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RESEARCH ARTICLE

Intra-articular injection of morphine to the horse: establishment of an *in vitro–in vivo* relationship

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Abstract

Background: In the area of parenteral depots, a strong need exists for the development of suitable *in vitro* drug release models that might enable establishment of *in vitro–in vivo* relations (IVIVRs).

Aim: The objective of this study was to investigate the possibility of establishing an IVIVR between morphine disappearance from the joint cavity and *in vitro* release data obtained employing the rotating dialysis cell model.

Method: *In vitro* release experiments were conducted using the rotating dialysis cell model. For establishment of an IVIVR, data from a previous study on pharmacokinetics of intra-articular (IA) morphine in horses with lipopolysaccharide-induced synovitis were used (Lindegaard et al., (2009). *Vet Anaesth Analg*, 37, 186–195).

Results: A rate constant of morphine disappearance from the donor phase of the *in vitro* model of 1.8×10^{-2} min^{-1} was calculated, independently of the different release media used. The *in vivo* synovial fluid disappearance rate constants were in the range of 1.0×10^{-2}–1.7×10^{-2} min^{-1}. An IVIVR ($R^2 = 0.89$) was established between the calculated disappearance data and the joint disappearance data.

Conclusion: The results indicate that the IA fate of morphine administered in the form of a solution can be predicted from the *in vitro* release data obtained in the rotating dialysis cell model. Thus, this model might be a valuable tool in the establishment of IVIVRs after IA administration of drugs with similar properties.

Keywords: Intra-articular administration, *in vitro in vivo* relations, IVIVR, morphine, pharmacokinetics

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Introduction

Opioids are frequently used for management of mild to moderate postoperative pain after minor arthroscopic joint surgery[1–3]. The oral and intravenous administration of this group of strong analgesics is associated with numerous side effects[4]. A means to minimize these side effects might be to inject the opioids directly into the disease affected joint (intra-articular (IA)), thereby significantly reducing the dose needed for achieving peripheral therapeutic drug concentrations[1,2,5,6]. After IA injection, drug residence time within the joint cavity is influenced by the transport of the solute out of the joint. In case of small-molecule drugs, the main route of disappearance is via the capillaries[5,7,8]. Due to the discontinuous nature of the synovial membrane, the diffusion process is driven by the drug concentration gradient between the blood and the synovial fluid (SF). For future research in the field of postoperative pain management and multimodal analgesia, establishment of an *in vitro–in vivo* relation (IVIVR) describing the relationship between an *in vitro* property and a relevant *in vivo* response would be a valuable tool. It has been suggested that the rotating dialysis cell model, consisting of a small, cylindrical donor compartment separated from a large acceptor compartment

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by a dialysis membrane, might be a useful *in vitro* model for the study of drug transport and equilibrium processes taking place in a synovial environment[9–11]. This *in vitro* model was recently used to establish an IVIVR for a small-molecule model drug (sodium diatrizoate, DTZ), administered intra-articularly in the form of a solution[12]. Demonstration of a more general application of the rotating dialysis cell model in the area of establishing IVIVRs after IA administration requires, however, similar relationships to be established for drugs differing from DTZ with respect to physiochemical properties including aqueous solubility, lipophilicity, electric charge state, protein binding and molecular size since these drug characteristics might influence the joint residence time[5]. Morphine differs from DTZ with respect to molecular mass as well as electric charge state, solubility, and protein binding at physiological conditions.

Hence, the objective of this study was to investigate the possibility of establishing an IVIVR between morphine disappearance from the joint cavity and *in vitro* release data obtained employing the rotating dialysis cell model. Morphine data was taken from a recent study of morphine pharmacokinetics after joint injection of drug solutions containing clinically relevant doses in horses with lipopolysaccharide-induced synovitis[6]. Expansion of the utility of the rotating dialysis cell model for prediction of rate of drug disappearance from the joint space appears to be of significant interest since, in addition to antibiotics and strong analgesics, IA administration is of relevance for local anesthetics and NSAID's as well as combination regimens to provide multimodal analgesia (Figure 1[13–16]).

Materials and methods

Materials

Morphine hydrochloride "DAK" 20 mg mL^{-1} was purchased from Nycomed (Roskilde, Denmark). Heparin "Leo" 5000 ui mL^{-1} was obtained from Leo Pharma (Ballerup, Denmark). Sodium Chloride "SAD", 9 mg mL^{-1} was purchased from Amgros I/S (Copenhagen, Denmark). Buffer substances and solvents were of analytical or reagent grade. Deionised or higher quality of water was used. Visking dialysis tubing size 27/32, 21.5 mm, MW cut-off 12–14 kDa purchased from VWR International (West Chester, PA) was employed in the rotating dialysis cell model experiments. Healthy equine SF (collected immediately postmortem) was obtained from the Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen (Taastrup, Denmark). A 67-mM (pH 7.4) phosphate buffer solution (PBS) and a solution, adjusted to pH 7.4, of equine SF and PBS (50% SF (v/v)) were used in the release experiments.

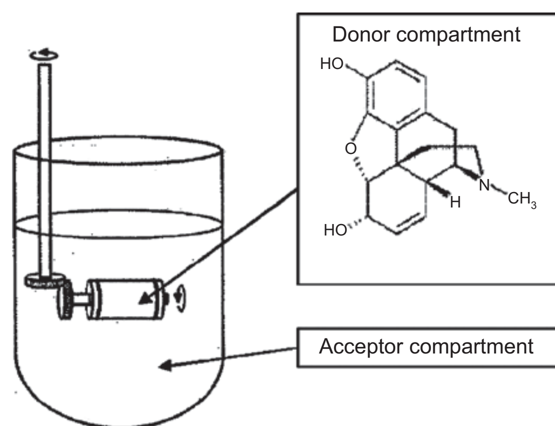

Figure 1. The rotating dialysis cell model. Release experiments were conducted by applying solutions of morphine in equine 50% synovial fluid, or phosphate buffer solution into the donor compartment.

In vitro experiments

For the *in vitro* release studies, the rotating dialysis cell model (Figure 1) was used as described elsewhere[11,17]. The cylindrical donor compartment is separated from the acceptor compartment by a dialysis membrane (interfacial area: 22 cm^2). Morphine solutions (2.0–2.6 mg mL^{-1}) were prepared by dissolving morphine in (i) 67 mM PBS pH 7.4 or (ii) healthy equine 50% SF to the donor compartment. At time zero, 5.00 mL of the solutions were instilled into the donor compartment, which was placed in a round-bottomed vessel containing 1000 mL of preheated PBS. Experiments were conducted at 37 ± 0.5°C with a stirring rate of 50 rounds per minute. Samples were withdrawn from the acceptor compartment and analyzed by High-performance liquid chromatography (HPLC). The release experiments were performed in triplicates and followed until equilibrium was attained in the system. The cumulative amount of morphine ($M_{A,t}$) released into the acceptor compartment at time t was calculated according to:

$$M_{A,t} = V_s \sum_{i=1}^{n} C_{i-1} + V_A C_n \quad (1)$$

where V_A and V_S are the volumes of the acceptor compartment and samples withdrawn from the acceptor compartment (V_S = 1.5 mL), respectively. C_i and C_n are the concentrations of morphine in sample i and n, respectively. Plots of the *in vitro* appearance of morphine in the acceptor compartment versus time were generated. The disappearance profile of morphine from the donor compartment was constructed based on mass balance considerations.

Analysis

Samples from the *in vitro* release experiments were analyzed by HPLC. The equipment consisted of a Merck-Hitachi L-6200 pump connected to a Merck-Hitachi L-4000 UV detector, a Merck-Hitachi L-7200 autosampler, and a Peltier Sample Cooler (10°C) (VWR International, Tokyo, Japan). Reversed phase chromatography was performed using a C18 Gemini® RP column (length 150 mm, inner diameter 4.6 mm, 5 μm particles) (Phenomenex,

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Torrance, CA) equipped with a Security Guard precolumn (Phenomenex, Torrance, CA). The flow rate was set at 1 mL min^{-1}, and the column effluent was monitored at 220 nm. The mobile phase consisted of acetonitrile: 0.1% (v/v) phosphoric acid 1:19 (v/v). Quantification of morphine was done from peak area measurements in relation to those of standards chromatographed under the same conditions.

In vivo experiments

Experimental details of the *in vivo* experiments employing eight horses with lipopolysaccharide-induced synovitis are reported elsewhere[6]. Briefly, the experiments conducted were arranged as a randomized, double dummy cross-over study. During each of the two study periods, blood and SF samples were collected at −4, 0, 2, 4, 8, 12, 16, 20, 24, 28, 44, 92 and 164 h after treatment with morphine administered either intra-articularly (or intravenously—data not available) dosing 0.05 mg/kg (dose range 17–30 mg). The SF data after IA administration are employed in this study. SF samples (approximately 3 mL) were collected by repeated arthrocentesis using standard aseptic techniques with a 21-gauge 40-mm needle, and immediately transferred into Ethylenediaminetetraacetic acid-stabilized tubes (BD Vacutainer®, BD, Franklin Lakes, NJ). The sample preparation method and the analytical procedures (LC-MS) used for quantification of morphine in the *in vivo* samples are reported elsewhere[6]. The protocol was pre-approved by the Danish Animal Experimentation Board, and all procedures were carried out according to the Danish Animal Testing Act.

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Pharmacokinetic analysis and IVIVR

A compartmental analysis was performed to obtain a mathematical expression adequately describing the *in vivo* data. SF morphine concentrations following IA administration were described using the following general polyexponential equation:

$$R(t) = \sum A_i \cdot e^{-\alpha_i \cdot t} \quad (2)$$

where $R(t)$ represents the concentration of morphine in the SF at time t, A_i is the coefficient of the ith exponential term and α_i is the exponent of the ith exponential term. The software WinNonlin® with IVIVC Toolkit (version 5.2, Pharsight Corporation, Mountain View, CA) was used for pharmacokinetic modeling. The Nelder-Mead algorithm was used for curve fitting[18]. Weighting and goodness-of-fit were based on visual inspection of the data presented in a semi-logarithmic plot. Model selection was based on the Akaike information criterion[19] and visual inspection. The SF data were weighted according to $1/y_j^2$ where y_j is the predicted value of the jth observation. IVIVR was confirmed by construction of Levy plots[20].

Results and discussion

The rotating dialysis cell model was selected for the present study since this model may mimic the conditions drug solute molecules may be exposed to following injection into a synovial joint[11,12]. In addition to opioids, the IA route of administration is also considered of interest for solutions containing local anesthetics[21], non-steroidal anti-inflammatory drugs[22,23] and antibiotics[24]. The *in vitro* results and the establishment of IVIVRs are dealt with below. Details related to the pharmacokinetic behavior of morphine in the horse were reported elsewhere[6].

In vitro study

The release experiments were initiated by placing the donor cell containing an aqueous buffer solution of morphine (7 mM) in the aqueous buffer acceptor compartment. Drug transport from the donor compartment across the membrane and into the acceptor compartment is driven by the concentration gradient existing between the two compartments. Similarly, a concentration gradient is most likely the driving force for the transport of small-molecule drugs out of the synovial space and into the systemic circulation[7,8]. In the *in vitro* model, mass transfer will continue until equilibrium is attained. The ratio of the employed volumes of the acceptor and donor phase, respectively, amounts to 200. Hence, 99.5% of the dose originally instilled in the donor cell is transferred to the acceptor compartment at equilibrium. In this case, therefore, the rate of attaining equilibrium between the donor and acceptor compartment, for practical purposes, represents the rate of morphine appearance in the acceptor compartment. First-order rate constants for appearance in the acceptor compartment were obtained from plots of cumulated amounts of morphine versus time[11]. Mean first-order rate constants of 1.8×10^{-2} min^{-1} ($n = 3$, relative standard deviations (RSDs) = 4.5%) and 1.8×10^{-2} min^{-1} ($n = 3$, RSDs = 5.3%) were calculated for morphine dissolved in PBS and equine SF, respectively. As apparent from Figure 2, almost identical *in vitro* appearance profiles were obtained from donor phases containing morphine dissolved in PBS and healthy equine SF (50% v/v), respectively. Since almost 100% morphine was released

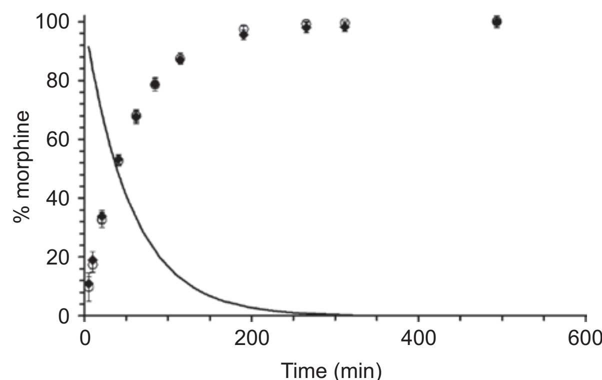

Figure 2. Appearance profiles of morphine in acceptor compartment of the rotating dialysis cell *in vitro* model (♦ = Equine 50% SF in donor compartment, ○ = PBS in donor compartment, $n = 3$, error bars represent the standard deviations). Calculated morphine disappearance profile (— = PBS in donor compartment).

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at equilibrium, using SF as donor medium (Figure 2), binding of morphine to SF proteins of healthy equines was neglectable. Overlapping appearance profiles (PBS and SF) were previously observed for the model drug substance DTZ[12], the protein binding of which is below 10%[25]. To this end, the degree of plasma protein binding of morphine, at therapeutic concentrations (about 10–30 ng mL^{-1}[26]), is reported to be of the order of 35%[27]. Thus, although the degree of protein binding of morphine is higher than that of the previously studied model drug substance DTZ, the observed *in vitro* release data can be ascribed to the relatively high drug dose added to the SF donor compartment resulting in saturation of morphine binding sites on the SF proteins. Hence, in the comparison to *in vivo* data, it was found acceptable to use *in vitro* data related to the least complex medium (PBS). In healthy horse SF, the albumin concentration amounts to about 2 g per 100 mL[28]. In the inflamed horse joint, a two-fold increase in the albumin concentration has been observed[28]. Under such conditions, the SF protein concentration approaches that of serum due to enhanced leakiness of the capillaries[5]. The albumin content in 5 mL (the volume added to the donor cell *in vitro*) of the latter inflamed SF corresponds to 1.5 µmol albumin. In comparison, 10 mg morphine added to 5 mL donor solution amounts to 35 µmol. Albumin constitutes the major binding protein of morphine[29]. In case morphine interacts with other SF proteins, saturation of the binding sites is to be expected since the albumin concentration exceeds that of the other SF proteins[5]. The morphine dose used for the *in vitro* experiments (10–13 mg per 5 mL) was comparable to those used in the *in vivo* experiments (17–30 mg per joint[6]) where the estimated SF volume of a normal equine radiocarpal joint is of the order of 13 mL[30]. The present findings indicate that protein binding may not be a key determinant of joint residence time in case such a massive dose of dissolved drug is injected. Since sampling from the donor compartment during the release experiment is not possible, the cumulated amount of drug transferred to the acceptor compartment at time t was calculated. From mass balance considerations, the amount of morphine remaining in the donor cell as function of time was obtained from knowledge of the total amount of morphine initially applied to the donor cell and cumulated amount of drug transferred to the acceptor compartment at time t (Figure 2).

IVIVRs

Pharmacokinetic data in SF were obtained from eight horses. A polyexponential expression was used for the eight horses in order to obtain an IVIVR for each animal (Figure 3).

The SF morphine concentration versus time profiles obtained after IA administration of 0.05 mg morphine/kg (dose range 17–30 mg) are presented in Figure 3 ($n = 8$). The disappearance of morphine from the joint cavity after IA administration was described by a two-compartment model with first-order elimination. As more than 90% of the dose administered was released initially (during the α_1 phase, ~0–300 min), the disappearance rate constant obtained from the initial phase was used for the establishment of an IVIVR. The obtained *in vivo* disappearance rate constants were in the range 0.9×10^{-2}–1.7×10^{-2} min^{-1}. The corresponding average half-life for disappearance of morphine from the joint cavity ($t_{½\alpha 1}$) was calculated to 52 min (range 40–78 min). In comparison, a mean serum half-life of the drug following intravenous administration to horses of 88 min has been reported[31]. In human, a similar pharmacokinetic behavior of morphine was observed ($t_{½} = 2$ h; range 1–8 h[32]).

The calculated apparent distribution volumes for the joint compartment (α_1 phase, V_{joint}) amounted to 82–282 mL. Normally, the volume of distribution is to be considered as a conversion factor between amounts and concentrations[33]. In the present case, the large V_{joint} values might reflect that the inflamed joint accumulate SF. In addition, enhanced protein content in the inflamed SF as well as the distribution of positively charged morphine into the negatively charged cartilage might result in an increased V_{joint}[33]. However, the extent to which the inflammation condition affects the magnitude of the apparent volume of distribution remains unknown since the corresponding V_{joint} values of morphine determined in healthy horses are not available.

In Figure 4, *in vivo* SF disappearance data after IA morphine administration are compared to the *in vitro* disappearance data from the donor cell by plotting the time points corresponding to 10, 20, 30,..., 90% disappearance of morphine from the joint cavity and the donor compartment, respectively. The IVIVR obtained is presented in Equation 4 in the form of a Levy plot.

$$t_{in\,vitro} = 1.40 \times t_{in\,vitro} - 0.85 \qquad (R^2 = 0.89;\ t \text{ in min}) \quad (4)$$

An even better correlation coefficient of 0.97 can be obtained in case data for H3 is excluded. From Equation 4, it appears that morphine disappearance from the joint cavity proceeds slightly slower than from the donor compartment due to a slope exceeding unity. Accomplishment

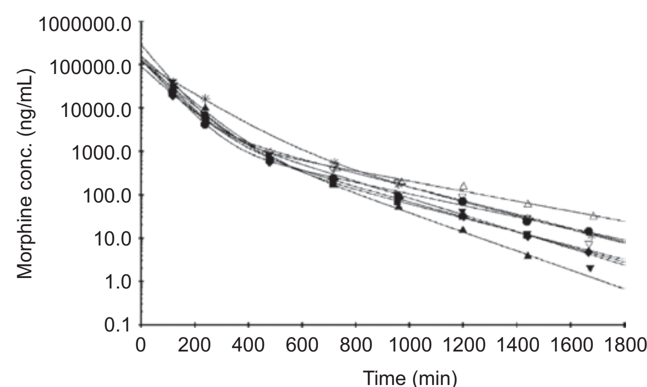

Figure 3. Estimated (—) and observed ($n = 8$, △ = H1, □ = H2, * = H3, ◇ = H4, ▲ = H5, ◆ = H6, ■ = H7, ● = H8) morphine concentrations in SF after intra-articular administration of 0.05 mg/kg to the radiocarpal joint of horses H1–H8.

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of a strict 1:1 correlation requires modification of the *in vitro* model setup for example through reduction of the dialysis cell surface area.

The observed morphine IA disappearance rate (disappearance rate constant in the range $0.9–1.7\times10^{-2}$ min^{-1}) is almost identical to that previously reported for sodium diatrizoate (DTZ) (rate constant in the range $0.8–1.8\times10^{-2}$ min^{-1}[12]). As opposed to cationic morphine, DTZ is negatively charged at physiological pH, and the degree of protein binding, amounting to approximately 10%[25], is significantly lower than that of morphine. The molar mass of DTZ (614 g mol^{-1}) exceeds that of morphine (285 g mol^{-1}). However, in the assessment of the ability of a solute molecule to permeate a semi-permeable membrane, molecular size or hydrodynamic volume rather than molar mass should be considered. The former parameter of the two species is reflected in the half-lives of disappearance from the donor cell compartment *in vitro,* which are 39 min (morphine) and 50 min (DTZ). From inspection of IA pharmacokinetic data[5], it seems that the electric charge state, *per se*, does not significantly influence IA residence time. Likewise, protein-binding effects on IA fate, under the conditions employed, are insignificant. The observed almost identical IA disappearance kinetics of morphine and DTZ may therefore suggest that the disappearance rate of DTZ was slightly overestimated due to the fact that high doses of DTZ was administered IA (about 2 g per joint) as discussed elsewhere[34]. Seemingly, the presented results suggest that under the conditions given the IA fate of morphine may be predicted from the *in vitro* disappearance data (Figure 4).

In summary, the data from the present study strongly support the suggestion that in the field of IA injections of aqueous formulations the rotating dialysis cell model is capable of predicting the *in vivo* fate of relatively large doses of small-molecule drugs (morphine and DTZ)

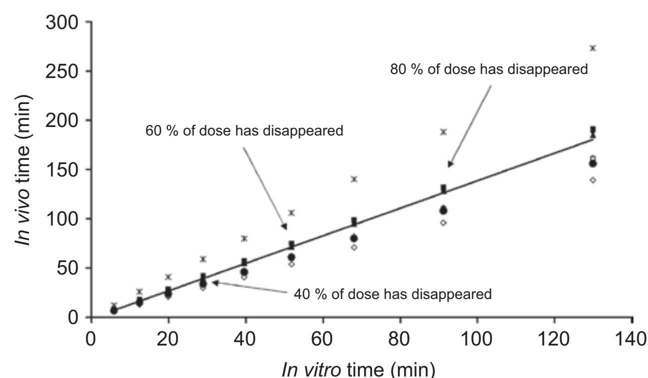

Figure 4. Levy plot produced from time points corresponding to 10, 20, 30,..., 90% of morphine disappearance from the joint cavity/donor compartment, respectively. *In vitro* disappearance data using phosphate buffer pH 7.4 in the donor compartment and *in vivo* disappearance data from the α phase of the synovial fluid versus time profiles were used (n = 8, △ = H1, □ = H2, * = H3, ◇ = H4, ▲ = H5, ◆ = H6, ■ = H7, ● = H8). The solid line (—) is the average regression line for all eight horses (Equation 4).

exhibiting first-order drug disappearance kinetics. Limited access to arthritic SF samples may, together with ethical and safety aspects, advocate for development of an artificial SF to be used in the *in vitro* release tests. The IA residence time of drugs administered as injectable depot formulations is governed by drug release from the depot as well as the transport of dissolved drug out of the joint. Hence, this *in vitro* model may also be of value in the study of *in vitro* drug release from certain sustained release principles.

Declaration of interest

This work was supported by the Danish Medicinal Research Council and the Drug Research Academy. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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