European Journal of Pharmacology 788 (2016) 98–103

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Endocrine pharmacology

Calcium effects and systemic exposure of vitamin D_3 analogues after topical treatment of active vitamin D_3-containing ointments in rats

Atsushi Hosomi [a,*], Maho Hirabe [a], Takuya Tokuda [a], Hiroaki Nakamura [a], Toru Amano [a], Tadao Okamoto [b]

[a] R&D division, Kyowa Hakko Kirin Co., Ltd., 1188, Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8731, Japan
[b] Scientific Affairs Department, LEO Pharma K.K., 3-11-6, Iwamoto-cho, Chiyoda-ku, Tokyo 101-0032, Japan

ARTICLE INFO

Article history:
Received 12 February 2016
Received in revised form
15 June 2016
Accepted 15 June 2016
Available online 16 June 2016

Keywords:
Vitamin D_3-containing ointment
Systemic exposure
Skin permeation
Hepatic metabolism
Calcemic effect

ABSTRACT

Topical agents containing vitamin D_3 (VD_3) analogues such as calcipotriol, maxacalcitol and tacalcitol and the combination of calcipotriol/betamethasone dipropionate (betamethasone) are prescribed for patients with psoriasis. However, they are known to occasionally cause hypercalcemia, and the frequency of hypercalcemia is suggested to vary according to the VD_3 analogue used. In this study, to address the reason for these differences, the calcemic effects of maxacalcitol-, calcipotriol- and calcipotriol/betamethasone-containing ointments in rats were evaluated. The serum calcium levels in rats treated with ointments containing maxacalcitol, but not calcipotriol or calcipotriol/betamethasone, were significantly elevated, which is consistent with clinical observations. The serum concentration of VD_3 analogue in rats treated with ointments containing calcipotriol and calcipotriol/betamethasone was lower than that in rats treated with maxacalcitol-containing ointment. Thus, the calcemic effects appear to be associated with the systemic exposure of VD_3 analogues in rats. To understand the mechanism underlying the different systemic exposures of VD_3 analogues, skin permeation and metabolic stability of VD_3 analogues were evaluated. The cumulative amount of calcipotriol permeated through rat skin was significantly lower than that of maxacalcitol. On the other hand, the metabolic clearance of calcipotriol in rat hepatocytes was higher than that of maxacalcitol. Similar results were obtained using human skin and human hepatocytes. The current study demonstrates that the lower calcemic effects of calcipotriol- and calcipotriol/betamethasone-containing ointments are caused by the low systemic exposure of calcipotriol according to low skin permeability and rapid hepatic elimination after topical application.

1. Introduction

Topical agents containing active vitamin D_3 (calcitriol, 1α, 25-dihydroxyvitamin D_3, VD_3) analogues such as tacalcitol, calcipotriol and maxacalcitol are widely used for psoriasis therapy. However, topical VD_3 agents are occasionally known to cause hypercalcemia in patients with psoriasis. The efficacy of these agents is considered to be due to the induction of cell differentiation, the inhibition of keratinocyte proliferation and the inhibition of cytokine production in T cells, dendritic cells and keratinocytes (Lovato et al., 2016) through vitamin D receptor, while the action *via* vitamin D receptor is likely to result in an elevation of the serum calcium level through modulating the expression levels of transient receptor potential vanilloid 6, calbindin, osteocalcin, and parathyroid hormone (Barthel et al., 2007; Jaaskelainen et al., 2003; Naveh-Many and Silver, 1993; Saito and Harada, 2014).

Some reports have referred to the side effect of calcitriol analogues in humans. Yamamoto et al. (2012) suggested that calcipotriol is associated with lower serum calcium levels than tacalcitol and maxacalcitol. Moreover, animal studies have been conducted following oral and intraperitoneal administration to evaluate the calcemic potential of several VD_3 analogues and the calcemic mechanisms. However, there are few reports about the calcemic actions/mechanisms after the transdermal application of VD_3 analogues, even though they are typically applied to the skin for psoriasis treatment. Hence, the calcemic potencies after topical application of VD_3 ointments in clinical situations have not yet been adequately investigated.

One reason for the calcemic effect is presumably due to the high systemic exposure of VD_3 analogues through transdermal application (Baroni et al., 2008; Saito and Harada, 2014). In general, the plasma concentration of a transdermal agent is dependent on the balance between the skin permeation profile and the

* Corresponding author.
E-mail address: atsushi.hosomi@kyowa-kirin.co.jp (A. Hosomi).

http://dx.doi.org/10.1016/j.ejphar.2016.06.028
0014-2999/

clearance of systemic circulating drugs (Nakamura et al., 2012). Therefore, *in vitro* studies using skin permeation with human skin and metabolic stability in human hepatocytes of transdermal agent are considered to be useful for predicting systemic exposure in humans.

The present study aimed to clarify the relationship between the systemic exposure of each VD_3 analogue and the following calcemic effect in rats and demonstrate the differences in systemic exposure among VD_3 analogues according to the pharmacokinetic, hepatic metabolism and skin permeation properties.

2. Materials and methods

2.1. Chemicals, tissues and experimental animals

Calcipotriol was purchased from Tocris Bioscience (Bristol, UK). Maxacalcitol was purchased from ChemScene, LLC. (Monmouth Junction, NJ, USA). Simvastatin was purchased from Wako Pure Chemical Industries (Osaka, Japan). Marketed ointment containing either 25 μg/g of maxacalcitol (maxacalcitol ointment), 50 μg/g of calcipotriol (calcipotriol ointment) or 50 μg/g of calcipotriol and 0.643 mg/g of betamethasone dipropionate (calcipotriol/betamethasone ointment) were used in this study. All other reagents were commercially available. Male Hairless Wistar Yagi (HWY)/Slc rats (5–6 weeks of age) were obtained from Japan SLC Inc. (Shizuoka, Japan). Male Sprague Dawley rats (7–9 weeks of age) were obtained from Charles River Laboratories Japan Inc. (Yokohama, Japan). The experimental protocols were reviewed and approved by the Kyowa Hakko Kirin Co., Ltd. Animal Care Committee in accordance with the "Company Policy on the Care and Use of Laboratory Animals".

Rat (Lot. OJK) and human (Lot. HDI) cryopreserved hepatocytes were purchased from Celsis In Vitro Technologies (Baltimore, MD, USA). Excised human skin (female, Caucasian, 34–70 years of age, Lot NO. TRA002001C014-C016, TRA002001C077-C079, TRA002001C083-C084) was obtained from Biopredic International (Saint-Gregoire, France) as frozen skin sets. The experimental protocols were maintained according to the "Company Policy on the Research Ethics Review Committee" by Kyowa Hakko Kirin Co., Ltd.

2.2. In vivo study

For the evaluation of the calcemic effects of VD_3 analogue-containing ointments after topical administration in rats, 200 mg of each ointment was applied on the abdominal skin of HWY/Slc rats anesthetized with isoflurane, followed by occlusion with Finn Chamber discs (18 mm diameter; Bio Diagnostics Ltd.), Tegaderm Transparent Dressing (3 M HealthCare) and bandages. In sham-treated rats, Finn Chamber discs without any ointment was applied on the skin, and then occluded with Tegaderm Transparent Dressing and bandages. Serum samples were collected from the abdominal vein of the rats under isoflurane anesthesia at 1, 2, 6, 10, 24 and 48 h after the application of each ointment and were stored at −20 °C until the measurement of calcipotriol, maxacalcitol and calcium. The serum calcium concentration was measured using the ortho-cresolphthalein complexone method with an automatic analyzer (7180, Hitachi High Technologies).

For the pharmacokinetic study in rats, calcipotriol and maxacalcitol were diluted in N, N-dimethylacetamide, and injected into the femoral vein at 1 mg/kg under isoflurane anesthesia. Plasma samples were obtained from the tail vein at 0.083, 0.25, 0.5, 1, 2, 4, 6 and 8 h after the dosing, and were stored at −20 °C.

The elimination half-life ($t_{1/2}$), area under the plasma concentration-time curve from 0 to infinity ($AUC_{0-\infty}$) and total plasma clearance (CL_{plasma}) were calculated using the Phoenix WinNonlin software program (version 6.1, Pharsight) based on a non-compartmental analysis.

2.3. In vitro metabolism

The metabolic stabilities of calcipotriol and maxacalcitol in rat and human cryopreserved hepatocytes were evaluated. The intrinsic clearance was determined using the substrate depletion approach. The study was performed using the methodologies described in a previous report (Sohlenius-Sternbeck et al., 2010). In brief, hepatocytes were diluted in William's medium E, and these viabilities were determined using the Trypan blue exclusion method. Hepatocytes were seeded into a 24-well plate at a cell density of 1×10^6 cells/ml. After pre-incubation for 15 min in an incubator at 5% CO_2, the metabolism was initiated by adding a substrate to each well. The hepatocytes were incubated with VD_3 analogues for 0, 15, 30, 60 and 90 min, and the reactions were stopped by the addition of ice-cold acetonitrile containing internal standard. The intrinsic metabolic clearance in hepatocytes ($CL_{int,\ H}$) was corrected by an unbound fraction of the test compound in the incubation medium (Kilford et al., 2008).

2.4. In vitro skin permeation

HWY/Slc rats were killed by CO_2 gas. The abdominal skin was carefully excised, and subcutaneous tissue was removed. Human skin was thawed at room temperature and cut into the appropriate size.

Franz-type diffusion cells with an effective diffusion area of 0.785 cm^2 (radius: 0.5 cm) and a receptor cell volume of 5 ml were used. Diffusion cells were filled with receptor fluid (phosphate-buffered saline containing 5 w/v% of bovine serum albumin). The skin was set dermis-side down on the diffusion cell, and the temperature of the receptor fluid was controlled to maintain the test system at 32 °C. Approximately 50 mg of each ointment was applied to the skin surface. Receptor fluid was sampled at 3, 18, 20, 22 and 24 h in HWY/Slc rat skin, and 3, 6, 18, 20, 22 and 24 h in human skin after application. The sampling volume was 2 ml, and 2 ml of fresh receptor fluid was added after each sampling.

2.5. Sample preparation for liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) measurement

The concentrations of calcipotriol and maxacalcitol in the samples were measured by LC-MS/MS. All samples were mixed with ice-cold acetonitrile containing internal standard. After centrifugation, the supernatant was collected and liquid-liquid extraction by ethyl acetate was performed. The extract was evaporated under nitrogen gas at 40 °C and reconstituted with methanol/water/5 mol/l ammonium acetate/1 mol/l ammonium formate (500/500/2/2, vol%) the same as the LC-MS/MS sample.

2.6. LC-MS/MS analysis

LC-MS/MS analyses were performed with the API5000 system (AB SCIEX) coupled with the Agilent 1200 series HPLC system (Agilent Technologies) and HTC PAL autosampler (CTC Analytics). The Analyst software program (version 1.6.1) was used for the calculation of the test compound concentrations in individual samples.

An XBridge C18 column (3.5 μm, 4.6 mm I.D. × 100 mm, Waters) with OPTI-GUARD (C18, Optimize Technologies) was used as the analytical column and the pre-column filter at room temperature, respectively. The mobile phases were A: methanol/water/5 mol/l ammonium acetate (200/800/2, v/v/v) and B: