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Research paper

Forecasting gastrointestinal precipitation and oral pharmacokinetics of dantrolene in dogs using an *in vitro* precipitation testing coupled with *in silico* modeling and simulation

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ABSTRACT

The aim of the current research was to determine the precipitation kinetics of dantrolene sodium using canine biorelevant *in vitro* testing and to model the precipitation kinetics by appropriately coupling the data with an *in silico* tool adapted for dogs.

The precipitation profiles of dantrolene sodium solutions were obtained with the *in vitro* paddle apparatus at a revolution rate of 50 rpm. The *in silico* prediction tool was designed using STELLA software and the predicted plasma concentration profiles of dantrolene using the *in vitro* precipitation data were compared with the observed *in vivo* pharmacokinetics in beagle dogs. The plasma profiles of dantrolene, which served as a model weakly acidic drug which precipitates in the upper gastrointestinal tract, was successfully predicted using the *in vitro* precipitation testing coupled with the *in silico* modeling and simulation approach. The approach was subsequently used to forecast the effect of pharmaceutical excipients (HPMC/PG) on the ability of the drug to supersaturate in the gut and the resulting pharmacokinetics. The agreement of the simulated pharmacokinetics with the observed values confirms the ability of canine biorelevant media to predict oral performance of enhanced dosage forms in dogs.

1. Introduction

Predicting oral performance of dosage forms is one of the most important steps in pharmaceutical development. Precise prediction methodologies would make it possible for the pharmaceutical industry to develop drugs more efficiently and effectively for the patients. Considering the large percentage of drug candidates which are poorly soluble and/or absorbable in the current pipelines of pharmaceutical companies, the importance of methodologies to predict oral performance of dosage forms containing such candidates cannot be overemphasized [1].

Many studies on predicting oral pharmacokinetics of poorly soluble drugs have been reported to date. Some "bottom-up" simulation models have been commercialized and are now widely implemented [2–4]. Successful combination of *in silico* modeling and simulation with *in vitro* biorelevant dissolution data has been reported [5–8], with an emphasis on predicting oral performance of dosage forms containing various poorly soluble drugs. Furthermore, precipitation, which has been shown to occur in the gastrointestinal tract of humans for dosage forms containing weakly basic and/or amorphous drug substances, has also been modeled recently using data obtained with *in vitro* precipitation testing and has resulted in successful prediction of intestinal concentration of weak bases [9–11].

For salts of weakly acidic drugs i.e. those with high pKa values, precipitation has also been observed during *in vitro* dissolution testing [12] and would potentially also occur in the gastrointestinal fluids in humans. Therefore, appropriate *in silico* modeling in conjunction with *in vitro* precipitation testing may also be a useful approach to predicting the oral performance of (enhanced) formulations of such drugs. An *in vitro* – *in silico* approach has already been reported for dantrolene sodium as a model weak acid drug, in which the marketed capsule formulations were used [13]. In that research, the precipitation kinetics were modeled rather simply by considering the late phase of the *in vitro* dissolution curve from a single initial concentration of the drug. Due to the relatively low fraction of drug which precipitated in the *in vivo* studies, a simple approach using a single initial concentration in each dissolution medium sufficed for the prediction. Further, Petrakis et al. were able to predict the oral pharmacokinetics of another weak

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acid compound using a similar *in vitro* and *in silico* approach [14]. However, both of these studies focused on one formulation for one compound. So the approach needs to be extended if predictions for a wider range of dosage forms with precipitating weak acid drugs are to be successful. In particular, it would be advantageous to be able to estimate the effects of pharmaceutical excipients to promote supersaturation of weak acids in the gastrointestinal tract.

The aims of the current research were therefore to understand the precipitation kinetics of dantrolene sodium with biorelevant *in vitro* testing using a wide range of initial drug concentrations, and to model the precipitation kinetics appropriately coupled with an *in silico* simulation. Further, the effect of using a combination of propylene glycol (PG) with hypromellose (HMPC) on the maintenance of the supersaturation of the drug was predicted using the *in vitro – in silico* approach and compared with the actual plasma profiles observed in dogs.

2. Materials and methods

2.1. Chemicals and reagents

Dantrolene sodium hydrate drug powder (Astellas Pharma Inc, lot 28030S) was used. SIF powder original (FaSSIF/FeSSIF/FaSSGF powder, lot 02-1405-09) and SIF powder FaSSIF-V2 (FaSSIF-V2 powder, lot 02-1311-03) were purchased from Biorelevant.com Ltd. (London, UK). Acetic acid, acetonitrile, ammonium acetate, hydrochloric acid solution (1 N HCl), maleic acid, propylene glycol, sodium chloride, sodium dihydrogen phosphate monohydrate, sodium hydroxide pellets, sodium hydroxide solution (1 N NaOH) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Egg lysolecithin (lot DT14007) was purchased from Kewpie Corporation (Tokyo, Japan). Lecithin (Lipoid E PC S, lot 510800-2140089-04/046) was purchased from Lipoid GmbH (Ludwigshafen, Germany). Pentagastrin (lot SLBL3719V), pepsin (lot SLBJ4999V), sodium oleate (lot SLBM1481V), sodium taurocholate (lot SLBL8177V), and sodium taurodeoxycholate (lot SLBL9929V) were purchased from Sigma Aldrich Co. LLC. (St. Louis, MO, USA). Hypromellose (HPMC; TC5E, Lot 2028037) was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan).

2.2. Methods

2.2.1. In vitro precipitation testing

2.2.1.1. Preparation of dissolution medium. The biorelevant dissolution media, Fasted State Simulated Gastric Fluid (FaSSGF), Fasted State Simulated Intestinal Fluid version 2 (FaSSIF-V2), and Fasted State Simulated Intestinal Fluid for canine (FaSSIFc) were used for *in vitro* precipitation testing. The composition and the preparation methods of the dissolution media have been described previously [15–17].

2.2.1.2. In vitro precipitation testing. A simplified (“dumping”) precipitation test [9] was applied to dantrolene sodium solutions.

Dantrolene sodium solutions for the *in vitro* precipitation testing were prepared by dissolving the drug substance in purified water or in a mixture of propylene glycol (PG)/1% HPMC solution (in a 2:3 v/v ratio). The concentration of dantrolene sodium in both solutions was 1 mg/mL. The dantrolene sodium solutions were added rapidly (“dumped”) into 300 mL of FaSSGF, FaSSIF-V2, or FaSSIFc in the dissolution vessels under the condition of the initial drug concentrations of 5–25 mg/300 mL.

A paddle dissolution apparatus (USP 2, NTR-6400, Toyama Sangyo Co., Ltd., Osaka, Japan) at a revolution rate of 50 rpm was used in this study. The temperature of the media in the vessels was maintained at 37 °C ± 0.5 °C throughout the precipitation testing. Approximately 5 mL samples were withdrawn using a plastic syringe connected with a stainless cannula at the following sampling times: 5, 10, 15, 20, 30, 45, and 60 min after dumping of the dantrolene sodium solutions into the biorelevant media.

Immediately after removal from the dissolution vessel, the samples were filtered using 0.45-μm PVDF (Whatman™ 13 mm GD/X, lot 7013410, GE Healthcare UK Limited, Buckinghamshire, UK). After discarding the first ca. 3 mL, the filtrate was immediately transferred into glass test tubes and mixed with the same volume of acetonitrile, in order to avoid further precipitation of dantrolene before the HPLC analysis.

All the precipitation experiments were conducted in triplicate for each condition.

2.2.1.3. Analytical methods for determining the concentration of dantrolene in the dissolution media. The analytical method used in the current research was slightly modified from the previous method [13]. The samples obtained from the *in vitro* precipitation testing were analyzed quantitatively for the concentrations of dantrolene using the following HPLC system: Alliance Separations Module 2695 with detector of type 2487 (Waters Corporation, Milford, MA). The analytical column was a TSKgel ODS-100Z 5 μm (4.6 mm i.d. × 150 mm, lot T00075) from Tosoh Corporation (Tokyo, Japan). The mobile phase was a mixture of acetonitrile and 100 mM ammonium acetate buffer (40/60) at a flow rate of 1 mL/min. The injection volume was 10 μL and the detecting wavelength was set at 385 nm. The chromatograms obtained in the HPLC analyses were evaluated with Empower 3 (Waters Corporation).

2.2.2. In vivo oral pharmacokinetic study in dogs

2.2.2.1. Oral pharmacokinetic study. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc. Furthermore, Astellas Pharma Inc., Yaizu Pharmaceutical Research Center was awarded Accreditation Status by the AAALAC International.

Four beagle dogs were fasted overnight before oral administration of the dantrolene sodium solutions, the compositions of which are described in Section 2.2.1.2. *Precipitation testing*. pH in the stomach and water intake during the study was controlled as follows. Pentagastrin was injected intramuscularly at the dose of 0.015 mg/kg at 0.5 h before and at 0.5 h and 1.5 h after oral dosing of dantrolene sodium in order to acidify the stomach of the dogs and make it similar to gastric pH conditions in fasted healthy humans [18]. Dogs were withheld access to water from 0.5 h before until 2 h after administration of the dantrolene sodium solution. The dogs were given free access to water thereafter and were fed commercial dog food at the conclusion of the study period (8 h after dosing).

25 mL of one of the solutions containing 25 mg of dantrolene sodium described above was administered via an oral catheter to the stomach of the dog, then the catheter was flushed with a further 25 mL of purified water. The 2.5 mL of blood samples were collected from the forelimb vein with a heparinized syringe before dosing and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h after dosing of the drug solution. Immediately after sample collection, the blood samples were centrifuged at 4 °C (1811 × *g*, 15 min) and the plasma samples obtained were stored frozen at −20 °C or less.

2.2.2.2. Analytical methods for determining the plasma concentration of dantrolene in dogs. The concentration of dantrolene parent drug was determined in the plasma samples as follows. 0.2 mL of the dog plasma sample was mixed with 10 ng of an internal standard (IS in 50% acetonitrile solution, dantrolene $^{13}C_3$, lot I2115, Santa Cruz Biotechnology, Dallas, TX, USA), 1 mL of 100 mM phosphate (Na) buffer (pH 2.1), and 5 mL of diethyl ether in a glass tube.