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Original Article

Effects of suberoylanilide hydroxamic acid on rat cytochrome P450 enzyme activities

Kezhi Lin[1*], Qingwei Zhang[2*], Zezheng Liu[2], Suping Yang[2], Yingying Lin[2], Congcong Wen[3], Yuancai Zheng[4]

[1]Medical Experimental Teaching Center, Wenzhou Medical University, Wenzhou 325035, China; [2]Shanghai Institute of Pharmaceutical Industry, Shanghai 200437, China; [3]Laboratory Animal Centre, Wenzhou Medical University, Wenzhou 325035, China; [4]The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325035, China. [*]Equal contributors.

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Abstract: Vorinostat (suberoylanilide hydroxamic acid, SAHA) is the first approved histone deacetylase (HDAC) inhibitor for the treatment of cutaneous T-cell lymphoma after progressive disease following two systemic therapies. The rats were randomly divided into SAHA groups (low, medium and high dosage) and control group. The SAHA group rats were given 12.3, 24.5, and 49 mg/kg SAHA, respectively, by continuous intragastric administration for 7 days. The influence of SAHA on the activities of CYP450 isoforms CYP2B6, CYP1A2, CYP2C19, CYP2D6 and CYP2C9 were evaluated by cocktail method, they were responsed by the changes of pharmacokinetic parameters of bupropion, phenacetin, tolbutamide, metroprolol and omeprazole. The five probe drugs were given to rats through intragastric administration, and the plasma concentration were determined by UPLC-MS/MS. The result of SAHA group compared to control group, there were statistical pharmacokinetics difference for bupropion, phenacetin, tolbutamide and metroprolol. Continuous intragastric administration for 7 days may induce the activities of CYP2C19 of rats, inhibit CYP1A2 and slightly inhibit CYP2B6 and CYP2D6 of rats. This may give advising for reasonable drug use after co-used with SAHA. The results indicated that drug co-administrated with SAHA may need dose adjustment. Furthermore, continuous intragastric administration of SAHA for 7 days, liver cell damaged, causing liver cell edema, in liver metabolism process.

Keywords: CYP450, SAHA, HDACi, cocktail, UPLC-MS/MS, rat

Introduction

During the drug discovery and development phase, inhibition of cytochrome P450 (CYP) family of enzymes is the most common cause of harmful clinical drug-drug interactions (DDIs) and has led to the removal of many drugs from the clinical trials [1, 2]. To avoid undesirable DDIs leading to severe adverse effects, it is necessary to preliminarily understand the potential effects of a new chemical entity on certain CYP metabolizing enzymes [3-5].

To date, several histone deacetylases inhibitors (HDACi) are currently undergoing clinical evaluation as anticancer agents and have been shown to regulate a variety of cellular responses including proliferation, differentiation, and apoptosis [6, 7]. Among all of these inhibitors, suberoylanilide hydroxamic acid (also known as SAHA or Vorinostat) has emerged as the first HDACi approved by the FDA in 2006 for the treatment of advanced cutaneous T-cell lymphoma (CTCL) [8]. Nevertheless, many HDACi including SAHA suffer from side effects, such as pulmonary embolism, deep vein thrombosis, gastrointestinal disturbances, or cardiovascular toxicity, and combined SAHA with coumarin-derivative anticoagulants could also lead to the drug-drug interactions [9, 10].

However, few studies on the effects of SAHA on CYP enzyme expressions were shown. Thus, the purpose of this study was to investigate the effects of SAHA on rat liver CYP enzyme protein expressions and the possible underlying mechanisms and further provide a pharmacological basis for its clinical application.

Material and methods

Chemicals

Bupropion, phenacetin, tolbutamide, metroprolol, omeprazole (all >98%) and the internal stan-

dard diazepam were obtained from Sigma-Aldrich Company (St. Louis, USA). Ultra-pure water was prepared by Millipore Milli-Q purification system (Bedford, USA). Methanol and acetonitrile (HPLC grade) were obtained from Merck Company (Darmstadt, Germany).

Animals

Sprague-Dawley rats (male, 220 ± 20 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. Animals were housed under a natural light-dark cycle conditions with controlled temperature (22°C). All forty rats were housed at Wenzhou Medical University Laboratory Animal Research Center. All experimental procedures were approved ethically by the Wenzhou Medical University Administration Committee of Experimental Animals.

UPLC-MS/MS conditions

UPLC-MS/MS with ACQUITY I-Class UPLC and a XEVO TQD triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface (Waters Corp., Milford, MA, USA) were used to analyze the compounds. The UPLC system was comprised of a Sample Manager with Flow-Through Needle (SM-FTN) and a Binary Solvent Manager (BSM). The Masslynx 4.1 software was used for data acquisition and instrument control (Waters Corp., Milford, MA, USA).

Bupropion, phenacetin, tolbutamide, metroprolol, omeprazole and diazepam (IS) were separated using a Waters BEH C18 column (2.1 mm ×100 mm, 1.7 μm) maintained at 40°C. The initial mobile phase consisted of 0.1% formic acid and acetonitrile with gradient elution at a flow rate of 0.4 mL/min and an injection volume of 2 μL. Elution was in a linear gradient, with the acetonitrile changing from 30 to 60% between 0.3 and 1.8 min and increasing up to 95% over 0.2 min. The acetonitrile content was maintained at 95% for 0.5 min and decreased to 30% within 0.1 min, and maintained at 30% for 0.4 min. The total run time of the analytes need 3 min.

The mass spectrometric detection was performed in a positive mode. Nitrogen was used as the cone gas (50 L/h) and desolvation gas (1000 L/h). The mass conditions were set as follows: source temperature 150°C; capillary voltage 2.5 kV; desolvation temperature 500°C. The multiple reaction monitoring (MRM) mode of m/z 180.1→109.9 for phenacetin, m/z 268.1→115.8 for metroprolol, m/z 346.1 →197.8 for omeprazole, m/z 271.2→155.1 for tolbutamide, m/z 240.1→184.1 for bupropion and m/z 285.1→193.1 for IS was used as quantitative analysis.

Pharmacokinetics

Forty rats (220 ± 20 g) were randomly divided to SAHA groups and control group. SAHA groups were give SAHA (12.3, 24.5, and 49.0 mg/kg, as low, medium, and high dosage, respectively) by continuous intragastric administration for 7 days. Control group were give saline by continuous intragastric administration for 7 days. After 8 days, the SAHA and control group intragastric administration of mixed five probe drugs (bupropion, phenacetin, tolbutamide, metroprolol and omeprazole were 10, 10, 1, 10 and 10 mg/kg).

Blood (0.3 mL) samples were collected at 0.0833, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 h from the tail vein into heparinized 1.5 mL polythene tubes after intragastric administration of five probe drugs. The 100 μL plasma was obtained from blood sample after centrifuged at 4000 *g* for 10 min. In a 1.5 mL centrifuge tube, 100 μL of collected plasma sample followed by the addition of 200 μL of acetonitrile (containing 50 ng/mL IS). After vortex-mixed for 1.0 min, the sample was centrifuged at 13000 *g* for 15 min. Then the 2 μL supernatant was injected into the UPLC-MS/MS system for analysis.

Plasma probe drugs concentration versus time was analyzed by Version 3.0 Data Analysis System (Wenzhou Medical University, China). The main pharmacokinetic parameters of the SAHA group and control group were analyzed by SPSS I8.0 statistical software.

Histopathology

After pharmacokinetics experiment, rats were deeply anesthetized with 10% chloral hydrate (i.p., 20 mg/kg). The liver were rapidly isolated and immersed in freshly prepared 4% w/v formaldehyde (0.1 phosphate buffer, pH 7.2) for 48 h and then embedded in paraffin. Then 4-μm-thick histological sections were prepared and stained with hematoxylin and eosin by routine HE method. The morphological changes were observed under light microscope.

Table 1. Pharmacokinetic parameters of bupropion, omeprazole, phenacetin, tolbutamide and metroprolol in control-group and SAHA-group rats (mean ± SD, n =10)

Drug	Group	$AUC_{(0-t)}$	AUC(0-∞)	t1/2z	Tmax	CLz/F	Vz/F	C_{max}
		μg/L*h	μg/L*h	h	h	L/h/kg	L/kg	ug/L
Bupropion	Low	77.2 ± 36.8	82.9 ± 40.0	0.9 ± 0.3	0.7 ± 0.3	149.6 ± 70.3	185.8 ± 90.4	48.7 ± 31.2
	Medium	88.2 ± 49.4	93.3 ± 56.9	0.8 ± 0.2	0.5**	135.7 ± 60.7	151.3 ± 78.1	66.1 ± 32.6**
	High	80.1 ± 27.9*	92.5 ± 36.5*	1.2 ± 0.7	0.6 ± 0.2	125.3 ± 50.9*	200.2 ± 91.2	48.3 ± 25.8*
	Control	53.2 ± 17.3	60.0 ± 23.4	1.0 ± 0.5	0.8 ± 0.3	189.3 ± 71.2	271.8 ± 168.4	27.8 ± 7.9
Omeprazole	Low	325.2 ± 138.1	327.4 ± 138.0	0.7 ± 0.2	0.5**	35.0 ± 12.6*	39.2 ± 23.0	293.2 ± 141.5
	Medium	431.0 ± 170.3	434.2 ± 170.2	0.7 ± 0.2	0.4 ± 0.2*	26.6 ± 11.0	30.6 ± 22.1	427.4 ± 214.4
	High	441.4 ± 114.8	445.8 ± 113.9	0.9 ± 0.2	0.4 ± 0.3	24.0 ± 7.5	30.6 ± 16.5	339.2 ± 159.5
	Control	428.3 ± 78.1	434.1 ± 77.6	1.0 ± 0.4	0.2 ± 0.2	23.7 ± 4.6	35.4 ± 14.8	313.1 ± 95.8
Phenacetin	Low	4641.4 ± 1932.6*	4642.4 ± 1932.7*	0.5 ± 0.2	0.6 ± 0.2	2.6 ± 1.3	2.1 ± 1.5	4005.0 ± 1342.7*
	Medium	4982.5 ± 2314.8*	4984.2 ± 2314.7*	0.4 ± 0.2	0.5	2.9 ± 2.6	1.8 ± 1.7	4242.9 ± 1661.7*
	High	4419.7 ± 1940.0*	4423.4 ± 1939.4*	0.7 ± 0.3	0.6 ± 0.3	3.0 ± 2.1	3.3 ± 3.8	3524.3 ± 1512.3*
	Control	2760.6 ± 1437.8	2763.9 ± 1437.9	0.7 ± 0.4	0.5 ± 0.2	4.7 ± 2.8	4.1 ± 2.5	2309.3 ± 983.0
Tolbutamide	Low	64613.1 ± 7322.5**	65079.8 ± 7284.4**	5.1 ± 0.9*	1.8 ± 0.7*	0.016 ± 0.002	0.115 ± 0.028	4988.5 ± 286.7**
	Medium	70655.0 ± 17511.4**	72341.5 ± 17613.2**	6.3 ± 2.9	1.8 ± 0.7*	0.014 ± 0.003	0.126 ± 0.044	5590.9 ± 542.9**
	High	79185.6 ± 16714.1*	80272.3 ± 16388.6*	5.2 ± 0.8*	2.0 ± 1.3	0.013 ± 0.003*	0.098 ± 0.025	6448.0 ± 1242.0*
	Control	99761.0 ± 14784.4	100196.9 ± 14802.9	6.1 ± 0.7	2.9 ± 1.4	0.010 ± 0.002	0.090 ± 0.024	7465.0 ± 1087.3
Metroprolol	Low	495.3 ± 137.1	501.8 ± 138.5	0.9 ± 0.1	0.8 ± 0.3	21.4 ± 6.0	26.3 ± 8.2	225.3 ± 74.1
	Medium	576.1 ± 110.4	581.3 ± 110.8	0.8 ± 0.1	0.7± 0.3	17.7± 3.2	20.4 ± 5.9	286.4 ± 51.6
	High	647.8 ± 130.6*	673.4 ± 143.5*	1.1 ± 0.7	0.8 ± 0.3	15.5 ± 3.3*	24.0 ± 12.4	313.6 ± 111.0
	Control	500.4 ± 91.4	510.3 ± 91.0	0.9 ± 0.4	0.6 ± 0.3	20.2 ± 4.1	27.4 ± 12.7	240.8 ± 72.0

Compared SAHA group with the control group, *: $P<0.05$, **: $P<0.01$.

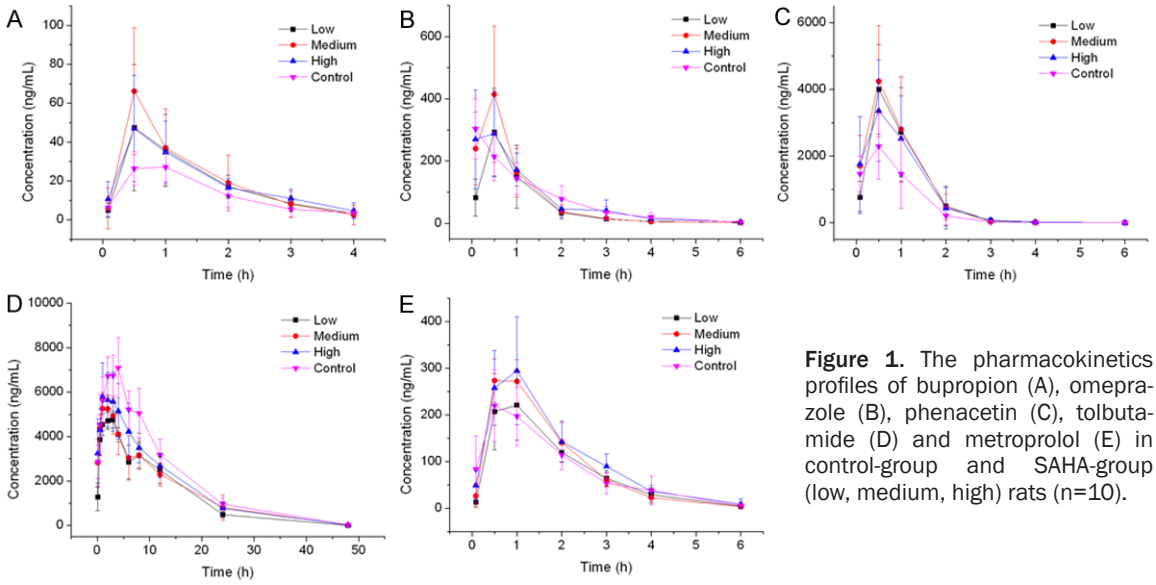

Figure 1. The pharmacokinetics profiles of bupropion (A), omeprazole (B), phenacetin (C), tolbutamide (D) and metroprolol (E) in control-group and SAHA-group (low, medium, high) rats (n=10).

Results

Method validation

The concentration of bupropion, phenacetin, tolbutamide, metroprolol and omeprazole in rat plasma was simultaneously determined by a sensitive and simple UPLC-MS/MS method. The LLOQ for each probe drug in plasma was 2 ng/mL. The RSD of the five probe drugs were less than 12%. The calibration plot of the probe drugs is in the range of 2-2000 ng/mL ($r>0.993$). The intra-day and inter-day accuracy ranged from 90% to 112%. The matrix effects were more than 85% or less than 114%. The extraction recoveries were better than 82%.

Pharmacokinetics

The main pharmacokinetic parameters of bupropion, phenacetin, tolbutamide, metroprolol and omeprazole were summarized from non-compartment model analysis in **Table 1**. The representative phenacetin, metroprolol, omeprazole, tolbutamide and bupropion concentration vs. time profiles were presented in **Figure 1**. As could be seen from **Figure 1**, the C_{max} and AUC of tolbutamide in SAHA group is lower than the control group, while the C_{max} and AUC of bupropion and phenacetin in SAHA group is higher than the control group.

As can be seen from **Table 1**, the pharmacokinetic parameters of tolbutamide have changed, $AUC_{(0-t)}$ decreased ($P<0.01$), CL increased ($P<0.05$, high), C_{max} decreased ($P<0.01$ or 0.05), compared SAHA group with the control group. It indicates that the continuous administration of SAHA may induce the activity of CYP2C19 enzyme of rats.

While compared SAHA group with the control group, there were no significant difference for AUC of some group of omeprazole (low, high and medium), metroprolol (low and medium) and bupropion (low and medium) ($P>0.05$), and here were significant difference for AUC increased, CL decreased and C_{max} increased of some group of phenacetin (low, high and medium), metroprolol (high) and bupropion (high) ($P<0.05$), it could show that the SAHA inhibit the activity of CYP1A2, and may slight inhibit the activity of CYP2D6 and CYP2B6 enzyme.

Morphological changes of liver

In low dose group, the structure of liver lobule is intact and the liver cells are arranged as funicular along with central veins at low magnification. At high magnification, the nucleus of liver cells is round, clear and fine luster, the cytoplasm become loose and slightly edema. In middle dose group, the structure of liver lobule is still can be recognized, the central veins are slightly dilated, some liver cells are arranged disorderly and hepatic sinusoid is dilated at low magnification. At high magnification, a few of inflammatory cells are observed. In high dose group, the structure of liver lobule is intact, liver cells are arranged in radiate form along with central veins and formed hepatic plate at low magnification. At high magnification, the liver

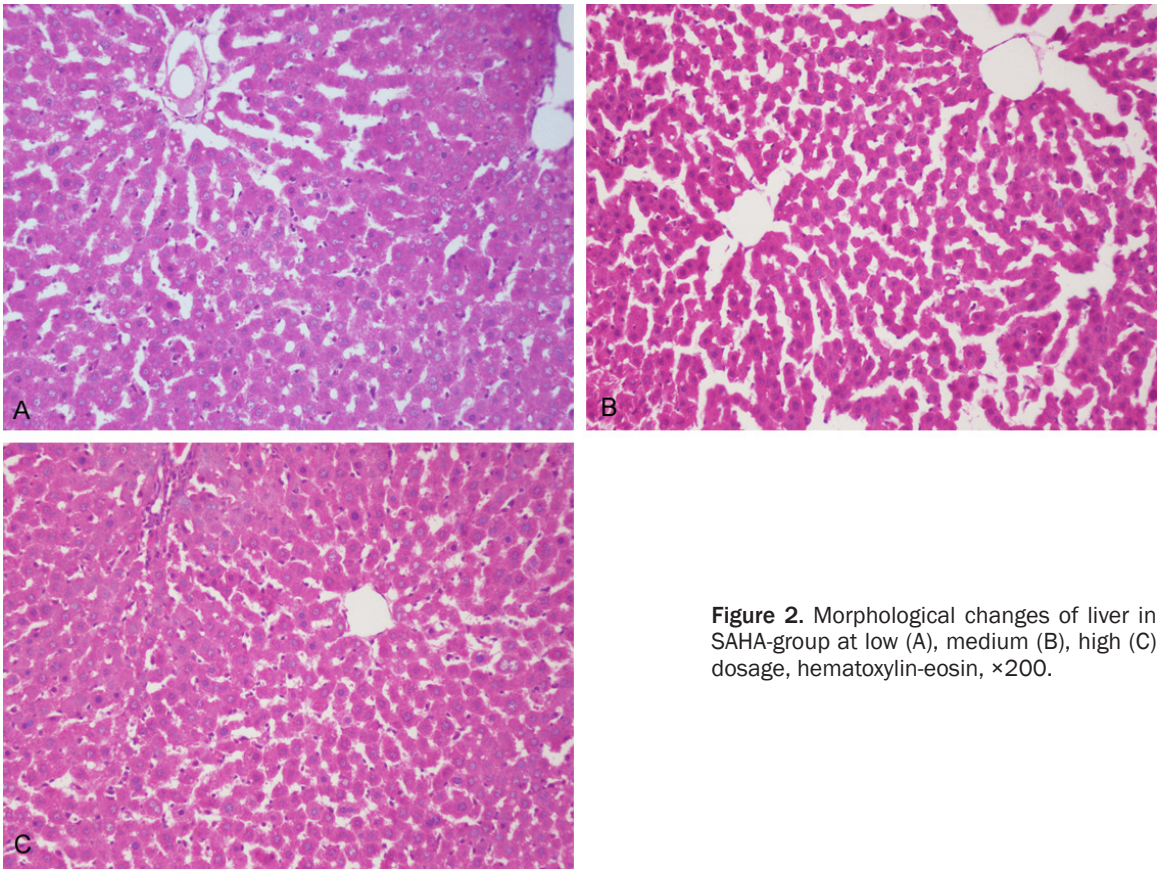

Figure 2. Morphological changes of liver in SAHA-group at low (A), medium (B), high (C) dosage, hematoxylin-eosin, ×200.

cell edema in high dose group is more obviously than that of middle dose group, liver cells arranged disorderly in some liver lobule,the gap of hepatic sinusoid decreased or disappeared and a few more inflammatory cells infiltrated. Continuous intragastric administration of SAHA for 7 days, liver cell damaged, causing liver cell edema, in liver metabolism process (**Figure 2**).

Discussion

Histone deacetylase inhibitors (HDIs) are a new class of promising anticancer agents that induce acetylation of the histones and non-histone proteins that are involved in the regulation of gene expression and various cellular pathways [11, 12]. SAHA, is a key second-generation hydroxamate HDACI of classes I and II for the treatment of refractory cutaneous T-cell lymphoma [11, 13]. Vorinostat can cause growth arrest and death of abroad of transformed cells and have little or no toxic effects on normal cell [11]. Recent evidence indicates that, vorinostat may interact with a variety of substrates including chromatin proteins transcription factors, metabolic enzymes, and cell structure proteins [14, 15].

As SAHA is always used combination with other drugs, interactions between SAHA and other drugs undertake the risk of either diminished efficacy or adverse effects. Drug-drug interactions often occur at the active site of these enzymes since CYP450 enzymes play a key role in the phase I metabolism of the majority of all marketed drugs.

In general, changes in pharmacokinetics are thought to be caused by drug-drug or drug-food interactions [16]. In pharmacokinetic interactions, approximately 65% of drug-drug interactions occur in metabolic sites [17], and drug metabolic enzymes are considered to be the most important interactive sites. A large number of drugs are metabolized by CYP enzymes in the liver, and more than 90% of drug-drug interactions occur at the CYP-catalyzed step [18, 19]. Similarly, supplement-drug interactions involving CYP activity are occasionally found to cause considerable adverse events. For these reasons, we evaluated the effects of intragastric administration of SAHA for 7 days on the activity of CYP enzymes in *vivo*. We selected CYP isoforms CYP1A2, CYP2D6, CYP2C19, CYP2C9 and CYP2B6 because more

than 50% of drugs are known to be metabolized by these 5 CYP enzymes [20, 21]. In present study, it indicates that the continuous administration of SAHA may induce the activity of CYP2C19 enzyme of rats, induction of drug metabolizing enzyme reduces the efficacy of drug; it could show that the SAHA inhibit the activity of CYP1A2, and may slight inhibit the activity of CYP2D6 and CYP2B6 enzyme, inhibition of drug metabolizing enzyme increase the concentration of plasma drug, it will bring the risk of adverse effects.

In conclusion, continuous intragastric administration of SAHA for 7 days may induce the activities of CYP450 isoforms CYP2C19 of rats, inhibit the activity of CYP1A2, and may slight inhibit the activity of CYP2D6 and CYP2B6 enzyme. Continuous intragastric administration of SAHA, liver cell damaged, causing liver cell edema, in liver metabolism process. These results would give us valuable information regarding the interactions of SAHA with drugs, induction of drug metabolizing enzyme reduces the efficacy of drug. Combination of SAHA with drugs might cause pharmacokinetic interactions, which required dose adjustment to avoid over dosage or reduced blood drug concentration.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yuancai Zheng, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang 325035, PR China. Tel: (86)577 86699156; E-mail: zhengyuancai2012@163.com

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