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Research paper

Cogrinding enhances the oral bioavailability of EMD 57033, a poorly water soluble drug, in dogs

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Abstract

The oral bioavailability of EMD 57033, a calcium sensitizing agent with poor solubility, was compared in dogs using four solid dosage form formulation approaches: a physical blend of the drug with excipients, micronization of the drug, preparation of coground mixtures and spray-drying of the drug from a nanocrystalline suspension. The formulations contained generally accepted excipients such as lactose, hydroxypropylmethyl cellulose and sodium lauryl sulphate in usual quantities. Drug micronization and cogrinding was realized by a jet-milling technique. Nanoparticles were created by media milling using a bead mill. All formulations were administered orally as dry powders in hard gelatine capsules. While micronization increased the absolute bioavailability of the solid drug significantly compared to crude material (from nondetectable to 20%), cogrinding with specific excipients was able to almost double this improvement (up to 39%). With an absolute bioavailability of 26%, spray-dried nanoparticular EMD 57033 failed to show the superior bioavailability that had been anticipated from *in vitro* data. The control solution prepared with cyclodextrin was shown to have an absolute bioavailability of 57% (vs. i.v. infusion). It was concluded that cogrinding can be a useful tool to improve the bioavailability of poorly soluble drugs from a solid dosage form format.

Keywords: Bioavailability; Cogrinding; *In vitro–in vivo* correlation; Jet-milling; Dissolution rate enhancement; Micronization; Particle size reduction; Poorly soluble drugs; Spray-drying

1. Introduction

During the last decade there has been an increasing trend in pharmaceutical research to produce drug candidates that exhibit high lipophilicity and poor water solubility [1]. Such physicochemical characteristics lead to problematic biopharmaceutical properties, which may diminish or even preclude success in the clinic [2].

The aim of the present canine study was to compare the effect of several processing techniques on the bioavailability of a poorly soluble drug in order to identify promising approaches for development of solid oral dosage forms for drugs with problematic dissolution kinetics. EMD 57033, a calcium sensitizing agent [3], was chosen as a typical example of a poorly soluble drug (aqueous solubility 5 μg/ml at 37 °C). The bioavailabilities of a physical mixture of EMD 57033 with lactose, a physical mixture of micronized drug with lactose, a coground mixture with lactose, a coground mixture with hydroxypropylmethyl cellulose as well as a formulation of EMD 57033 with lactose and sodium lauryl sulphate (SLS) prepared by spray-drying from a nanoparticulate drug suspension were compared.

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All formulations were administered orally to dogs as dry powders in hard gelatine capsules. Hydroxypropyl-β-cyclodextrin solutions served as oral and intravenous controls. The *in vivo* results were then compared to *in vitro* dissolution studies of the formulations in biorelevant media [4] using established *in vitro–in vivo* correlation (IVIVC) techniques.

2. Materials and methods

2.1. Chemicals

EMD 57033 (see Fig. 1) was a development candidate from Merck KGaA (Darmstadt, Germany). The drug was synthesized by alkaline hydrolysis of its chemically modified prodrug EMD 82571 (lot 02/HH/31). Lactose monohydrate, HPMC 2910/5 and SLS were from Merck KGaA (Darmstadt, Germany). Sodium taurocholate was obtained from Prodotti Chimici E Alimentari S.P.A. (Basaluzzo, Italy). Egg-phosphatidylcholine, Lipoid E PC, was purchased from Lipoid GmbH (Ludwigshafen, Germany). Hydroxypropyl-β-cyclodextrin was purchased from Sigma–Aldrich (Steinheim, Germany). All other chemicals used were of HPLC grade or analytical grade.

2.2. Preparation of the test formulations

Formulation A was a dry powder mixture and was prepared by physically blending EMD 57033 (10%) with lactose (90%), and then manually filling the blend into Coni-Snap Supro A hard gelatine capsules (Capsugel, Belgium). Formulations B through D were prepared by milling either the drug substance alone or as a physical mixture with specific excipients using an Alpine 50 AS jet-mill (Hosokawa Alpine AG, Germany) operated at 5 bar air pressure and a feed rate of 0.5–1.0 g/min. The milled powder was then manually filled into Coni-Snap Supro A hard gelatine capsules, after blending with lactose, if necessary, to obtain a final drug concentration of 10%. Correspondingly, Formulation B contained jet-milled drug in a physical mixture with 90% lactose. Formulation C comprised a coground mixture of EMD 57033 with lactose in a 10:90 ratio. Formulation D consisted of EMD 57033 coground with hydroxypropylmethyl cellulose in a ratio of 50:50, which was then physically blended with 80% lactose. Homogeneity of the mixtures was investigated by quantitative HPLC determination of the drug content after accurate weighing of aliquots of powder, dissolving and diluting with mobile phase ($n = 3$).

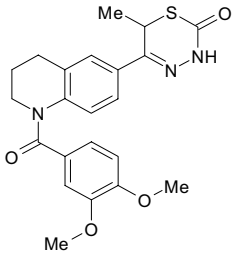

Fig. 1. Chemical structure of EMD 57033 (MW = 425.5).

Formulation E, a nanoparticulate dispersion of EMD 57033, was prepared by a media milling process using a Dyno Mill (Willy A. Bachofen AG Maschinenfabrik, Switzerland) operated in the circulation mode. A 300 ml cylindrical steel vessel with inside coating was filled with 0.1 mm grinding spheres to approximately 85% of the volume. A 600 ml suspension containing 30 g EMD 57033, 30 g lactose and 3 g SLS in water was pre-treated in an Ultra-Turrax at 20,500 min^{-1} before processing in the mill for 90 min. The resulting nanoparticulate suspension was diluted with 300 ml water immediately prior to feeding to the 0.7 mm pneumatic spray nozzle of a Büchi Mini Spray Dryer B-191 (Büchi Labortechnik AG, Switzerland). The mill was kept operating during the spray-drying process in order to maintain homogeneity of the suspension. The spray dryer was operated at 6 bar air pressure, 11 ml/min pump speed, 600 l/h air flow rate, 80% aspirator level and 150 °C inlet temperature.

2.3. Particle size measurement

Particle size was determined by laser light diffraction using a Malvern Mastersizer 2000 (Malvern Instruments, Germany) including a Scirocco 2000 module for dry measurement purposes operating at 3.0 bar air pressure for dispersion. Data were evaluated with Malvern software version 4.0 using the Fraunhofer approximation as the evaluation algorithm [5].

2.4. HPLC analysis

The system consisted of a Merck Hitachi pump L-6200A, a Merck Column Thermostat T-6300 operating at 36 °C, a Merck Hitachi Interface D-6000A, a Merck Hitachi UV–Vis Detector L-4250 and a Merck Hitachi Autosampler AS-4000A. Data acquisition and evaluation was performed with Merck Hitachi D-7000 Chromatography Data Station Software version 4.0. Using a LiChrospher 60 RP select B 125-3 (5 μm) column and a mobile phase consisting of 65% of pure water and 35% of acetonitrile at a flow rate of 1 ml/min, EMD 57033 was eluted at approximately 5 min. The detection wavelength was set at 321 nm.

2.5. Solid state characterization by X-ray diffraction

Powder X-ray patterns were recorded using a Bruker AXS diffractometer (Bruker AXS GmbH, Germany) with a PSD-50M detector and EVA Application Software version 6. Measurements were performed with a Cu Kα radiation source at 40 kV voltage, 30 mA current and a maximum scanning speed of 2°/min.