European Journal of Pharmaceutical Sciences 101 (2017) 107–114

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Insight into stereoselective disposition of enantiomers of a potent antithrombotic agent, S002-333 following administration of the racemic compound to mice

Manisha Bhateria [a,b,1], Ramakrishna Rachumallu [a,b,1], Sahithi Yerrabelli [a,c], Anil K. Saxena [d], Rabi Sankar Bhatta [a,b,*]

[a] Pharmacokinetics and Metabolism Division, CSIR-Central Drug Research Institute, Lucknow 226032, Uttar Pradesh, India
[b] Academy of Scientific and Innovative Research, New Delhi 110001, India
[c] Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), Rae Bareli 229010, India
[d] Medicinal and Process Chemistry Division, CSIR-Central Drug Research Institute, Lucknow 226032, Uttar Pradesh, India

ARTICLE INFO

Article history:
Received 26 September 2016
Received in revised form 6 February 2017
Accepted 6 February 2017
Available online 09 February 2017

Keywords:
S002-333
Stereoselective pharmacokinetics
Tissue distribution
Plasma protein binding
In vitro metabolism
Enantiomer-enantiomer interaction

ABSTRACT

S002-333 [2-(4-methoxy-benzenesulfonyl)-2,3,4,9-tetrahydro-1H-b-carboxylic acid amide], a potent antithrombotic agent developed by CSIR-CDRI, is a racemic mixture of two enantiomers (S004-1032 (*R*)-isomer and S007-1558 (*S*)-isomer). Despite extensive research, little is known about the pharmacokinetics of S002-333 enantiomers. Given that mouse is an established model for anti-platelet/antithrombotic activity and inter-species differences exists in the direction of stereoselectivity in pharmacokinetic processes, we investigated the pharmacokinetic disposition of S002-333 enantiomers in mice. Whereas the pharmacokinetics of S002-333 was non-stereoselective after intravenous (i.v.) administration, substantial stereoselectivity was observed after oral administration of the racemate. The oral $AUC_{0-\infty}$ of (*R*)-isomer (1228.21 ± 97.55 h∗ng/mL) was higher than that of (*S*)-isomer (861.55 ± 182.07 h∗ng/mL) whereas it was comparable after i.v. administration. The absolute oral bioavailability of (*R*)-isomer was ~1.7 times higher than that of its antipode. On incubating the racemic mixture or individual isomers with mice liver microsomes, (*S*)-isomer depleted significantly faster than (*R*)-isomer. Thus, low absolute oral bioavailability of (*S*)-isomer in comparison to (*R*)-isomer could be associated to stereoselective hepatic metabolism of (*S*)-isomer. Furthermore, no metabolic interaction between the enantiomers was observed. Tissue distribution analysis revealed that the highest amount of the enantiomers was localized in small intestine and liver which could be due to first pass metabolism in these organs. Stereoselectivity in the distribution of S002-333 was observed in liver, kidney, spleen and brain; however no significant differences between the plasma protein binding of the enantiomers were observed. The information revealed in the present work might prove valuable in deciding the development of S002-333 as racemic mixture and/or single enantiomer.

1. Introduction

Around 127 new chemical entities (NCEs) have been approved by US Food and Drug Administration (USFDA) from January 2010 to December 2014, out of which chiral NCEs constitutes 64% (81 out of 127 NCEs) and single enantiomers were the great majority among the approved chiral NCEs. Nowadays, scientists are encouraged to conduct research on enantiomers in pharmacokinetics, pharmacology and toxicology separately early in drug development phase since it would aid in deciding whether to develop a recemate or an individual enantiomer. Stereoselectivity has been well documented in all the pharmacokinetic processes such as absorption, distribution, metabolism and excretion (Evans et al., 1988) owing to enantioselective interaction with biological macromolecules (Testa, 1988). This necessitates the careful evaluation of these phenomenons in order to understand the stereoselective implications, therapeutic use and toxicokinetics of a racemic compound in clinical applications.

S002-333 [2-(4-methoxy-benzenesulfonyl)-2,3,4,9-tetrahydro-1H-b-carboxylic acid amide] is a novel and potent antithrombotic agent developed by CSIR-Central Drug Research Institute (CSIR-CDRI), India, for the treatment of intravascular thrombosis such as myocardial ischemia and stroke. S002-333 exhibited marked anti-thrombotic activity in

* Corresponding author at: Pharmacokinetics & Metabolism Division, CSIR-Central Drug Research Institute (CSIR-CDRI), B.S. 10/1, Sector 10, Jankipuram Extension, Sitapur Road, Lucknow 226032, India.
E-mail addresses: rabi_bhatta@cdri.res.in, rabi.cdri@gmail.com (R.S. Bhatta).
[1] Authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ejps.2017.02.012
0928-0987/

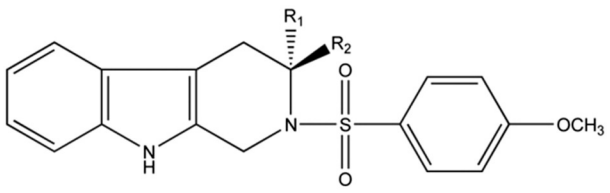
collagen- and epinephrine-induced thrombosis in mice and reduced human platelet aggression (patent granted: WO2006070385 A1). However, S002-333 is defined as the racemic mixture of two enantiomers: S004-1032 (*R*-isomer) and S007-1558 (*S*-isomer) (Bhatta et al., 2011). Stereoselectivity in the Phase I metabolism of S002-333 enantiomers has previously been published where CYP2C19 was found to be the major isoform involved in the metabolism of (*S*)-isomer while CYP3A4 was found responsible for the metabolism of (*R*)-isomer (Saxena et al., 2014). Besides, stereoselectivity in the inhibition of CYP-mediated drug metabolism by S002-333 has recently been documented (Bhateria et al., 2016a). Lately, report demonstrating the pharmacokinetic and tissue distribution behavior of S002-333 in mice has been published but pharmacokinetic and biodistribution of individual enantiomers was not taken into consideration (Bhateria et al., 2016b). However, pharmacokinetic characterization of (*R*)- and (*S*)-isomer after oral administration of racemate in rabbits has been reported earlier where no significant differences in the bioavailability and pharmacokinetic parameters of enantiomers were observed (Saxena et al., 2015). Despite extensive research on S002-333 till date, none of the studies investigated the pharmacokinetics along with tissue distribution of S002-333 enantiomers in mice. Since, it is well acknowledged that mice is an established model for anti-platelet/antithrombotic activity (Kohler et al., 1976) and some drugs exhibit interspecies differences in the direction of stereoselectivity in pharmacokinetic processes (Brocks, 2006), it becomes evident to determine the stereoselective pharmacokinetics and tissue distribution of S002-333 enantiomers in mouse model.

In the present study, we intend to determine the pharmacokinetics of S002-333 enantiomers in mice after oral and i.v. administration of racemate. We further examined whether S002-333 enantiomers exhibit stereoselective distribution in various mouse tissues. An in-depth examination to determine the metabolic behavior and plasma protein binding capacities of the enantiomers was also conducted which might assist in delineating the enantioselective pharmacokinetics and tissue distribution of the racemate in mice.

2. Experimental

2.1. Chemical and reagents

S002-333 (racemic mixture), S004-1032 (*R*-isomer) and S007-1558 (*S*-isomer) (purity > 99%) (Fig. 1) were synthesized and purified at Medicinal Process Chemistry Division of CSIR-CDRI. Fluconazole (purity > 99%) was used as an internal standard (IS) and purchased from Sigma Aldrich (St. Louis, MO, US). HPLC-grade ethyl acetate and *n*-Hexane were procured from Spectrochem Pvt. Ltd. (Mumbai, India). Chromatographic grade acetonitrile was obtained from Merck Chemicals (Darmstadt, Germany). Formic acid was purchased from Sigma-Aldrich (St. Louis, US). Heparin was purchased from Gland Pharma Ltd. (Hyderabad, India). Ultrapure water was prepared in-house by Milli-Q PLUS PF water purifying system (Millipore, MA).



Enantiomers of S002-333	R_1	R_2
S004-1032 (*R*-isomer)	H	$CONH_2$
S007-1558 (*S*-isomer)	$CONH_2$	H

Fig. 1. Chemical structure of the S002-333 enantiomers.

Other reagents were of analytical grade and purchased from standard chemical suppliers.

2.2. Animals

Male *Swiss-albino* mice, weighing 25–30 g were obtained from National Laboratory Animal Division (NLAC), CSIR-CDRI. The animal study was conducted as per the guidelines of Institutional Animal Ethical Committee (IAEC approval no: IAEC/2014/155) at CSIR-CDRI, India. Before commencing the study, animals were housed under standard laboratory conditions with maintained temperature (23–25 °C), humidity (50–70%) and day/night cycle (12/12 h) in ventilated polypropylene cages. A standard chow diet was fed to animals with free access to water *ad libitum*. Drug-free mouse plasma containing heparin as an anticoagulant was collected from adult healthy mice.

2.2.1. Pharmacokinetics and tissue distribution study

Before the drug (S002-333) administration, mice were fasted overnight. Animals were divided into two groups, each consisted of 9 subgroups (n = 5 for each subgroup); first group received an i.v. dose (5.3 μMol/kg body weight) through caudal vein; whereas, the second group received an oral dose (30 μMol/kg body weight) suspended in 0.5% w/v sodium carboxymethylcellulose through oral gavage (Bhateria et al., 2016c). Blood samples were collected *via oculi chorioideae* using sparse sampling technique at 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24 and 48 h after oral administration; and at 0.083, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12 and 24 h after i.v. administration into heparin containing polypropylene tubes under light anesthesia. Animals were divided into nine subgroups such that from each subgroup blood samples were collected for not more than two time points and after the second time point the mouse was sacrificed for tissue collection. Since nine time points (0.5, 1, 3, 4, 6, 10, 12, 24 and 48 h) contributed in the tissue distribution profile of the enantiomers, nine subgroups were made. For each time point, a maximum of 100 μL was collected which was < 10% of the circulating blood volume (~2 mL) thereby precluding any chances of disturbing the normal physiological activity of mouse (Hoff, 2000; Parasuraman et al., 2010). Animals were allowed for free access to food and water 4 h post dosing. Plasma was harvested by centrifuging the blood samples at 4000 × *g* for 10 min after each time point and stored at −80 °C until analysis.

After animal sacrifice at respective time points, organs (brain, lungs, heart, liver, small intestine, kidney and spleen) were removed carefully, washed with normal saline thoroughly to remove blood and other contents and blot dry with filter paper. Tissues were weighed and homogenized in deionized water (1:3, w/v) using tissue homogenizer (IKA, Germany) and stored at −80 °C till analysis.

2.3. Preparation of microsomes from mouse liver

Six untreated mice were sacrificed after 12-h fasting period. Their livers were quickly removed and microsomes (MLMs) were prepared using differential centrifugation method as described elsewhere (Ramakrishna et al., 2016a; Singh et al., 2013). Briefly, livers were perfused with ice-cold normal saline, excised, weighed and homogenized in five volumes of ice-cold buffer consisting of Tris-HCl buffer (50 mM, pH 7.4), KCl (0.15 M), EDTA (1 mM) and sucrose (0.25 M) using Potter-Elvjhem type homogenizer. The homogenate was then centrifuged at 10,000 × *g* for 30 min at 4 °C. The obtained supernatant was further centrifuged at 105,000 × *g* for 60 min at 4 °C and resuspended in microsomal buffer containing Tris-HCl (50 mM, pH 7.4), KCl (0.15 M), EDTA (1 mM), glycerol (20%, v/v). The microsomal suspension was divided into aliquots and stored at −80 °C until use. Lowry method was used for the estimation of protein content in the microsomal fraction (Lowry et al., 1951) using bovine serum albumin (BSA) as calibration standard. Absorbance was measured at 660 nm on multimode microplate reader (Tecan, Switzerland) using Magellan ™ software.