. Partial *AUC*s were generated from t = 0 to each time point using the linear trapezoidal method. The geometric mean of each *AUC* value was used, along with the mean pharmacokinetic parameters derived from the intravenous data, in Wagner-Nelson deconvolution calculations to obtain the absorption profiles of metoprolol and alprenolol from the different formulations. Deconvolution allows the plasma concentration time curve to be separated mathematically into its absorption component and its distribution/elimination component. The calculated fraction absorbed was then plotted against time to give the absorption profile of each

compound from the subcutaneous site. Absorption rate constants at different time intervals were then calculated from the slopes of the curves.

Statistical analyses

Statistical operations were carried out using SPSS 14.0 for Windows (SPSS Inc.). Repeated measures ANOVA was used to determine the influence of lipophilicity and of formulation type on the fraction absorbed with time profiles. Students t test was used to compare the drug absorption rates.

Results and Discussion

HIP absorption from aqueous solutions

The mean plasma concentration-time profiles of the two hydrophobic ion pairs, following their subcutaneous administration in aqueous solutions, are shown in Figure 1. The profiles are as expected for extravascularly administered compounds - a rapid absorption phase, followed by the elimination phase characterised by a gradual decline in plasma drug concentration. A plot of fraction of drug absorbed versus time showed a greater extent of drug absorption for the more lipophilic alprenolol (Figure 2). Repeated measures ANOVA, conducted to explore the impact of drug nature (lipophilicity) on fraction of drug absorbed over time, confirmed that the two profiles were significantly different ($F(1,6) = 26.1$, $p = 0.002$), with a large effect size (eta squared = 0.8). To compare the 2 drugs' absorption rates, approximate absorption rates between time intervals 0 - 5, 5 - 90 and 90 - 360 minutes were calculated as the gradient of the straight lines drawn between these points. An independent-samples *t*-Test showed that alprenolol absorption rates were significantly higher ($p < 0.005$)

than those of metoprolol (0.03 ± 0.005 vs 0.01± 0.004 at 0-5 min and 0.005 ± 0.0010 vs 0.002 ± 0.0008 at 5-90 minutes) for the first 90 minutes, after which the absorption rates for both compounds were approximately equal to zero, indicating that no further drug absorption occurred after this time. As expected, the more lipophilic alprenolol (log P 1.25) was absorbed to a much greater extent and at a faster rate than metoprolol (log P 0.6), due to its greater partitioning from the aqueous vehicle, into and across the lipophilic epithelial wall of blood vessels.

HIP absorption from PLGA microspheres and from in situ forming implants

As expected, HIP loading into PLGA microspheres and in situ forming implant provided a sustained release of drug compared to the aqueous solutions and drug levels were detected in the blood for more than one week, as shown in Figures 3 and 4 for PLGA microspheres and in situ forming implants respectively. The fraction of drug absorbed with time is shown in Figure 5, while Cmax and tmax are shown in Table 1.

From Figures 3 and 4, it can be seen that both microspheres and in situ forming implants showed burst release of the 2 drugs. Burst release from microspheres is a common problem and has been associated with drug localisation (due to trapping at and/or migration to) at the microsphere surface as a result of the manufacturing, drying and storage processes and/or with porous polymeric matrices [12]. The burst release from in situ forming implant arises as a direct result of the implant formation/solidificaton in situ. Following injection of the formulation (a drug and polymer solution in an organic solvent), the water-miscible solvent dissipates away from the site of injection and the polymer precipitates entrapping the drug. During

dissipation of the solvent away from the injection site, a portion of the drug dissolved in the solution is taken away by the dissipating solvent [13]. In addition to this solvent drag effect, the burst release has also been assigned to the lag time between administration of the liquid formulation and the formation of a release-controlling solid polymeric implant, during which drug release is not totally controlled [8]. While the burst release (often defined as the drug released in the first 8 or 24h as a percentage of total drug released) can be seen in Figures 3-5, it was not quantified due to the fact that in our studies, total drug release was not seen by the end of the 10-day investigation. However, an indication of the burst release is given in the rates of drug absorption within the first 8 hours, which is discussed later.

Duration of drug release from PLGA microspheres and in situ forming implants

Following the initial burst, drug levels in plasma decreased for both microspheres and in situ forming implants, as drug release was controlled by diffusion through the polymer matrix and the latter's erosion (Figures 3-4), increasing once more at day 10 in the case of alprenolol microspheres, (Figures 3) which suggested the onset of particle degradation. Such triphasic plasma drug profile is often associated with PLGA microspheres [2]. In contrast to the microspheres, plasma drug levels remained low in Figure 4, which shows that the in situ forming implants were still controlling drug release at day 10 of the study. This correlates with previous in vivo studies which have shown fairly long residence and efficacy of in situ forming depots. For example, Ravivarapu et al were able to retrieve implants 105 days after administration of in situ forming implants in rats [9]. The shorter residence time of microspheres compared to in situ forming implants shown in our studies is likely to be

a result of faster hydrolytic degradation of the microspheres, due to the much larger surface area exposed to interstitial fluid and available for erosion.

Extent of drug absorption from PLGA microspheres and in situ forming implants

When the fraction of dose absorbed profiles of PLGA microspheres and in situ forming implants were compared (Figure 5), they appeared to be quite different. Firstly, larger error bars are associated with the *in situ* forming depots, indicating higher variability. Such high variability has previously been reported, and is attributed to the irregular and variable size and shape of the implant formed following administration of the drug/polymer solution and dissipation of the organic solvent [13]. Secondly, more drug was absorbed from the in situ forming depots than from the microspheres. Repeated measures ANOVA, showed significant differences between the profiles for the microspheres and the in situ forming depots for both drugs: ($F(1,7) = 32.8$, $p = 0.001$, eta squared = 0.8 for alprenolol, and ($F(1,8) = 11.5$, $p = 0.009$, eta squared = 0.6). The significantly greater drug absorption from the in situ forming implants compared to the microspheres is most likely to be due to the fact that much less polymer (the release-rate controlling material) was used in the formulation of the in situ forming implants (each animal received 15 mg drug and 75 mg PLGA (in situ forming implant) or 150 mg PLGA (microspheres)). As mentioned earlier, the smaller amount of PLGA used in the in situ forming implant formulation was related to the fact that 0.3 ml of a 50%w/v PLGA in NMP solution was required to administer 150mg PLGA, and this volume was found to be irritating to experimental animals. While the same drug loading in the 2 depot systems would have been desirable, the amount of polymer that can be used in the in situ forming implant formulation was found to be limited by the nature of the solvent.

When the influence of drug lipophilicity on the fraction absorbed profile was examined, no statistical difference was found between the profiles of the 2 drugs, for both microspheres and in situ forming depots ($F(1,8) = 1.4$, $p > 0.05$, eta squared = 0.15 for microspheres, and ($F(1,7) = 0.4$, $p > 0.05$, eta squared = 0.06, for in situ depot). Drug absorption must be preceded by drug release from the polymeric systems (microspheres/in situ forming depots) into the interstitial fluid. The lack of difference between the two drugs suggests that drug lipophilicity did not have a great influence on drug release from the 2 depot systems. However, the low power of these studies (less than 80%, due to the large differences between the drug absorption profiles of the different animals and the small number of animals - 5 - used per group) means that a larger study should be conducted in future.

Rates of drug absorption from PLGA microspheres and in situ forming implants

To compare the absorption rates from the 2 depot systems, approximate absorption rates were calculated as the slope of the lines drawn between times 0 - 8 h, 8 h - 7 days and 7 - 10 days, and are shown in Table 2. Students t tests showed that drug lipophilicity did not have an impact on the 3 rates of absorption from both microspheres and from the in situ forming depot ($p>0.05$). This implies that the rate of drug partitioning out of the polymeric matrix into the interstitial fluid did not influence the rate of drug absorption.

The formulation type was found to be important, especially for the first 0-8h time period. The initial absorption rates from *in situ*-forming depots were significantly faster (Student's t test, $p<0.05$) than from microspheres for both alprenolol and

metoprolol. The lower polymer concentration in the in situ forming depot is an obvious reason for the faster rate. However, the main reason for the faster absorption rates from the in situ forming implant (compared to the pre-formed microspheres) is likely to be due to lag time between administration of the in situ forming depot and polymer precipitation into a solid implant. The kinetics of in situ polymer precipitation and depot formation has not been reported in any detail, however, Royals et al reported that NMP was released over approximately 24 hours following injection of the polymer solution in rats [3]. Lambert and Peck suggested that a significant amount of the solvent remained in the in situ formed depots 13 days after their formation in vitro in Phosphate Buffered Saline at 37^{o}C [4]. During solvent movement out of the in situ forming implant, the latter is not as good a barrier to drug release as the pre-formed microspheres. Furthermore, solvent movement out of the forming implant is likely to enhance drug movement out of the implant, further contributing to drug release.

Conclusions

Both PLGA microspheres and *in situ*-forming implants provided a sustained drug release over the 10-day study period, after a burst release. Drug lipophilicity did not influence the rate of drug absorption or the extent of drug absorption (fraction of dose absorbed) from PLGA microspheres and from in situ forming depots. There were significant differences between PLGA microspheres and the in situ forming implants, in terms of plasma drug profiles, and the rate and extent of drug absorption. Greater variability was found in the drug absorption profiles of the in situ forming implants, assigned to the variable shape and size of implants formed in vivo. The microspheres

showed smaller rates and extent of drug absorption, which was thought to be, in part (but not totally), due to their lower drug:polymer ratio.

Acknowledgements

AstraZeneca and the University of London School of Pharmacy are gratefully acknowledged for their financial contribution.

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Table 1: Cmax and tmax from solutions, microspheres and in situ forming implants. Means and S.D are shown; n=5.

	HIP aqueous solution (1mg HIP dosed)		PLGA microspheres (10mg HIP dosed)		In situ forming depot (10mg HIP dosed)	
	alprenolol	metoprolol	alprenolol	metoprolol	alprenolol	metoprolol
Cmax (ng/mL)	262 ± 61	21 ± 4	115 ± 31	32 ± 18	368 ± 38	41 ± 30
tmax (min)	60	15	120	0-30	Within 120	30

Table 2: Absorption rates following subcutaneous administration of microspheres and in situ forming depots

Time	Absorption rates (min^{-1} x 10^{-6})			
	PLGA microspheres		In situ forming depot	
	Alprenolol	Metoprolol	Alprenolol	Metoprolol
0-8 h	83 ± 11	83 ± 31	323 ± 183	345 ± 162
8h – 7 days	6 ± 4	3 ± 1	10 ± 4	17 ± 8
7-10 days	15 ± 10	4 ± 3	12 ± 17	3 ± 2

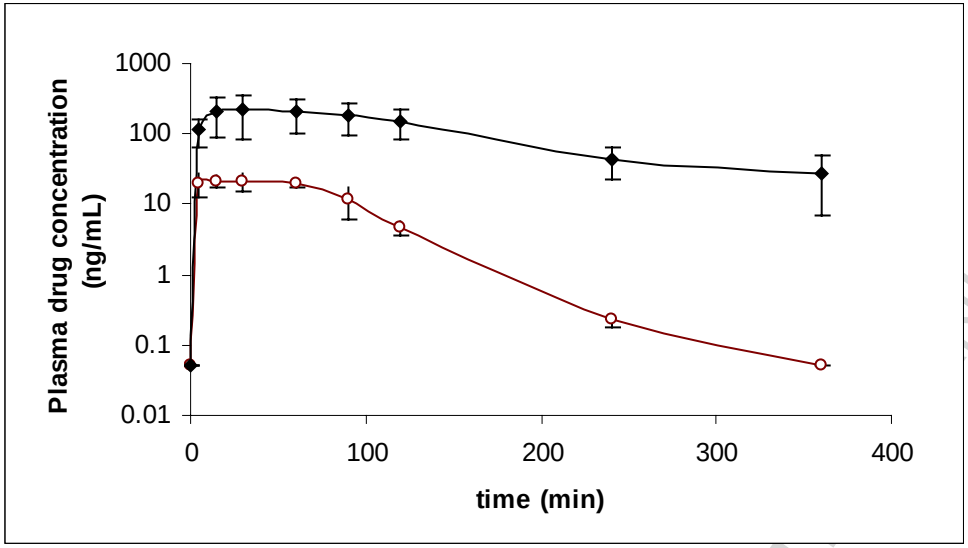

Figure 1: Weight normalised alprenolol (♦) and metoprolol (○)plasma concentration-time curve following a 1 mg (free base equivalent) subcutaneous dose of alprenolol or metoprolol HIP in aqueous solutions. Means ± SD are shown. N=5.

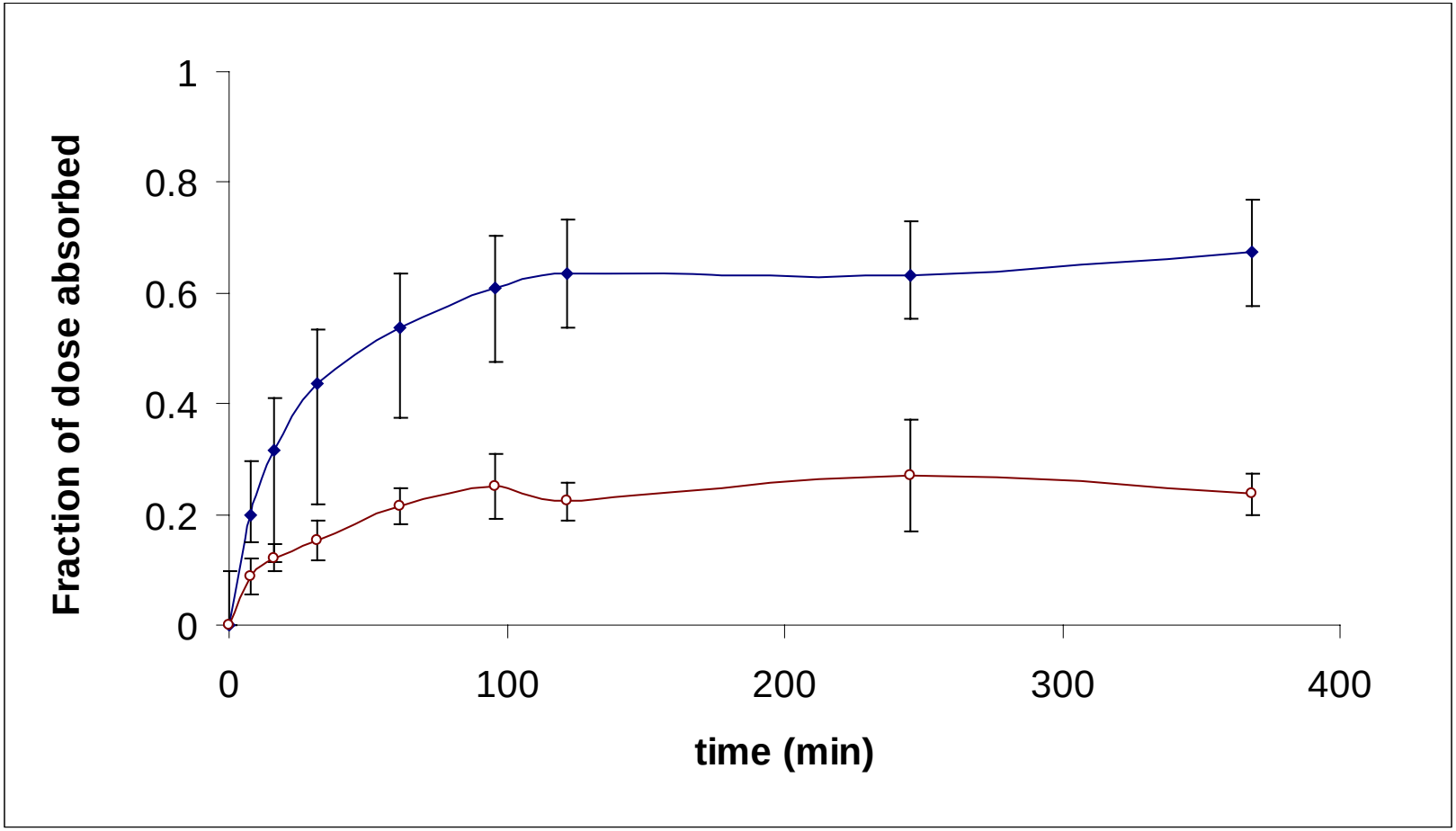

Figure 2 Fraction absorbed *vs* time curves of aqueous alprenolol (♦) and metoprolol (○) subcutaneous injection. Means ± SD are shown. N=5.

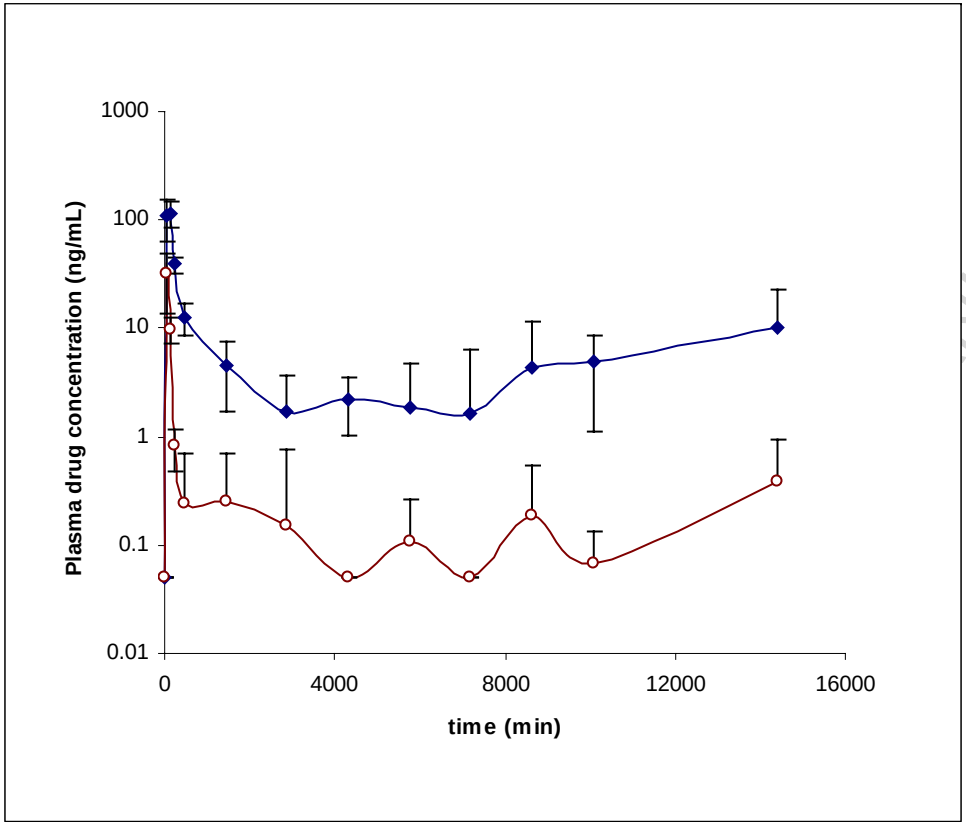

Figure 3 Weight normalised alprenolol (♦) and metoprolol (○) plasma concentration *vs* time curves following subcutaneous dosing of microspheres. Means ± SD are shown. N=5.

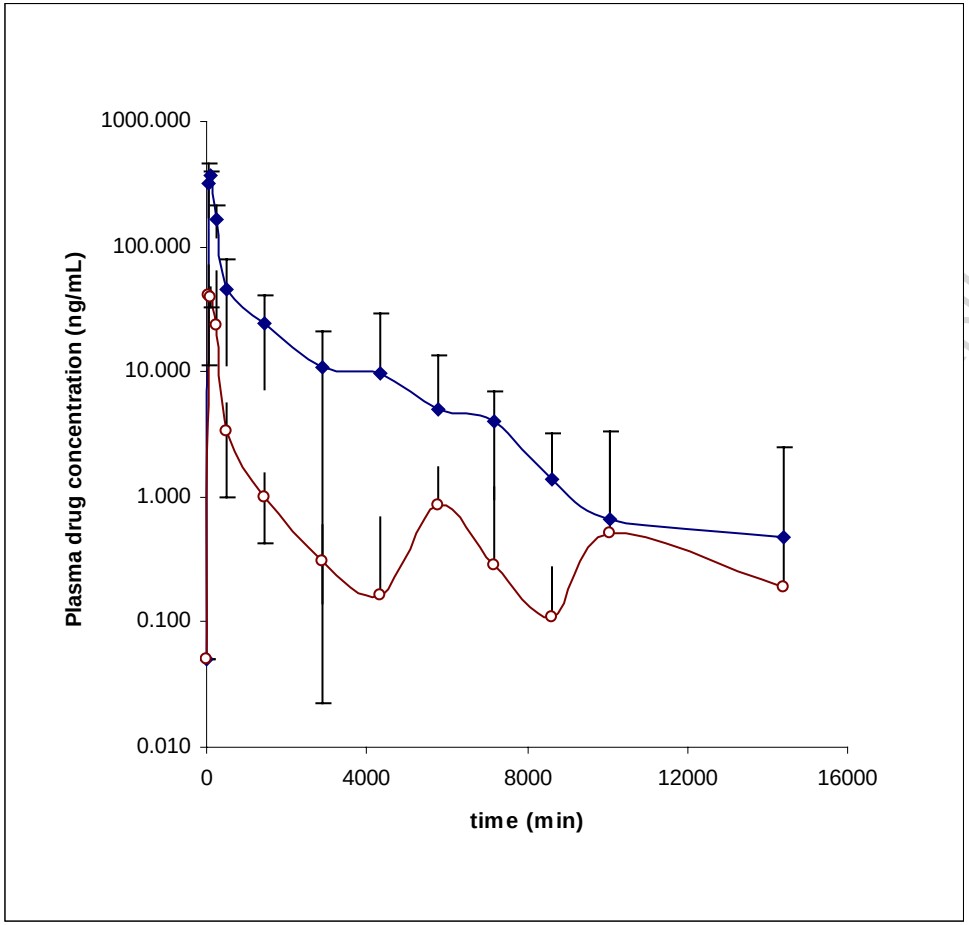

Figure 4 Weight normalised alprenolol (♦) and metoprolol (○) plasma concentration *vs* time curves following subcutaneous dosing of *in situ*-forming depots. Means ± SD are shown. N=5.

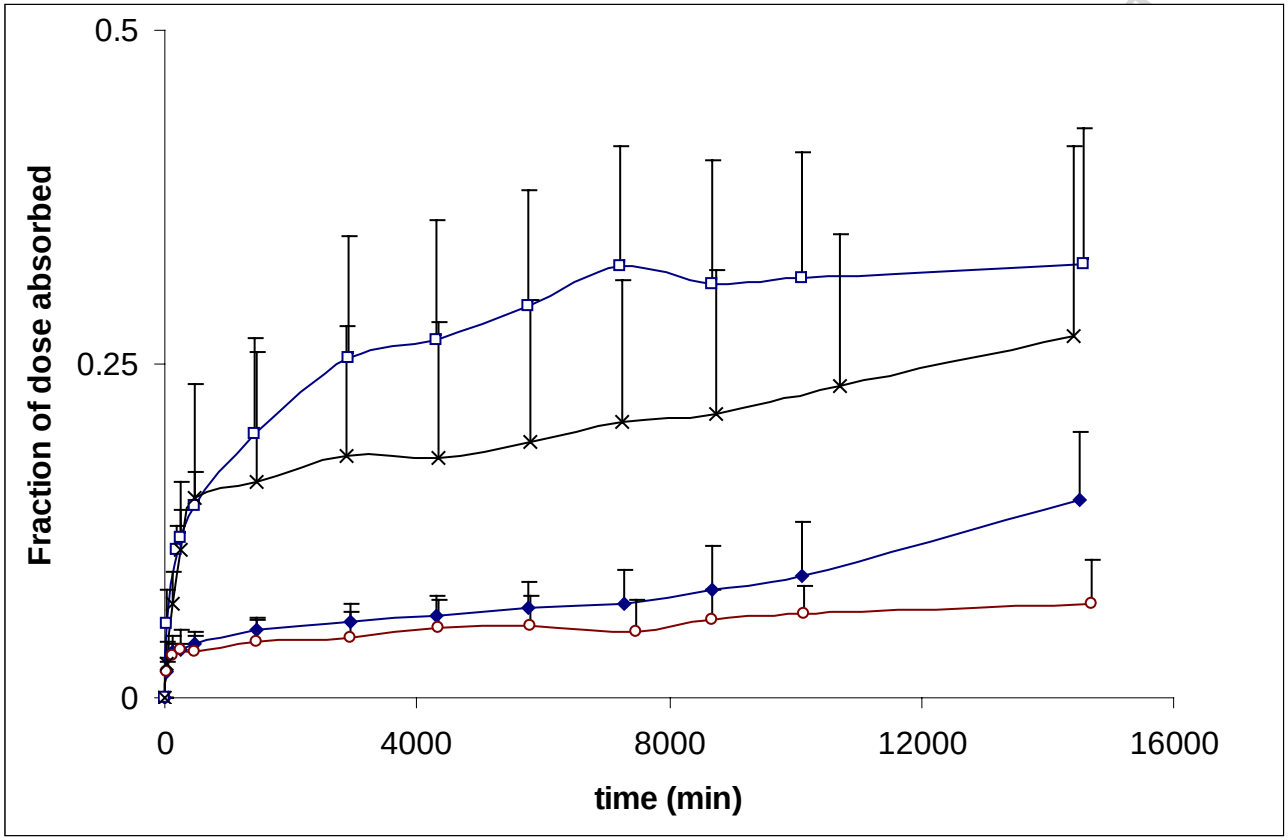

Figure 5 Fraction absorbed *vs* time curves of subcutaneously administered alprenolol in situ forming depot (□), metoprolol in situ forming depot (x), alprenolol microspheres (♦) and metoprolol microspheres (○). Means ± SD are shown. N=5.