European Journal of Pharmaceutical Sciences 53 (2014) 28–34

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

# Pharmacokinetic interactions between rebamipide and selected nonsteroidal anti-inflammatory drugs in rats

Dustin L. Cooper [a], Robert C. Wood III [b], Jarrett E. Wyatt [b], Sam Harirforoosh [a,*]

[a] Department of Pharmaceutical Sciences, Gatton College of Pharmacy, East Tennessee State University, Johnson City, TN 37614, United States
[b] Gatton College of Pharmacy, East Tennessee State University, Johnson City, TN 37614, United States

ARTICLE INFO

*Article history:*
Received 3 July 2013
Received in revised form 1 November 2013
Accepted 5 December 2013
Available online 14 December 2013

*Keywords:*
NSAIDs
Rebamipide
Pharmacokinetics
HPLC
Celecoxib
Diclofenac

ABSTRACT

Nonsteroidal anti-inflammatory drugs (NSAIDs) cause gastrointestinal and renal side effects. Rebamipide is a mucoprotective agent that reduces gastrointenstinal side effects when administered concomitantly with NSAIDs. In this study, we investigated the pharmacokinetic drug interactions of rebamipide with two selected NSAIDs, celecoxib or diclofenac. Rats were randomly divided into five groups. Two groups received placebo and three groups were administered rebamipide (30 mg/kg) orally twice daily for two days. On day 3, the animals treated with placebo received celecoxib (40 mg/kg) or diclofenac (10 mg/kg) and rats receiving rebamipide were administerd rebamipide followed by a single dose of placebo, celecoxib, or diclofenac. To investigate drug protein interactions, blank rat plasma was spiked with known concentrations of rebamipide, diclofenac plus rebamipide, or celecoxib plus rebamipide then dialyzed through a Rapid Equilibrium Dialysis device. AUC (139.70 ± 24.97 μg h/mL), $C_{max}$ (42.99 ± 2.98 μg/mL), and $CL_{oral}$ (0.08 ± 0.02 L/h/kg) values of diclofenac in diclofenac plus rebamipide group altered when compared to those of diclofenac treated groups. Treatment with rebamipide showed no significant change in pharmacokinetic parameters of celecoxib treated rats. $C_{max}$ (7.80 ± 1.22 μg/mL), AUC (56.46 ± 7.30 μg h/mL), $V_d/F$ (7.55 ± 1.37 L/kg), and $CL_{oral}$ (0.58 ± 0.09 L/h/kg) of rebamipide were significantly altered when diclofenac was co-administered with rebamipide. Pharmacokinetic parameters of rebamipide plus celecoxib group were not significantly different from those of rebamipide group. Plasma protein binding was not affected by concomitant administration of another drug. These results indicate alteration of pharmacokinetic parameters of both rebamipide and diclofenac when co-administered and cannot be explained by a variation in plasma protein binding.



## 1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase enzymes and block downstream prostaglandin synthesis, effectively blocking key inflammatory pathways (Harirforoosh and Jamali, 2009). NSAIDs have become extensively used in acute or chronic inflammatory diseases such as arthritis and acute gout (Green, 2001). However, this widespread use has facilitated the development of NSAID associated adverse drug reactions that manifest themselves most commonly in the form of gastrointestinal, renal, and cardiovascular side effects (Green, 2001).

The quinolone derivative drug, rebamipide, is commonly used to facilitate gastro-mucosal protection, prevent gastric or duodenal ulcer formation and offset the formation of gastritis (Arakawa et al., 1995). Rebamipide functions by enhancing mucosal defenses such as the induction of prostaglandin synthesis, the scavenging of free radicals and increases in gene expression of the cyclooxygenase-2 enzyme (Tarnawski et al., 2004). It has been discovered that concomitant administration of rebamipide aids in the prevention of NSAID induced mucosal damage and small intestinal injuries (Nagano et al., 2012; Tanigawa et al., 2011). Recently, rebamipide has shown the ability to suppress diclofenac induced intestinal damage and prevent celecoxib based gastric mucosal cell apoptosis; further elucidating the drug's potential effectiveness in NSAID based drug protocols (Diao et al., 2012; Ishihara et al., 2010). The elucidated ability of rebamipide has led to a rise in rebamipide use for patients undergoing prolonged or highly concentrated NSAID protocols (Kim et al., 2007; Mizukami et al., 2011).

It has been shown that some drugs may alter the blood levels of other drugs when taken together. These alterations may change the clinical effect of a drug on the body. In this study, we investigated the potential drug interactions of rebamipide during co-administration with celecoxib or diclofenac, using plasma samples collected from a previous study (Wood et al., 2013).

* Corresponding author. Address: Department of Pharmaceutical Sciences, Gatton College of Pharmacy, East Tennessee State University, Box 70594, Johnson City, TN 37614-1708, United States. Tel.: +1 423 439 2074; fax: +1 423 439 6350.
*E-mail address:* harirfor@etsu.edu (S. Harirforoosh).

0928-0987/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejps.2013.12.002

## 2. Materials and methods

### 2.1. Chemicals

Celecoxib powder was obtained from Toronto Research Chemicals Corporation (North York, ON, Canada). Ibuprofen, ofloxacin, and flufenamic acid was purchased from Sigma–Aldrich (St. Louis, MO, USA). Diclofenac was obtained from MP Biomedicals (Solon, OH, USA). Rebamipide was purchased from Tokyo Chemical Industry, Co., Ltd. (Tokyo, Japan). HPLC grade acetonitrile, water, acetic acid, sulfuric acid and triethylamine were purchased from Fischer Scientific Laboratory (Fair Lawn, NJ, USA).

Methyl cellulose 4000 was purchased from Science Stuff, Inc. (Austin, TX, USA). Calcium carboxymethyl cellulose was purchased from Spectrum Chemical Mfg. Corp. (Gardena, CA, USA). BD Microtainer Tubes was purchased from Becton, Dickinson and Company (Franklin Lakes, NJ, USA). Sodium chloride 0.9% injection was purchased from VWR International, LLC (Suwanee, GA, USA).

### 2.2. Animals and drug administration

All experiments are performed on Male Sprague-Dawley rats (250–280 g) fitted with jugular vein cannula carried out within the guidelines of the Animal Care Committee of East Tennessee State University. The animals were housed in ambient temperature and humidity with a 12 h light-dark cycle. Celecoxib or diclofenac were dissolved in 0.5% methyl cellulose and rebamipide was suspended in 0.5% carboxymethyl cellulose. The doses of rebamipide (30 mg/kg), celecoxib (40 mg/kg), and diclofenac (10 mg/kg) in this study were chosen base on previous studies (Harirforoosh and Jamali, 2005; Suzuki et al., 2008).

### 2.3. The study design

Rats were randomly divided into five groups ($n \geqslant 5$). Two groups were administered placebo, and three groups received rebamipide (30 mg/kg) twice daily for two days. On day 3, rats treated with placebo received a dose of placebo followed by a single dose of celecoxib (40 mg/kg) or diclofenac (10 mg/kg) with a 10 min interval. Rats treated with rebamipide were given a dose of rebamipide followed by a single dose of placebo, celecoxib, or diclofenac. Serial blood samples were collected at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h in BD Microntainer tubes. Blood was replaced by saline solution.

### 2.4. Data treatment and statistical analysis

Comparisons were made between groups by the Student's *t*-test. The non-compartment component of Phoenix® WinNonlin® 6.3 (Pharsight, CA, USA) was used to calculate pharmacokinetic parameters. Statistical significance is set at $p < 0.05$. Data are presented as mean ± standard error of the mean (S.E.M.).

### 2.5. Drug concentration analysis

Drug concentrations were determined by high performance liquid chromotography (HPLC). The HPLC system (Shimadzu, Japan) consisted of LC020AB solvent delivery system, SIL-20A HT auto-sampler with a SPD-M20A photodiode array detector, CBM-20A communication bus, DGU-20A3 vacuum degasser, RF20A fluorescence detector, and CTO-20A column oven.

#### 2.5.1. Celecoxib assay

Celecoxib concentrations were determined by a previously described method (Guirguis et al., 2001). Celecoxib mobile phase was filtered through a 0.45 μm nylon filter before use and consisted of acetonitrile:water:acetic acid:triethylamine (47:53:0.1:0.03). Mobile phase was pumped at a flow rate of 1 mL/min through a C18 analytical column (100 × 4.6 mm, 2.6 μm; Phenomenex, Torrance, CA, USA).

Celecoxib and ibuprofen (internal standard) stock solutions were prepared by dissolving 10 mg of each drug in 100 mL mobile phase (100,000 ng/mL). Stock solution ibuprofen (100 μL) and specific volumes celecoxib stock solution were added to 100 μL blank rat plasma. Calibration curve concentrations of celecoxib solution ranged 25–100,000 ng/mL. To each standard solution was added 0.2 mL of 0.6 M sulfuric acid and 5 mL isooctane-iso-propanol (95:5). Samples were then vortex mixed for 30 s and centrifuged at 2500*g* for 5 min. Supernatant was transferred to new tubes, then evaporated with a CentriVap concentrator (Lab Conoco, Kansas City, MO, USA) set at 50 °C. Sample residue was re-constituted with 200 μL mobile phase solution. After re-constitution, 115 μL of solution was transferred to clean HPLC injection vials. Sample aliquots of 100 μL were injected into the chromatographic system with a UV detector set at 254 nm. The chromatogram was quantified based on the area ratios of celecoxib to ibuprofen. The minimum detectable concentration of celecoxib was 25 ng/mL and the coefficient of variation (CV) was 7.8%.

#### 2.5.2. Diclofenac assay

Diclofenac concentrations were determined by a method described previously (Mohamed et al., 1988). Diclofenac mobile phase consisted of acetonitrile:water:acetic acid (50:50:0.25) and was prepared similarly as celecoxib with a flow rate of 0.75 mL/min through a C18 analytical column (50 × 4.6, 2.6 μm; Shimadzu, Japan) for separation.

Diclofenac stock solution was prepared by dissolving 10 mg raw powder into 200 mL of methanol for a 50,000 ng/mL concentration. Flufenamic acid (internal standard) stock solution was prepared by dissolving 10 mg raw powder into 10 mL of acetonitrile, then diluted 100 fold for a 10 μg/mL concentration. Using methanol, diclofenac stock solution was diluted into titrating concentrations consisting of 50–50,000 ng/mL. Flufenamic acid stock solution (50 μL) and diclofenac (100 μL) were added to blank rat plasma, followed by acetonitrile (2 mL). Solutions were then vortex mixed for 1 min followed by centrifugation for 15 min at 4000 rpm. Organic phase was separated and transferred to clean tubes, then evaporated to dryness with a CentriVap concentrator (Lab Conoco, Kansas City, MO, USA) set at 56 °C. Sample residue was re-constituted with 200 μL mobile phase solution. After re-constitution, 115 μL was transferred to clean HPLC injection vials. Sample aliquots of 100 μL were injected into the chromatographic system with a UV detector set at 280 nm. The chromatogram was quantified based on the height ratios of diclofenac to flufenamic acid. The minimum detectable concentration of diclofenac was 50 ng/mL and the CV was 5.8%.

#### 2.5.3. Rebamipide assay

Rebamipide concentrations were determined according to a method described previously (Jeoung et al., 2004). Rebamipide mobile phase consisted of acetonitrile:water:acetic acid (30:70:5). Separation for Rebamipide was performed using a C18 analytical column (100 × 4.6 mm, 2.6 μm; ACE, Aberdeen, Scotland) with a flow rate of 0.5 mL/min.

Rebamipide stock solution was prepared by dissolving 4 mg of raw powder into 200 mL of methanol for a 20,000 ng/mL concentration. Ofloxacin solution was prepared by dissolving 100 μg raw powder into 200 mL of acetonitrile for a final concentration of 500 ng/mL. Using methanol, rebamipide calibration curves were obtained by diluting solutions ranged 25–20,000 ng/mL. In clean glass tubes, 100 μL of stock solution ofloxacin and 100 μL of