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In vitro and in vivo release of estradiol from an intra-muscular microsphere formulation

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Abstract

In vitro 17β-estradiol release from controlled-release microspheres intended for intra-muscular administration was investigated using a rotating bottle apparatus. Prior to intra-muscular administration of 17β-estradiol loaded microspheres the in vivo pharmacokinetics of 17β-estradiol in NZW rabbits were determined: the clearance value was 50.0 ± 8.1 l/d/kg and the elimination half-life was 7.7 ± 2.2 min ($n = 6 \pm$ S.D.). The release of 17β-estradiol from the microspheres in vivo was determined and compared to the release profile obtained in vitro using two release protocols. There was a linear relationship from day 0 until day 30 irrespective of which in vitro test protocol was used. It is suggested that the rotating bottle apparatus is a good starting point for the development of an in vitro release test method for controlled-released intra-muscular dosage forms. Possible strategies for the improvement of an in vitro test to predict in vivo release are discussed. © 1997 Elsevier Science B.V.

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1. Introduction

The correlation of in vitro drug release data of a pharmaceutical dosage form to the drug release profile after in vivo administration is important. Such correlations will permit a more rapid rational dosage form selection. There is an abundance of literature in which attempts have been made to correlate the in vitro release profile of an orally administered dosage form to that seen in vivo (Aoki et al., 1992; Murthy and Ghebre-Sellassie, 1993; Bonny and Kyowa, 1995). It is unlikely that methods used to determine the release profile of

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the orally administered dosage forms will be suitable for intra-muscular preparations due to the different milieu which are encountered by the two dosage forms in vivo. Indeed, there is a paucity of reports where an in vitro drug release procedure for intra-muscular dosage forms accurately predicts the in vivo release profile

As a preliminary investigation into the possibility of developing an in vitro dissolution apparatus that will enable the prediction of the in vivo release profile of a sustained release intra-muscular preparation the release of a model drug, 17β-estradiol hemihydate (E2), from poly-(lactide-co-glycolide-50/50)-microspheres in vitro and in vivo was studied.

2. Materials and methods

2.1. E2-microsphere preparation

Microsphere formation was achieved by means of a spray drying process. In particular a solution of poly-(lactide-co-glycolide-50/50)-Polymer, Mw 30 kDa (RG 503, Boehringer Ingelheim, Germany) and specific amount of 17β-estradiol hemihydate (Diosynth, Netherlands) intended to produce a drug load of 20% w/w, in a mixture of methylene chloride and acetone (7:3) was sprayed through the nozzle (0.7 mm) of a laboratory spray drier (Büchi Mini Spray 190, Büchi, Flawil, CH). The inlet temperature was set to 60°C, the spray flow pressure was 600 Nl/h and the spray flow rate was maintained at approximately 5 ml/min using an aspirator setting of 18 SKT. The measured outlet temperature was between 37 and 39°C. The resulting microspheres were collected from the cyclone and dried under vacuum (20 mbar) at 25°C for 48 h.

2.2. Determination of in vitro release of 17β-estradiol from E2-microspheres

In vitro release studies were performed on 20 mg of microspheres (equivalent to 3.984 mg E2) using the rotating bottle apparatus (NF XIII), consisting of 80-ml screw capped glass bottles containing the release medium rotated at 10 rpm in a temperature controlled water bath. The release medium, maintained at 37°C, was phosphate buffered saline pH 7.4 containing sodium azide 0.05%, to prevent microbial contamination, and Pluronic F68™ 0.05%, as a wetting agent. The buffer was completely replaced at 2, 8, 24 h and daily thereafter (Protocol A) or 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 24 h and daily thereafter (Protocol B).

2.3. Determination of 17β-estradiol pharmacokinetics by infusion of 17β-estradiol to steady-state

Six male New Zealand White (NZW) rabbits (3.72–4.01 kg) were anaesthetised by intra-muscular (i.m.) injection of fentanyl citrate 0.032 mg/kg and fluanisone 1 mg/kg (Hypnorm™, Janssen Pharmaceutical, Oxford, U.K.). Anaesthesia was maintained by i.m. administration of fentanyl citrate 0.016 mg/kg and fluanisone 0.5 mg/kg when required. The left and right peripheral ear veins were cannulated (Jelco™ 22G i.v. catheter, Critikon, Johnson and Johnson, Bracknell, U.K.). 17β-estradiol was administered as heparinised isotonic saline solution (10 U/ml heparin, 0.05% v/v ethanol).

Three rabbits were administered, as a zero-order infusion, 17β-estradiol 24 μg/day (5 ml/h, 75 min) (low dose) followed by 17β-estradiol 142.8 μg/day (5 ml/h, 75 min)(high dose), using an IMED 800 syringe pump (Kabi Pharmacia, Milton Keynes, U.K.), via the left ear vein. The remaining three rabbits were administered 17β-estradiol in a similar manner except the high dose preceded the low dose.

Blood samples (1 ml) were taken from the right ear vein prior to the commencement of the infusions (× 2) and at 10, 25, 40, 60, 70 and 75 min after the commencement of each infusion. Plasma was separated and stored at −20°C until analysis.

2.4. Determination of 17β-estradiol release after intra-muscular administration of E2-microspheres

The same six male NZW rabbits were anaesthetised, on Day 0, as above and the right peripheral ear vein was cannulated (Jelco™ 22G i.v. catheter).

The E2-microspheres (20 mg equivalent to 3.984 mg E2) were dispersed in the injection vehicle (isotonic Tylopur C30, Synperonic F68 saline) and immediately administered into the right adductor muscle.

Blood samples (1 ml) were taken from the right ear vein prior to the administration of the microspheres and at 1, 2, 4, 8, 24, 48, 72 h and 5, 10, 15, 20, 22, 24, 26, 28 and 30 days ($\times 2$) post administration. After Day 0 the rabbits were lightly anaesthetised by administration of fentanyl citrate 0.016 mg/kg and fluanisone 0.5 mg/kg intra-muscularly and blood samples were taken from the right peripheral ear vein. Plasma was separated and stored at -20°C until analysis.

At the end of the experiments the adductor muscle was removed from three rabbits and analysed for 17β-estradiol content.

2.5. Assay of 17β-estradiol

Concentrations of 17β-estradiol in the release medium were determined using an isocratic reverse-phase HPLC assay with detection at 280 nm: Promis II Injection System (Spark Holland, Emmen, Netherlands); Constametric 3000 solvent delivery system (Laboratory Data Control, Stone, UK); LDC Spectromonitor 5000; Spectra-Physics SP4270 integrator (San Jose, USA). A C18 spherisorb S5ODS (Hichrom, Reading, UK) was used at ambient temperature. The mobile phase was 550 ml of acetonitrile, with water to 1000 ml. The flow rate was 2.0 ml/min.

17β-estradiol plasma concentrations were determined by L.A.B. (Wegenstraße 13, D-89231, Neu-Ulm, Germany) utilising a GC-MS technique. Briefly, 0.5 ml of plasma was spiked with 25 μl of internal standard (deuterated 17β-estradiol and estrone) solution and then extracted with a diethyl ether/pentane mixture. The solvent was then evaporated and the 17β-estradiol and estrone derivatised. GC/MS analysis was then performed using a Carlo Erba 2000 gas chromatograph and a Nermag R10-10C mass spectrometer run in the temperature programmed mode and negative chemical isolation for mass selective detection. Selected mass/charge ratios (for the derivatives) were m/z 608 for 17β-estradiol and m/z 510 for estrone and 612/514 for the internal standards.

2.6. Pharmacokinetic analysis

Plasma concentrations were corrected for endogenous 17β-estradiol plasma concentration, determined immediately prior to the commencement of the infusion. A 1-compartment disposition pharmacokinetic model was then fitted to the plasma 17β-estradiol concentration time profiles using a non-linear least squares regression program (Minim, Purves, R.D., University of Otago, New Zealand). The plasma clearance (CL, ml/d) of 17β-estradiol was determined for both infusion rates using $CL = R_0/Css$ where R_0 is the infusion rate (pg/d) and Css is the mean corrected plasma concentration at steady-state (pg/ml).

The amount of 17β-estradiol released from the microspheres for each rabbit was determined using CL $\times$ AUC where the AUC (area under the plasma concentration-time curve) was determined using the trapezoidal rule after the trimmed mean of all endogenous 17β-estradiol plasma concentrations had been subtracted from the plasma concentrations measured. The clearance used was that previously determined for that rabbit at the plasma concentration closest to that observed after microsphere administration.

3. Results

3.1. Determination of in vitro release of 17β-estradiol from E2-microspheres

On addition of the microspheres to the release medium there was an initial burst of 17β-estradiol which was maximal during the first day, independent of the experimental protocol used, the release rate then decreased until approximately day 9, after which it remained constant until day 20 when it increased gradually until day 28 (Fig. 1). The release on the first day of the study when Protocol B was utilised was approximately twice when Protocol A was followed.

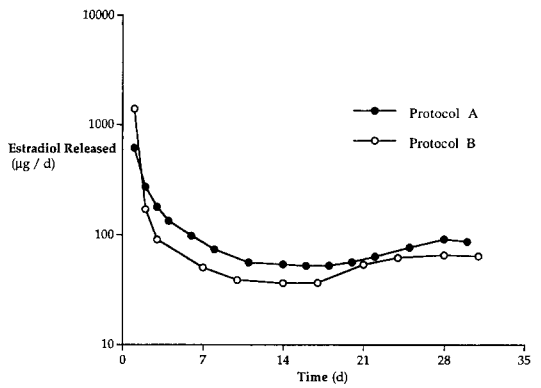

Fig. 1. 17β-estradiol released in vitro from E2-microspheres vs. time in release apparatus ($n = 3 \pm$ S.D.).

3.2. Determination of 17β-estradiol pharmacokinetics by infusion of 17β-estradiol to steady-state

During the first infusions the 17β-estradiol plasma concentration increased rapidly reaching a plateau within 50 min. After changing the infusion rate at 75 min, a second plateau was achieved by 125 min (Fig. 2). The elimination half-life was 7.67 ± 2.19 min ($n = 6 \pm$ S.D.), the plasma clearance of E2 was 51.2 ± 9.6 and 49.0 ± 7.5 l/d/kg ($n = 6 \pm$ S.D.) for low and high dose 17β-estradiol infusion, respectively, which are not significantly different (paired t-test, $p < 0.05$).

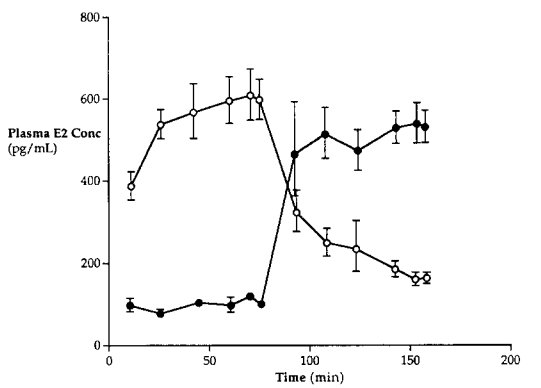

Fig. 2. Geometric mean 17β-estradiol plasma concentration-time profiles after i.v. infusion of E2 21.6 ± 0.5 μg/day for 75 min, followed by E2 106.9 ± 5.5 μg/day for 75 min ($n = 3 \pm$ S.E.M.) or E2 100.7 ± 3.1 μg/day for 75 min, followed by E2 22.3 ± 1.1 μg/day for 75 min ($n = 3 \pm$ S.E.M.).

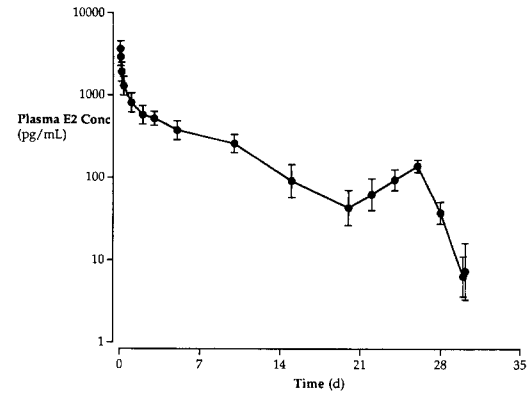

Fig. 3. Geometric mean 17β-estradiol plasma concentration-time profiles after i.m. administration of E2-microspheres (equivalent to 17β-estradiol 4.35 ± 0.28 mg) ($n = 6 \pm$ S.E.M.).

3.3. Determination of 17β-estradiol release after intra-muscular administration of E2-microspheres

After intra-muscular administration of the E2-microspheres the highest plasma 17β-estradiol concentrations were measured at 1 h post administration the plasma concentration then decreased rapidly throughout the first day then more slowly until day 10. The 17β-estradiol plasma concentrations then remained relatively constant until day 28 when they started to decrease again (Fig. 3). The amount of 17β-estradiol released, and the percentage of 17β-estradiol administered accounted for is shown in Table 1.

Table 1
Amount of 17β-estradiol released from intra-muscularly administered E2-microspheres in 30 days

Rabbit	17β-Estradiol released	
	mg[a]	% of dose
1	1.86	46.7[b]
2	1.53	38.6
3	1.30	45.6[b]
4	0.62	15.6
5	0.98	24.6[b]
6	1.79	44.9
Mean ($\pm$ S.D.)	1.35 ($\pm$ 0.48)	36.0 ($\pm$ 13.0)

[a]Normalised to a 20 mg dose of microspheres (equivalent to 3.984 mg E2).
[b]Including E2 recovered from the adductor muscle at the end of the study.

4. Discussion

The fact that the initial burst in the release of 17β-estradiol from the microspheres in vitro was dependent on the frequency of the media change suggests that, at least for Protocol A, the release of 17β-estradiol on day 1 was limited by the aqueous solubility of 17β-estradiol (i.e. sink conditions were not maintained). The reduction in 17β-estradiol release due to saturation of the release medium was obviously less for Protocol B but may not have been totally abolished. This highlights the need to select carefully the time intervals for sampling of the release medium.

A preliminary investigation of 17β-estradiol pharmacokinetics in rabbits by intravenous infusion was necessary to allow more accurate determination of 17β-estradiol release from the E2-microspheres administered to each rabbit as only limited and variable reports of 17β-estradiol pharmacokinetics in rabbits exist in the literature. A total body clearance comparable to that reported here (45 l/d/kg) has been measured previously (Bourget et al., 1984), however these are in contrast to earlier published values of 78 l/d/kg (Tremblay et al., 1977) and 88 l/d/kg (Fraser et al., 1976), although the latter was in chinchilla rabbits, and values of 150 l/d/kg and up to 170 l/d/kg have been estimated from more recently available data (Hermens et al., 1990; Lee et al., 1993). There are apparently no published values for the elimination half-life of 17β-estradiol in lagomorphs, although steady-state has been achieved within 1 h, suggesting an elimination half-life of less than 12 min (Tremblay et al., 1977).

The mass balance calculations (Table 1) showed that between 15 and 47% of the dose administered to the rabbits could be accounted for; this is despite the assay of the injection site, in three rabbits, for 17β-estradiol remaining at the end of the study. Possible reasons for this discrepancy between 17β-estradiol administered and that recovered are: firstly, the peak 17β-estradiol plasma concentration was reached before the first sample post administration was taken. It is therefore impossible to accurately assess the amount of 17β-estradiol released from the microspheres in the first hour after administration, which was probably more than that estimated using the CL × AUC method. Secondly, any first-pass extraction by the muscle tissue before the 17β-estradiol enters the systemic circulation could not be quantified (the 17β-estradiol to estrone ratio after microsphere administration was smaller than after intravenous administration possibly suggesting first-pass metabolism—data not shown). One method to overcome this complication of possible first-pass metabolism would be to administer 17β-estradiol solution intra-muscularly, the in vivo release of 17β-estradiol from the E2-microspheres may then be calculated using numerical deconvolution (Lagenbucher and Mysicka, 1985; Bonny and Kyowa, 1995). It should be noted that the numerical deconvolution technique will only allow the calculation of the in vivo dissolution rate if the pharmacokinetics of the drug under investigation are release rate limited. The determination of amount of 17β-estradiol released by the microspheres in vivo using the CL × AUC method assumes that the pharmacokinetics of 17β-estradiol are release rate limited. This condition is likely to apply to 17β-estradiol as it is a lipophilic compound that is rapidly absorbed when administered via other routes (Patel et al., 1995).

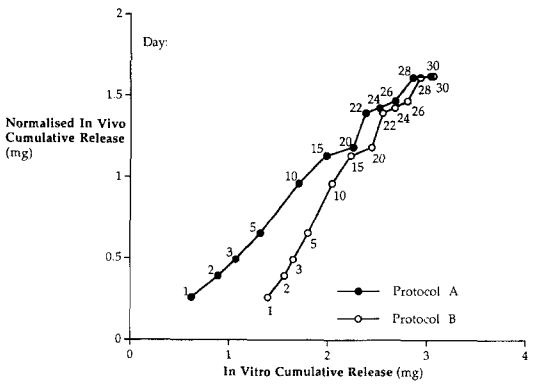

Fig. 4. Mean cumulative 17β-estradiol release from microspheres (normalised to 20 mg E2-microsphere dose) in vivo vs. cumulative release determined in vitro.

The plots of in vivo cumulative release versus in vitro release (Fig. 4) are approximately linear (Protocol A: $r = 0.995$, slope = 0.601; Protocol B: $r = 0.990$, slope = 0.856), suggesting that the

choice of the in vitro technique for the prediction of drug release in vivo is valid.

The data presented above suggests that the rotating bottle apparatus is a reasonable starting point for the development of an in vitro release test procedure for intra-muscular microsphere delivery systems containing a steroid. This is especially true when Protocol B was followed for the in vitro release test as the slope of the in vivo/in vitro plot is close to the ideal of 1 (if the release only up to day 26 is considered the slope was 0.906). The curve describing the in vivo/in vitro plot using Protocol B for the in vitro release experiment had a large negative intercept (−0.978). This suggests that the mass balance calculations for the dose recovered in vivo are biased due to 17β-estradiol not accounted for, before the first plasma sample (1 h). That is if plasma samples had been taken which enabled the characterisation of the 17β-estradiol peak then the intercept would be reduced.

An issue raised by this work is whether it is desirable for the in vitro release profile to mimic the in vivo profile on a real-time basis. That is, is it really desirable for in vitro tests, which are supposed to provide relatively simple and rapid indication of the likely effect of changes in formulation on in vivo performance, to take up to 30 days? This time period could be unacceptable, especially as the in vitro release test may well be included in the subsequent quality control of the final commercial preparation (Skelley et al., 1990; Murthy and Ghebre-Sellassie, 1993). In future, the possibility of changing the in vitro release test so that, for example the release of drug per hour in vitro equates to the release in vivo per day, should be considered. This approach will necessitate more extreme test conditions which may have the disadvantage of masking changes in the dosage forms characteristics (Murthy and Ghebre-Sellassie, 1993). It should be possible to reduce the in vitro release time by employing the methods used to increase release of drugs from oral controlled-release dosage forms during in vitro release testing. These include strategies such as: the inclusion of surfactant in the release medium (Skelley et al., 1990), although surfactant was already present the concentration or type may not be optimal; and increasing agitation, either via faster stirring or the inclusion of polystyrene beads (Aoki et al., 1992). A further possibility is the inclusion of a more lipophilic liquid in the release medium to more accurately mimic the lipophilic nature of the tissue, this step may also have the advantage of reducing the problems associated with saturation of the release medium with 17β-estradiol.

In conclusion the rotating bottle apparatus may be useful for the investigation of the in vitro release of drug from controlled release intra-muscular microspheres to enable more rational selection of the dosage forms to be investigated in vivo.

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