Saudi Pharmaceutical Journal 31 (2023) 101819

HOSTED BY

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Herb-drug interaction: Effect of sinapic acid on the pharmacokinetics of dasatinib in rats

Mudassar Shahid [a], Ajaz Ahmad [b], Mohammad Raish [a,*], Yousef A Bin Jardan [a], Khalid M. Alkharfy [b], Abdul Ahad [a], Mohd Abul Kalam [a], Mushtaq Ahmad Ansari [c], Muzaffer Iqbal [d], Naushad Ali [e], Fahad I. Al-Jenoobi [a]

[a] Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia
[b] Department of Clinical Pharmacy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia
[c] Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia
[d] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia
[e] Quality Assurance Unit, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:
Received 31 August 2023
Accepted 2 October 2023
Available online 5 October 2023

Keywords:
Herb drug interactions
Sinapic acid
Dasatinib
Pgp/MDR1
BCPR/ABCG2
CYP3A2
Pharmacokinetics

ABSTRACT

Dasatinib (DAS) is a narrow therapeutic index drug and novel oral multitarget inhibitor of tyrosine kinase and approved for the first-line therapy for chronic myelogenous leukemia (CML) and Philadelphia chromosome (Ph +) acute lymphoblastic leukemia (ALL). DAS, a known potent substrate of cytochrome (CYP) 3A, P-glycoprotein (Pgp) and breast cancer resistance protein (BCRP) and is subject to auto-induction. The dietary supplementation of sinapic acid (SA) or concomitant use of SA containing herbs/foods may alter the pharmacokinetics as well as pharmacodynamics of DAS, that may probably lead to potential interactions. Protein expression in rat hepatic and intestinal tissues, as well as the *in vivo* pharmacokinetics of DAS and the roles of CYP3 A2 and drug transporters Pgp-MDR1 and BCPR/ABCG2, suggested a likely interaction mechanism. The single dose of DAS (25 mg/kg) was given orally to rats with or without SA pretreatment (20 mg/kg p.o. per day for 7 days, n = 6). The plasma concentration of DAS was estimated by using Ultra-High-Performance Liquid Chromatography Mass spectrometry (UHPLC-MS/MS). The *in vivo* pharmacokinetics and protein expression study demonstrate that SA pretreatment has potential to alter the DAS pharmacokinetics. The increase in C_{max}, AUC and AUMC proposes increase in bioavailability and rate of absorption *via* modulation of CYP3 A2, PgP-MDR1 and BCPR/ABCG2 protein expression. Thus, the concomitant use of SA alone or with DAS may cause serious life-threatening drug interactions.

1. Introduction

Tyrosine kinase (TK), a key regulator of a cell cycle signaling pathways and have been implicated in the regulation of cell growth, cell division, apoptosis, and metabolism in reaction to stimuli (Paul and Mukhopadhyay 2004). TKs are often dysregulated (either overexpression or through somatic mutations) in various cancers (Teo et al., 2015). Tyrosine kinase inhibitors (TKIs) are renowned as targeted cancer therapies for several malignancies and have lower toxicities than traditional chemotherapies (Hartmann et al., 2009). DAS is a novel TKI of the breakpoint cluster region-Abelson gene (BCR-ABL) and Src family, which are proto-oncogenes that play key roles in cell morphology, motility, proliferation, and survival. Unite States food and drug administration (USFDA) permitted the use of the DAS for the treatment of chronic myeloid leukemia (CML) (Bonvin et al., 2008). TKIs are comprehensively metabolized by CYP3A4 in liver and intestine (Rochat et al., 2008). TKIs are also widely influenced by the drug transporters P-glycoprotein (P-gp; coded by ABCB1) and Breast Cancer Resistance Protein (BCRP; ABCG2) (Chen et al., 2009, van Erp et al., 2009, Haouala et al., 2011). DAS broadly undergoes CYP3A4-facilitated metabolism (Kamath et al., 2008, Keam 2008, Squibb 2017). The previous studies have demonstrated that DAS, a known potent substrate for P-gp-MDR1 and BCPR/ABCG2 (Chen et al., 2009). DAS a narrow therapeutic index drug is linked with fatal toxicities such

* Corresponding author.
E-mail address: mraish@ksu.edu.sa (M. Raish).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2023.101819
1319-0164/

as QT prolongation, heart failure, hypertension, peripheral arterial occlusive disease (PAOD), thrombosis, hyperglycemia, hypocalcemia, myelosuppression, cytopenias, hyperlipidemia, pneumonitis, pulmonary hypertension, and pleural effusion (Herviou et al., 2016, Steegmann et al., 2016).

Sinapic acid (SA), a phytoconstituent belongs to the Brassicaceae family with good oral bioavailability, present in cereals, spices, fruits, and vegetables and poses no risk of harm to human health (Chen 2016). The widespread use of SA in traditional and modern herbal medicine owing to its phytotherapeutic properties such as chemo-preventive, antioxidant, anti-mutagenic, anti-hypertensive, anti-aging, anti-inflammatory, anti-hyperglycemic, anti-hyperlipidemic, antibacterial, hepatoprotective, anti-depressant, gastroprotective and neuroprotective activities (Kikuzaki et al., 2002, Zou et al., 2002, Yoon et al., 2007, Chen 2016). DAS is a substrate of CYP3A, P-gp and BCRP and is subject to auto-induction. Our previous studies have demonstrated that SA exhibits inhibitory effects on cytochrome P450 enzymes, specifically CYP3A2 and CYP2C11, as well as P-glycoprotein in liver and intestine. These findings were observed in the context of other drugs, including carbamazepine and aripiprazole, which share metabolic pathways and transport mechanisms with DAS (Raish et al., 2019, Raish et al., 2019). Therefore, it is necessary to scrutinize the safety concerns of such concurrent usage with SA (Kamath et al., 2008, van Erp et al., 2009, Abdelgalil et al., 2019, Raish et al., 2019, Raish et al., 2019, Raish et al., 2023). DAS interactions may take place due to concomitant use of SA/herb containing SA may lead to alter the pharmacokinetics (PK) and pharmacodynamics (PD) of DAS. Thus, the primary objective of this study is to comprehensively evaluate the PK interaction between DAS and SA in animal models. Specifically, we aim to investigate the alterations in the PK profile of DAS when administered concomitantly with SA. To achieve this objective, we will assess the mRNA expression levels of key drug-metabolizing enzymes, namely cytochrome P450 3A2 (CYP3A2), as well as efflux transporters such as P-gp and BCRP, in both hepatic and intestinal tissues. The rationale for this objective is rooted in the understanding that DAS undergoes significant metabolism in the liver and intestine, with these tissues playing a pivotal role in its biotransformation. As approximately 95 % of DAS metabolism occurs within the liver and intestine, it is crucial to investigate how co-administration with SA may modulate the expression of key enzymes and transporters involved in its disposition. This research will provide valuable insights into the potential pharmacokinetic interactions between DAS and SA, contributing to a deeper understanding of the safety and efficacy of concurrent use.

2. Material and methods

2.1. Materials

DAS, SA, and Ibrutinib (IBR), were procured from Sigma-Aldrich (St. Louis MO, USA). Acetonitrile, ammonium acetate formic acid and methanol (HPLC grade) were acquired from BDH (Poole, UK) and antibodies Anti-CYP3 A2 (Santa Cruz Biotechnology, Inc. Texas USA; sc-271033), anti-P-glycoprotein/MDR1/ABCB1 (Santa Cruz Biotechnology, Inc. Texas USA; sc-390883), Anti-BCRP/ABCG2 ((Santa Cruz Biotechnology, Inc. Texas USA; sc-58222) and anti-β-actin (Santa Cruz Biotechnology, Inc. Texas USA; LS-C147034).

2.2. Animals

In vivo PK studies were carried out in Wistar rats (204 ± 7 gm). The rats were acquired from Central Animal House Facility of King Saud University and were kept in plastic cages with 12 h' light and dark cycle at 25 ± 2 °C as per animal facility guidelines. The rats were fed on standard rat chow and provided water ad libitum. The dose of SA and DAS were selected based on our preliminary and published data (Raish et al., 2019, Raish et al., 2023). The study protocol was approved by the King Saud University Research Ethics Committee (KSU-SE-21––58).

2.3. Pharmacokinetic studies

Animals were divided in to four groups (n = 6) in each group. The study was carried out on overnight fasted rats. The animals were treated by means of using the intragastric gavage technique and were grouped as: Group 1 served as vehicle control was treated with normal saline orally for seven days. Animals in group II was given normal saline orally for six days, followed by a DAS solution (25 mg/kg; p.o) on day seven. Animals in group III were administered DAS (25 mg/kg p.o) on day seven, two hours after SA 40 mg/kg was administered, and animals in group IV were administered SA 40 mg/kg for seven days. Food and water were allowed to ad libitum and to all groups before the study. After the administration of DAS, blood sample (0.5 mL) was collected in heparinized tubes at different time point intervals (0, 0.5, 1, 1.5, 2, 3, 4, 6, and 12 h). The plasma was separated by centrifuge the blood for 10 min at 3000 × g. The plasma was then transferred to 1.5 mL tubes for ultra-high-performance liquid chromatography mass spectrometry (UHPLC-MS/MS) analysis. After decapitating the rats, liver and intestine tissue samples were harvested for protein estimation followed by western blotting.

2.4. Mass spectrometry and UPLC chromatographic conditions

DAS was quantified in rat plasma samples by UHPLC-MS/MS. DAS and IBR (used as internal standard; IS) were separated on Acquity BEH C_{18} column (100 × 2.1 mm; 1.7 µm). Both DAS and IS were chromatographically eluted by composition of acetonitrile (with 0.1 % formic acid) and 20 mM ammonium acetate in ratio of 95:5 (*v/v*). The flow rate of mobile phase was 0.25 mLmin-1 and the total run time of the analysis was 2.5 min. The column oven temperature was fixed to 40 °C whereas the temperature of autosampler was 10 °C. The sample ionization of the molecules was performed by electrospray ionization (ESI) method which was operated in positive ionization mode. The calibration curves were achieved for concentrations between 5 ng/mL and 2500 ng/mL. The parent to daughter ion transition of 488.06 > 401.1 and 441.16 > 84.04 were used for quantification of analyte (DA) and IS, respectively in multiple reaction monitoring mode. The optimized mass spectrometry parameters were; capillary voltage (3.4 kV), source temperature (150 °C), desolvation temperature (350 °C) were used for sample ionization. The nitrogen gas was used for desolvation gas flow at the rate of 600 Lh^{-1} whereas sample collision was performed by using argon gas at flow of 0.18 mLmin-1. A cone voltage of 46 V and 48 V and collision energy of 28 eV and 40 eV were used as compound specific parameters for analyte and IS, respectively. Before actual sample analysis, the method was partially validated in terms of precision and accuracy parameters by following international guideline for bioanalytical method validation (FDA 2018, Meesters and Voswinkel 2018). The samples were acquired using Masslynx software version 4.1 SCN 805, and then processed using TargetLynks. The method has been used after some modification of previously reported study (Thappali et al., 2012, Ezzeldin et al., 2020).

2.5. Sample preparation

For sample preparation, an aliquot of 0.15 mL plasma was transferred into 2 mL of Eppendorf tube. The 20 µL of IS (250 ng/mL)

were spiked into each tube followed by vortex-mixing to all tube adequately. Then 1 mL of ethyl acetate (used as extracting agent) was transferred into each tube for liquid based partition extraction. The samples were vortex-mixed gently for 1 min and were loaded to shaker for 15 min. All samples were put on cold centrifugation (4 °C) at 10,500 rpm for 10 min. After that, 0.8 mL of upper organic layer was transferred to 1.5 mL capacity of Eppendorf tube and transferred to for sample drying in sample concentrator by maintaining moderate temperature. The dried residues were reconstituted with 0.15 mL of acetonitrile for UHPLC-MS/MS analysis.

2.6. Pharmacokinetic analysis

By utilizing a non-compartmental analysis model, PK Solver software (version 1.0) was used to calculate PK parameters. The calculated parameters were as follows: half-life ($T_{1/2}$), elimination rate constant (Kel), time to maximum concentration (T max), maximum concentration (C max), area under the moment curve (AUMC); area under the concentration–time curve (AUC), the volume of distribution (Vd), clearance (CL) and the mean residence time (MRT) were determined.

2.7. Protein expression analysis

The total cytosolic protein concentrations were examined in hepatic and intestinal tissues by using a Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, USA) (Smith et al., 1985). Western blots were carried as per the procedure of Towbin et al., 1979 (Towbin et al., 1979). Briefly, 25 g of protein were electrophoresed on PAGE with 10 % SDS, transferred to activated PVDF membranes, and blocked in a blocking solution. The membrane was then incubated overnight (4 °C) with antibodies CYP3A2, P-gp/MDR1, BCRP/ABCG2, and β-actin for hepatic and intestinal protein expression. The membrane was incubated with the appropriate secondary antibodies for 2 h after being repeatedly washed four times with 1 % Tween TBS and TBS for five minutes. Bands were visualized using Luminata™ Western Chemiluminescent HRP Substrates (Millipore, Billerica, MA, United States), followed by densitometric analysis of the immunoblots.

2.8. Statistical analysis

All results are presented as the average ± SD. Data were analyzed using a one-way analysis of variance followed by Dennett's test by using Graph Pad Prism V8.

3. Results

3.1. Pharmacokinetic interaction

PK analysis and concentration of plasma versus time plots of DAS (25 mg/kg) and SA (40 mg/kg) after oral administration in rats were analyzed. The alteration in PK parameters of single dose of DAS 25 mg/kg for 12 h were evaluated with or without SA pretreatment. Change of PK parameters were calculated by using non-compartmental model and results are summarized in Table 1. The mean plasma concentration–time curves after oral administration of DAS with SA in rats are shown in Fig. 1. The plasma concentration of DAS was enhanced several folds after SA pretreatment than vehicle control. The mean plasma concentration–time profiles for DAS with or without SA pretreatment demonstrate that co-administration of SA markedly increase the plasma DAS levels (Fig. 1). $T_{1/2}$, 28.82 % ($p < 0.05$), Tmax 100 % ($p < 0.05$), Cmax 62.01 % ($p < 0.05$), AUC 28.98 % ($p < 0.05$), AUMC 53.62 % ($p < 0.05$) and MRT 12.08 % ($p < 0.05$), respectively, were increased significantly as compared to DAS alone treated animals. Kel 22.32 % ($p < 0.05$), Vd 5.96 % ($p < 0.05$) and Cl 26.97 % ($p < 0.05$) decreased significantly as compared to DAS alone. Increased systemic bioavailability of DAS in plasma was observed in SA 40 mg/kg pretreated rats as measured by a decrease in Kel, Vd, and Cl and an increase in $T_{1/2}$, Cmax, $AUC_{0\text{-}t}$, $AUMC_{0\text{-}inf}$, and MRT. This was attributable to a significant inhibition of CYP3A2, Pgp/MDR1, and BCPR/ABCG2 mediated metabolism of DAS in the liver and intestines. The PK parameters of TKIs depends on the function of Pgp/MDR1, BCPR/ABCG2 transporters as well as the CYP3A metabolizing enzymes (van Leeuwen et al., 2014, Herbrink et al., 2015).

3.2. Effect of SA on hepatic and intestinal CYP3A2 protein expression

As shown in Fig. 2 (A, D), the hepatic and intestinal CYP3A2 ($p < 0.05$) protein expression was significantly increased 92.03 % and 92.97 % change in DAS administered rats as compared to normal rats. Chronic administration of SA (40 mg/kg b. w.) pretreatment for seven days to DAS administered rats significantly inhibits 57.11 % and 47.15 % hepatic and intestinal CYP3A2 protein expression as compared to DAS administered rats. SA (40 mg/kg) alone pretreatment led to the inhibition of 88.91 % and 89.47 % of hepatic and intestinal CYP3A2 protein expression as compared to normal rats.

3.3. Effect of SA on hepatic and intestinal Pgp/MDR1 protein expression

The inhibitory potential of SA on Pgp/MDR1 protein expression was investigated and shown in Fig. 2 (B, E). Hepatic and intestinal

Table 1
Non-compartmental pharmacokinetic parameters of dasatinib and dasatinib along with sinapic acid following an oral administration in rats.

PK Parameter (Unit)	DAS (25 mg/kg)	SA 40 mg/kg + DAS (25 mg/kg)	% Change
	Mean ± SD	Mean ± SD	
Kel (1/h)	0.16 ± 0.00	0.12 ± 0.00*	22.33
T1/2 (h)	4.34 ± 0.11	5.59 ± 0.20*	28.82
Tmax (h)	1.00 ± 0.00	2.00 ± 0.00*	100.00
Cmax (ng/mL)	258.77 ± 9.71	419.25 ± 18.81*	62.02
AUC 0-t (ng/mL*h)	1716.24 ± 70.62	2213.64 ± 96.29*	28.98
AUMC 0-_{∞} (ng/mL*h^2)	15341.83 ± 607.98	23569.00 ± 1418.58*	53.63
MRT 0-inf_obs (h)	7.31 ± 0.17	8.20 ± 0.25*	12.08
Vd (mg/kg)/(ng/mL)	0.075 ± 0.003	0.070 ± 0.004*	5.96
Cl (mg/kg)/(ng/mL)/h	0.012 ± 0.001	0.009 ± 0.00*	26.97

All results are presented as the average ± SD. *$p < 0.05$ (DAS).

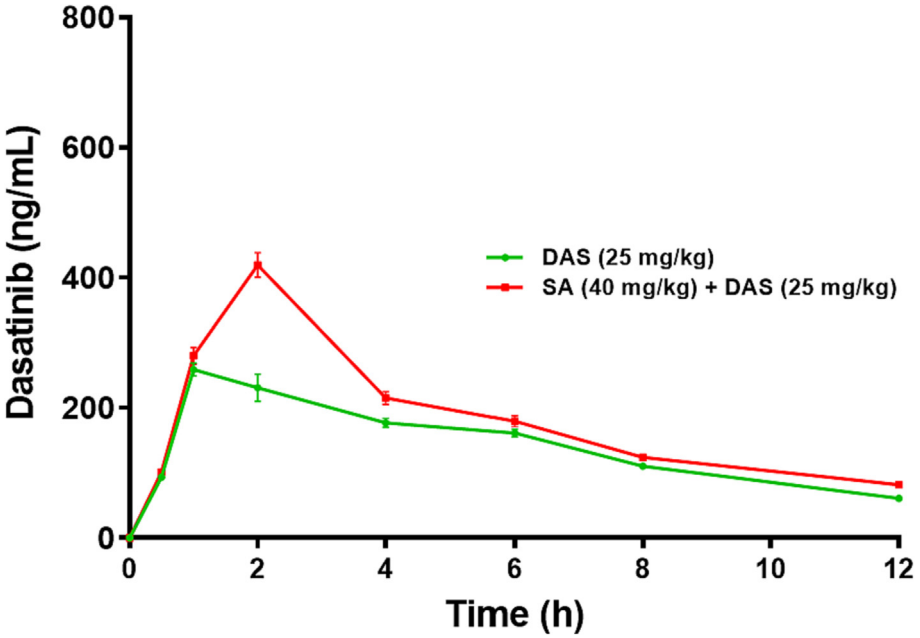

Fig. 1. A representative plasma concentration–time curve of DAS and DAS along with SA (40 mg/kg p.o. for seven days) following an oral administration in rats ($p < 0.05$).

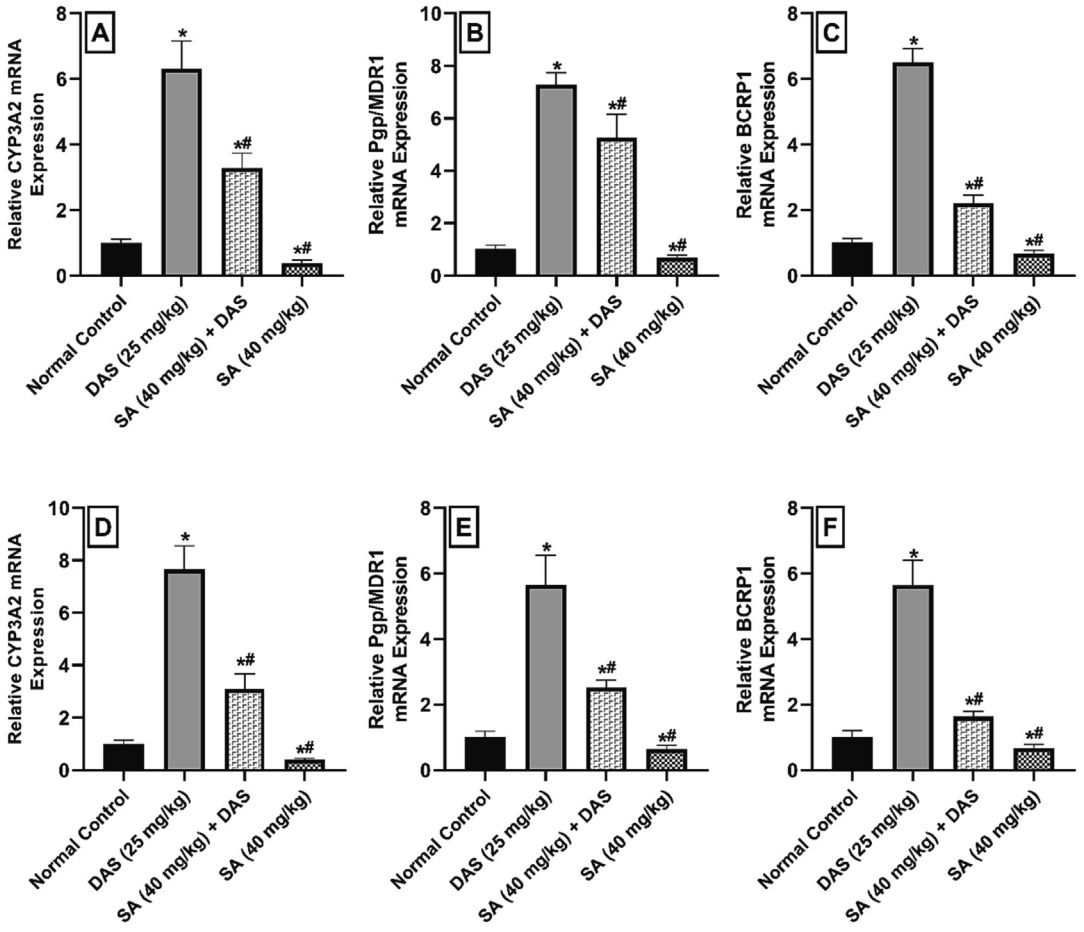

Fig. 2. Hepatic CYP3A2 (A), Pgp glycoprotein/MDR1 (B), and BCRP/ABCG2 (C); Intestinal CYP3A2 (D), Pgp glycoprotein/MDR1 (E), and BCRP/ABCG2 (F) mRNA expression in rats after DAS administration with or without sinapic acid (SA) pretreatment. All results are presented as the average ± SD. *$p < 0.05$ (Normal control); #$p < 0.05$ (DAS).

Pgp/MDR1 protein expressions were significantly decreased after SA pretreatment as compared to the normal control group. DAS administration caused significant induction of 72.30 % and 76.42 %, respectively ($p < 0.05$) in hepatic and intestinal protein expression of Pgp/MDR1 compared to that in normal rats. The SA-pretreated rats showed significant inhibition of the Pgp/MDR1 in hepatic and intestinal protein expression that is (44.06 % and 47.43 % inhibition, respectively) as compared to DAS alone ($p < 0.05$). SA (40 mg/kg) alone pretreatment led to the inhibition of 84.93 % and 76.93 % of hepatic and intestinal Pgp/MDR1 protein expression as compared to normal rats.

3.4. Effect of SA on hepatic and intestinal BCPR/ABCG2 protein expression

The inhibitory potential of SA on BCPR/ABCG2 protein expression was investigated and shown in Fig. 2 (C, F). hepatic and intestinal BCPR/ABCG2 protein expression was significantly inhibited after SA pretreatment compared to that in normal rats. DAS per-oral administration caused significant induction of (75.09 % and 73.55 %, respectively) ($p < 0.05$) hepatic and intestinal protein expression of BCPR/ABCG2 compared to that in normal rats. The SA-pretreated rats showed significant inhibition of the BCPR/ABCG2 protein that is (51.44 % and 50.48 % inhibition, respectively) as compared to DAS alone ($p < 0.05$). SA (40 mg/kg) alone pretreatment led to the inhibition of 80.99 % and 83.20 % of hepatic and intestinal Pgp/MDR1 protein expression as compared to normal rats.

4. Discussion

The UPLC MS/MS technique was used to analyze the DAS. Specificity and sensitivity of the analytes were optimized by modifying the spectroscopic conditions. Internal standard ibrutinib and DAS were separated on an Acquity BEH C_{18} column using a mobile phase ratio of (95:5) acetonitrile, 0.1 % formic acid, and 20 mM ammonium acetate at a flow rate of 0.25 mL/min. The concentration ranges for calibration curves of DAS between 5 and 2500 $ngmL^{-1}$ were found to be linear throughout the analysis. Quantification of DAS and IS (ibrutinib) in MRM mode involved using a precursor to product ion transition of 488.06 and gt; 401.1 and 441.16 and gt; 84.04, respectively. Variation in precision and accuracy was measured at the end of each day and found to be within the allowable range of 15 %. It is estimated that between ~60 percent of the people in the United States who have a chronic illness also take a dietary supplements, and another ~25 percent of those who take prescription medicines also take a dietary supplements (Gardiner et al., 2008). Patients takes dietary supplements with prescribed medicines having narrow therapeutic index may result in a potential drug interaction (DI). The parallel use of dietary supplements containing pharmacologically active ingredients may mimic, heighten, or diminish the pharmacological effect of medicines (Fugh-Berman and Ernst 2001). Several potential herb/drug interactions (HDIs) have been reported with DAS (Durmus et al., 2015, Fleisher et al., 2015, Abdelgalil et al., 2019, Alzoman et al., 2019).

DAS broadly undergoes CYP3A4 facilitated biotransformation in liver to form main metabolites as M4, M5, M6, M20 and M24 (Christopher et al., 2008, Wang et al., 2008, Di Gion et al., 2011). The main metabolite M4 is biotransformation by N-dealkylation with CYP3A4, the predominant catalyzing enzyme responsible hydroxylation to M20 and M24 (Wang et al., 2008, Di Gion et al., 2011). These metabolites are transported out from body via drug transporters such as Pgp/MDRI/ABCB1 and BCRP/ABCG2 with the contribution of other enzymes to a partial extent (Wang et al., 2008, Di Gion et al., 2011). Systemic bioavailability of DAS is affected by various factors including intestinal metabolism, enterocytic efflux and uptake transport, gastrointestinal residence time, hepatic metabolism and secretion. The dissolution of DAS is however the first step allowing the intestinal absorption and is dependent on gastric pH. Acidic pH may increase intestinal absorption of DAS and vice versa (Eley et al., 2009). The parallel usage of DAS along with food supplement/herbs may lead to potential DAS drug/herb interactions (DDHIs).

SA, a nutraceutical with good oral bioavailability is easily available in foods with a long history of human use and poses little risk of harm (Chen 2016). SA is used in various therapeutic conditions in traditional and modern herbal medicine owing to its medicinal properties (Kikuzaki et al., 2002, Zou et al., 2002, Yoon et al., 2007, Chen 2016). Previously our group has reported that SA 40 mg/kg modulates CYP3A2 and enhances the bioavailability of carbamazepine and aripiprazole PK in rats and modulate Pgp/MDR1 in liver and intestine of rats (Raish et al., 2019). Pharmacokinetic interactions may occur when DAS and SA are used concomitantly, therefore, it is obligatory to investigate the safety apprehensions of such simultaneous or parallel usage. The alteration in PK parameters of DAS 25 mg/kg were evaluated with or without SA pretreatment. The plasma concentration of DAS was increased several folds after SA pretreatment as compared to normal control animals. The mean plasma concentration–time profiles of DAS with or without SA pretreatment demonstrated that co-administration of SA markedly enhanced systemic bioavailability of DAS as indicated by increase in $T_{1/2}$, Tmax, Cmax, $AUC_{0\text{-}t}$, $AUMC_{0\text{-}inf}$, and MRT. This change in PK parameters may be due to the significant inhibition of CYP3 A2, Pgp/MDR1 and BCPR/ABCG2 mediated metabolism of DAS in the liver and intestine, thereby increase in rate of absorption. This enhanced rate of absorption was indicated by sharp increase in Tmax (100 %) from 1 h to 2 h as compared to DAS alone group. The PK parameters obtained from our study are similar to previous reports where pretreatment of St. John's wort, fruit juices and moderate/strong CYP3A inhibitors have found to increase in Cmax, AUC and AUMC and diminish the Vd, and Cl thereby increasing bioavailability (Kamath et al., 2008, Haouala et al., 2011, Fleisher et al., 2015, Squibb 2017, Abdelgalil et al., 2019). There are several research studies reported that showed DAS enhances the exposure of CYP3A4 substrates of simvastatin and midazolam, respectively, in humans and cyclosporine in rats (Chen et al., 2009, Haouala et al., 2011, Abdelgalil et al., 2019). The increased intestinal absorption of DAS may be due to decrease in pH and inhibition of drug transporters Pgp/MDR11, BCPR/ABCG2, which further corroborate with our results (Horinkova et al., 2019).

The western blot experiment performed in liver and intestinal tissues were performed to observe the protein expression behavior of CYP3 A2, Pgp-MDR1 and BCPR/ABCG2. Cytochrome enzymes (CYP-P450-CYP3A2) and drug transporters (P-gp/MDR1 and BCRP/ABCG2) are inducible genes, involved in xenobiotic metabolism, expression and, their substrates induce the expression of these inducible genes (Porter and Coon 1991, Breedveld et al., 2006, Jigorel et al., 2006, Olinga et al., 2008). The protein expression results demonstrate the upregulation of CYP3 A2, Pgp-MDR1 and BCPR/ABCG2 in DAS treated animals as compared to normal control. DAS is substrate for CYP3 A2, PGP-MDR1 and BCPR/ABCG2 is responsible for inducing the expression of these enzymes and drug transporters. The upregulation of protein expression to several folds after a single dose of DAS indicating auto induction of CYP3 A2, Pgp-MDR1 and BCPR/ABCG2 as compared to normal

control in hepatic and intestinal tissues. Conversely, SA alone group has less inhibitory effect on these enzymes and drug transporters. The SA pretreatment in animals along with the DAS significantly inhibits this upregulated protein expression, suggest that SA is an inhibitor of CYP3 A2, Pgp-MDR1 and BCPR/ABCG2 protein expression in the liver and in the intestine of rats. The results of the study also demonstrate that SA has ability to modulate the PK/PD of DAS. The present results further corroborate PK investigation, where DAS concentration augmented by interactions with CYP3 A, Pgp and BCPR/ABCG2 inhibitors (Dutreix et al., 2004, Johnson et al., 2010, Fleisher et al., 2015, Abdelgalil et al., 2019, Alzoman et al., 2019). Oral bioactive polyphenol SA widely consumed as food, food supplements and herbal medicine has ability to modulate CYP3 A2, Pgp-MDR1 and BCPR/ABCG2 protein expression thereby increasing DAS absorption (Chen 2016).

5. Conclusion

The PK interaction of SA with DAS in rat plasma following single and co-oral dosing was successfully studied using UHPLC–MS/MS method. SA may change the PK profile of DAS in rats, potentially resulting in DAS and SA interaction thereby increasing bioavailability and rate of absorption enhanced via modulation of CYP3A2, Pgp-MDR1 and BCPR/ABCG2 protein expression. The concomitant consumption of SA containing food or traditional herb with DAS may cause serious life-threatening drug interactions. Further studies are required to determine the clinical relevance of these observation.

6. Institutional review board statement

The research was approved by the Research Ethics Committee of King Saud University College of Pharmacy Riyadh, Saudi Arabia (KSU-SE-21–58).

CRediT authorship contribution statement

Mudassar Shahid: Data curation, Formal analysis, Methodology. **Ajaz Ahmad:** Conceptualization, Investigation, Methodology, Project administration, Resources, Software, Writing – original draft, Writing – review & editing. **Mohammad Raish:** Conceptualization, Investigation, Methodology, Project administration, Resources, Software, Writing – original draft, Writing – review & editing. **Yousef A Bin Jardan:** Methodology, Resources. **Khalid M. Alkharfy:** Writing – review & editing. **Abdul Ahad:** Data curation, Formal analysis. **Mohd Abul Kalam:** Methodology. **Mushtaq Ahmad Ansari:** Formal analysis, Methodology. **Muzaffer Iqbal:** Formal analysis, Methodology, Writing – review & editing. **Naushad Ali:** Data curation. **Fahad I. Al-Jenoobi:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project no. (IFKSUOR3-550).

References

Abdelgalil, A.A., Alam, M.A., Raish, M., et al., 2019. Dasatinib significantly reduced in vivo exposure to cyclosporine in a rat model: The possible involvement of CYP3A induction. Pharmacol. Rep. 71, 201–205. https://doi.org/10.1016/j.pharep.2018.10.018.

Alzoman, N.Z., Maher, H.M., Shehata, S.M., et al., 2019. UPLC-MS/MS study of the effect of dandelion root extract on the plasma levels of the selected irreversible tyrosine kinase inhibitors dasatinib, imatinib and nilotinib in rats: Potential risk of pharmacokinetic interactions. Biomed. Chromatogr. 33, e4674.

Bonvin, A., Mesnil, A., Nicolini, F.E., et al., 2008. Dasatinib-induced acute hepatitis. Leuk. Lymphoma 49, 1630–1632. https://doi.org/10.1080/10428190802136384.

Breedveld, P., Beijnen, J.H., Schellens, J.H., 2006. Use of P-glycoprotein and BCRP inhibitors to improve oral bioavailability and CNS penetration of anticancer drugs. Trends Pharmacol. Sci. 27, 17–24. https://doi.org/10.1016/j.tips.2005.11.009.

Chen, C., 2016. Sinapic acid and its derivatives as medicine in oxidative stress-induced diseases and aging. Oxid. Med. Cell. Longev. 2016, 3571614. https://doi.org/10.1155/2016/3571614.

Chen, Y., Agarwal, S., Shaik, N.M., et al., 2009. P-glycoprotein and breast cancer resistance protein influence brain distribution of dasatinib. J. Pharmacol. Exp. Ther. 330, 956–963. https://doi.org/10.1124/jpet.109.154781.

Christopher, L.J., Cui, D., Wu, C., et al., 2008. Metabolism and disposition of dasatinib after oral administration to humans. Drug Metab. Dispos. 36, 1357–1364. https://doi.org/10.1124/dmd.107.018267.

Di Gion, P., Kanefendt, F., Lindauer, A., et al., 2011. Clinical pharmacokinetics of tyrosine kinase inhibitors: focus on pyrimidines, pyridines and pyrroles. Clin. Pharmacokinet. 50, 551–603. https://doi.org/10.2165/11593320-000000000-00000.

Durmus, S., Hendrikx, J.J., Schinkel, A.H., 2015. Apical ABC transporters and cancer chemotherapeutic drug disposition. Adv. Cancer Res. 125, 1–41.

Dutreix, C., Peng, B., Mehring, G., et al., 2004. Pharmacokinetic interaction between ketoconazole and imatinib mesylate (Glivec) in healthy subjects. Cancer Chemother. Pharmacol. 54, 290–294. https://doi.org/10.1007/s00280-004-0832-z.

Eley, T., Luo, F.R., Agrawal, S., et al., 2009. Phase I study of the effect of gastric acid pH modulators on the bioavailability of oral dasatinib in healthy subjects. J. Clin. Pharmacol. 49, 700–709. https://doi.org/10.1177/0091270009333854.

Ezzeldin, E., Iqbal, M., Herqash, R.N., et al., 2020. Simultaneous quantitative determination of seven novel tyrosine kinase inhibitors in plasma by a validated UPLC-MS/MS method and its application to human microsomal metabolic stability study. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1136,. https://doi.org/10.1016/j.jchromb.2019.121851 121851.

FDA, 2018. Guidance for industry: bioanalytical method validation. Food Drug Administration U. FDA.

Fleisher, B., Unum, J., Shao, J., et al., 2015. Ingredients in fruit juices interact with dasatinib through inhibition of BCRP: a new mechanism of beverage-drug interaction. J. Pharm. Sci. 104, 266–275.

Fugh-Berman, A., Ernst, E., 2001. Herb-drug interactions: review and assessment of report reliability. Br. J. Clin. Pharmacol. 52, 587–595. https://doi.org/10.1046/j.0306-5251.2001.01469.x.

Gardiner, P., R. S. Phillips and A. F. J. A. f. p. Shaughnessy, 2008. Herbal and dietary supplement-drug interactions in patients with chronic illnesses. American family physician. 77, 73-78.

Haouala, A., Widmer, N., Duchosal, M.A., et al., 2011. Drug interactions with the tyrosine kinase inhibitors imatinib, dasatinib, and nilotinib. Blood 117, e75–e87. https://doi.org/10.1182/blood-2010-07-294330.

Hartmann, J.T., Haap, M., Kopp, H.G., et al., 2009. Tyrosine kinase inhibitors - a review on pharmacology, metabolism and side effects. Curr. Drug Metab. 10, 470–481. https://doi.org/10.2174/138920009788897975.

Herbrink, M., Nuijen, B., Schellens, J.H., et al., 2015. Variability in bioavailability of small molecular tyrosine kinase inhibitors. Cancer Treat. Rev. 41, 412–422. https://doi.org/10.1016/j.ctrv.2015.03.005.

Herviou, P., Thivat, E., Richard, D., et al., 2016. Therapeutic drug monitoring and tyrosine kinase inhibitors. Oncol. Lett. 12, 1223–1232. https://doi.org/10.3892/ol.2016.4780.

Horinkova, J., Sima, M., Slanar, O., 2019. Pharmacokinetics of dasatinib. Prague Med. Rep. 120, 52–63. https://doi.org/10.14712/23362936.2019.10.

Jigorel, E., Le Vee, M., Boursier-Neyret, C., et al., 2006. Differential regulation of sinusoidal and canalicular hepatic drug transporter expression by xenobiotics activating drug-sensing receptors in primary human hepatocytes. Drug Metab. Dispos. 34, 1756–1763. https://doi.org/10.1124/dmd.106.010033.

Johnson, F.M., Agrawal, S., Burris, H., et al., 2010. Phase 1 pharmacokinetic and drug-interaction study of dasatinib in patients with advanced solid tumors. Cancer 116, 1582–1591. https://doi.org/10.1002/cncr.24927.

Kamath, A.V., Wang, J., Lee, F.Y., et al., 2008. Preclinical pharmacokinetics and in vitro metabolism of dasatinib (BMS-354825): a potent oral multi-targeted kinase inhibitor against SRC and BCR-ABL. Cancer Chemother. Pharmacol. 61, 365–376. https://doi.org/10.1007/s00280-007-0478-8.

Keam, S., 2008. Dasatinib. Bio-Drugs. 22, 59-69

Kikuzaki, H., Hisamoto, M., Hirose, K., et al., 2002. Antioxidant properties of ferulic acid and its related compounds. J. Agric. Food Chem. 50, 2161–2168.

Meesters, R. and S. J. J. A. B. Voswinkel, 2018. Bioanalytical Method Development and Validation: from the USFDA 2001 to the USFDA 2018 Guidance for Industry. 4, 67-73.

Olinga, P., Elferink, M.G., Draaisma, A.L., et al., 2008. Coordinated induction of drug transporters and phase I and II metabolism in human liver slices. Eur. J. Pharm. Sci. 33, 380–389. https://doi.org/10.1016/j.ejps.2008.01.008.

Paul, M.K., Mukhopadhyay, A.K., 2004. Tyrosine kinase - Role and significance in cancer. Int. J. Med. Sci. 1, 101–115. https://doi.org/10.7150/ijms.1.101.

Porter, T.D., Coon, M.J., 1991. Cytochrome P-450. Multiplicity of isoforms, substrates, and catalytic and regulatory mechanisms. J. Biol. Chem. 266, 13469–13472.

Raish, M., Ahmad, A., Ansari, M.A., et al., 2019. Effects of sinapic acid on hepatic cytochrome P450 3A2, 2C11, and intestinal P-glycoprotein on the pharmacokinetics of oral carbamazepine in rats: Potential food/herb-drug interaction. Epilepsy Res. 153, 14–18. https://doi.org/10.1016/j.eplepsyres.2019.03.012.

Raish, M., Ahmad, A., Ansari, M.A., et al., 2019. Effect of sinapic acid on aripiprazole pharmacokinetics in rats: Possible food drug interaction. J. Food Drug Anal. 27, 332–338. https://doi.org/10.1016/j.jfda.2018.06.002.

Raish, M., Ahmad, A., Shahid, M., et al., 2023. Effects of apigenin on pharmacokinetics of dasatinib and probable interaction mechanism. Molecules 28, 1602.

Rochat, B., Fayet, A., Widmer, N., et al., 2008. Imatinib metabolite profiling in parallel to imatinib quantification in plasma of treated patients using liquid chromatography-mass spectrometry. J. Mass Spectrom. 43, 736–752. https://doi.org/10.1002/jms.1369.

Smith, P.K., Krohn, R.I., Hermanson, G.T., et al., 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150, 76–85. https://doi.org/10.1016/0003-2697(85)90442-7.

Squibb, B.-M. J. P., NJ, Bristol-Myers Squibb, 2017. Sprycel (dasatinib).

Steegmann, J.L., Baccarani, M., Breccia, M., et al., 2016. European LeukemiaNet recommendations for the management and avoidance of adverse events of treatment in chronic myeloid leukaemia. Leukemia 30, 1648–1671. https://doi.org/10.1038/leu.2016.104.

Teo, Y.L., Ho, H.K., Chan, A., 2015. Metabolism-related pharmacokinetic drug-drug interactions with tyrosine kinase inhibitors: current understanding, challenges and recommendations. Br. J. Clin. Pharmacol. 79, 241–253. https://doi.org/10.1111/bcp.12496.

Thappali, S., Varanasi, K., Veeraraghavan, S., et al., 2012. Simultaneous determination of methotrexate, dasatinib and its active metabolite N-deshydroxyethyl dasatinib in rat plasma by LC-MS/MS: method validation and application to pharmacokinetic study. Arzneimittelforschung 62, 624–630.

Towbin, H., Staehelin, T., Gordon, J., 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. PNAS 76, 4350–4354. https://doi.org/10.1073/pnas.76.9.4350.

van Erp, N.P., Gelderblom, H., Guchelaar, H.J., 2009. Clinical pharmacokinetics of tyrosine kinase inhibitors. Cancer Treat. Rev. 35, 692–706. https://doi.org/10.1016/j.ctrv.2009.08.004.

van Leeuwen, R.W., van Gelder, T., Mathijssen, R.H., et al., 2014. Drug-drug interactions with tyrosine-kinase inhibitors: a clinical perspective. Lancet Oncol. 15, e315–e326. https://doi.org/10.1016/S1470-2045(13)70579-5.

Wang, L., Christopher, L.J., Cui, D., et al., 2008. Identification of the human enzymes involved in the oxidative metabolism of dasatinib: an effective approach for determining metabolite formation kinetics. Drug Metab. Dispos. 36, 1828–1839. https://doi.org/10.1124/dmd.107.020255.

Yoon, B.H., Jung, J.W., Lee, J.-J., et al., 2007. Anxiolytic-like effects of sinapic acid in mice. Life Sci. 81, 234–240.

Zou, Y.-N., A.-R. Kim, J.-E. Kim, et al., 2002. Peroxynitrite Scavenging Activity of Sinapic Acid. Proceedings of the PSK Conference, The Pharmaceutical Society of Korea.