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Simultaneous magnetic resonance imaging and pharmacokinetic analysis of intramuscular depots

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ABSTRACT

The present pilot study introduces a method that might give novel insights in drug absorption processes from intramuscularly administered depots.
An oily suspension or an aqueous solution of paracetamol (6 mg/kg body mass), prednisolone or its hemisuccinate sodium salt for the aqueous solutions (10 mg/kg body mass) or diclofenac (10 mg/kg body mass) was injected into the muscle tissue of the hind leg of female Lewis-rats (n = 47). For the oily suspensions the micronized particles were suspended in medium-chain triglycerides. The aqueous solutions were buffered to a pH of 7.4 ± 0.5. Polyethylene glycol was added as a cosolvent in the formulations containing paracetamol (acetaminophen) and diclofenac and sodium chloride was added to the aqueous solutions of prednisolone hemisuccinate sodium to achieve nearly isotonic formulations. The formed depot was visualized by magnetic resonance imaging (MRI) and characterized with regard to volume and surface area. A 7 T-small animal scanner was used and T1-weighted and T2-weighted sequences including a fat saturation were performed. Simultaneously blood samples were taken and the drugs were quantitatively analyzed.
The water based solvent and the oily dispersion agent were visible in the MRI images without the use of contrast agents. Since a free hand injection mostly led to an application directly into the fascia, resulting in a fast removal of the depot, MRI-guided injection was conducted. Comparing pharmacokinetic data with MRI data it was observed that maximal blood levels occurred before the solvent and the dispersion agent were removed from the muscle tissue. Thus, the drug is not absorbed together with the depot. Furthermore, no correlation was found between the shape of the depot and the rate of absorption. Consequently, a higher surface area or volume of the depot did not result in a faster release or absorption of the drugs from the tested formulations. In contrast to the paracetamol and prednisolone formulations the formulations containing diclofenac led to a massive accumulation of interstitial fluid around the injection area being a sign for an acute local reaction. Histological analysis of the muscle tissue revealed a clear correspondence between the amount of interstitial fluid and the extent of infiltrating lymphocytes and granulocytes indicating a tissue response.
In conclusion combining MRI with pharmacokinetic data is a suitable method to gain deeper insights into drug absorption processes from intramuscular depots. Furthermore, MRI offers a great possibility detecting local side effects caused by an intramuscularly applied dosage form. This might be very useful in preclinical phases during the development of new intramuscular formulations.

1. Introduction

Despite the increasing interest in the intramuscular route of administration, the in vivo processes following injection into the muscle tissue are not fully understood to-date. Most work that has been performed to identify factors influencing drug absorption after intramuscular injection was carried out in the 1980s and early 1990s [1,2]. It was observed that the formulation itself exerts a major influence on the release and absorption kinetics of the intramuscularly applied dosage forms [3–5]. Furthermore, the injection depth and technique have an impact as well as body movement and blood supply [6–8]. From these studies it is known that in vivo conditions possess considerable effects on the release and absorption behavior of API's (active pharmaceutical

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ingredients) after intramuscular injection. For example local reactions caused by the injection [9–11] can have an effect on the uptake of the drug into the systemic circulation [12]. However, a detailed understanding of the in vivo processes and their variability which might explain the wide variability of the pharmacokinetic data for the same formulation does not exist [13,14]. A visualization technique providing more information about the site of injection may support further investigations. Magnetic resonance imaging (MRI) is one possibility as it contributes many features characterizing the site of application of pharmaceutical dosage forms and their in vivo behavior. It enables the visualization of depots after intramuscular injection in a high resolution at different time points in the same subject without the use of contrast agents [15–17].

It was the goal of the presented study to investigate for the first time whether the combination of MRI based visualization of the temporal development of an intramuscular depot together with the determination of the resulting blood concentration profile provides novel insights in the processes of drug absorption from intramuscular depots. For this purpose intramuscular water based solutions and oily suspensions of the probe drugs paracetamol, diclofenac and prednisolone were prepared and injected into the muscle tissue of the thigh of rats using high resolution (7 T) MRI and simultaneous blood sampling. In this way the impact of the formulation containing the same API's and of different API's characterized by different pKa and lipophilicity in the same formulation on the absorption behavior and the spreading behavior of the depot within the muscle tissue was investigated.

2. Methods

All animal care and experimental procedures of this prospective study were approved by the responsible local authorities (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern) under the protocol number 7221.3-1.1-091/12.

2.1. Animals

Forty-seven female Lewis rats with a weight of 190–300 g were enrolled in this study. Animals were kept under a 12 h light/dark cycle at a constant temperature (22 ± 1 °C) and had unlimited access to water and food.

2.2. Materials

Raw materials were obtained from the following sources and were used as received unless otherwise described: diclofenac sodium (Unique Chemicals, India), paracetamol, prednisolone and sodium hydroxide (all Caesar & Loretz GmbH, Germany), prednisolone-21-hemisuccinate sodium salt (Sigma-Aldrich Chemie, Germany) potassium dihydrogen phosphate, sodium chloride, disodium hydrogen phosphate dihydrate (all Merck KGaA, Germany), medium-chain triglycerides, polyethylene glycol 400 (both Fagron GmbH & Co.KG, Germany), formaline 4.5% (Pharmacy of the University Medicine Greifswald, Germany), isoflurane (CP-Pharma, Switzerland), Oxygen (Air Liquide, Germany). All chemicals and solvents used for chromatography were of analytical grade.

2.3. Study medication

Intramuscular injection was performed using two formulations of three different drugs each; an aqueous solution and an oily suspension (Table 1). They were produced shortly before the administration to exclude problems of instability and aggregation. API's used in this study were diclofenac sodium, paracetamol and prednisolone or its hemisuccinate-sodium salt. They were chosen in order to investigate exemplarily API's with different properties regarding their pK_a and lipophilicity. The dose was chosen with respect to analytical detectability and solubility in the aqueous formulations and is not necessarily clinically relevant. It was adapted for the individual animal based on the body mass while maintaining the same injection volume of 100 μL for each animal.

Table 1
Overview of the different formulations and the drug dose.

Group	Formulation	Drug	Dosage (per kg body mass)
1	Aqueous solution	Paracetamol	6 mg
2		Diclofenac sodium	10 mg/1 mg[a]
3		Prednisolone-21-hemisuccinate-sodium	10 mg calculated on prednisolone
4	Oily suspension	Paracetamol	6 mg
5		Diclofenac sodium	10 mg
6		Prednisolone	10 mg

[a] Three animals received a lower dose of diclofenac in order to investigate if 1 mg/kg body mass diclofenac also leads to a local reaction like it was observed with the higher dose.

For the aqueous solutions of paracetamol and diclofenac a mixture of 9 parts of water for injection and 1 part polyethylene glycol 400 (PEG 400) was used as a solvent. The pH of the solution was measured using a pH meter (Five Easy, Mettler Toledo, Switzerland) and adjusted to pH 7.4 ± 0.2 by the addition of sodium hydroxide and potassium dihydrogen phosphate. Prednisolone-hemisuccinate sodium salt was dissolved in water for injection. Sodium chloride was added to increase the osmotic pressure and disodium hydrogen phosphate dihydrate and potassium dihydrogen phosphate were added for the adjustment of the pH 7.4 ± 0.2. The osmolality of each aqueous solution that was administered was determined using a freezing point depression osmometer (Semi-Micro Osmometer K-7400, Knauer, Germany). In the oily formulations microcrystalline diclofenac sodium, paracetamol and microcrystalline prednisolone were each dispersed in 5 mL of medium-chain triglycerides. While prednisolone and diclofenac were dispersed in the ultrasonic bath (USR18H Merck eurolab N. V., Belgium) for one minute, paracetamol was pretreated two minutes in the ball mill (Narva Vibrator GM 9458, Germany), suspended and placed in the ultrasonic bath for 30 min. The API's were practically insoluble in medium-chain triglycerides. The suspensions were characterized regarding their particle size during the development process using a MasterSizer (MS 20, Malvern Instruments, England). Some selected characteristics of the API's and the resulting formulations are listed in Table 2.

The medium-chain triglycerides and the aqueous solvents including all excipients without the drug were used as placebo formulations. The osmolality of the solvent for the aqueous solution of paracetamol and diclofenac was 410 ± 15 mosmol/kg and the pH 7.46 ± 0.04. The placebo of the prednisolone solution showed a pH of 7.55 ± 0.01 and an osmolality of 180 ± 2 mosmol/kg. All formulations were prepared aseptically under a clean bench (Safe 2020, Thermo Scientific, Germany). The solvents and dispersion agents were filtered through sterilized membranes prior to the addition of the drug. The aqueous solvents were filtered through a polyethersulfone membrane (0.22 μm pore size, MILLEX®GP, MILLIPORE, Ireland) and for the medium-chain triglycerides a polytetrafluorethylene filter (0.2 μm pore size, REZIST 30, Whatman, England) was used.

2.4. Study protocol

A timeline giving an overview of the events during the study is given in Fig. 1.

Prior to any experiment each rat was anesthetized with an isoflurane/oxygen mixture. The animals were then laid onto the coil of the MRI and continuously inhaled the gas mixture. MRI was performed using a 7.1 T animal scanner with a 16-channel rat whole body coil (ClinScan, Bruker, Ettlingen, Germany). MRI sequence techniques were chosen to visualize the aqueous solutions and oily suspensions