Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2015, Volume 3, Issue 10
ISSN: 2321–3647(online)

Pharmacokinetic Differences of the Glucuronide-Conjugated Metabolites of Magnoflorine, and Jatrorrhizine between Healthy Chinese and African Volunteers

Raphael N. Alolga[1*], Assogba G. Assanhou[2*], Vitus Onoja[2], Moses Muyaba[2], Sarah W. Amadi[2], Said Abasse Kassim[2]

1. State Key Laboratory of Natural Medicines, China Pharmaceutical University, No. 24 Tongjia Lane, 210009, Xuanwumen, Nanjing.

2. School of Pharmacy, China Pharmaceutical University, No. 24 Tongjia Lane, 210009, Xuanwumen, Nanjing.

ABSTRACT

This short communication reports the pharmacokinetic differences of the glucuronide-conjugated metabolites of magnoflorine and jatrorrhizine between Chinese and African male volunteers. From an earlier report, glucuronidation was determined to be one of the main metabolic pathways and these two compounds were reported to differ significantly between the two races. Pharmacokinetic parameters of half-life,$t_{1/2}$, time to reach maximum concentration, T_{max}, maximum plasma concentration, C_{max}, volume of distribution, Vd, area under the concentration-time curve, AUC and clearance, CL were considered. Statistically significant differences were observed in almost all the parameters studied in terms of their glucuronide-conjugated metabolites. The findings indicate the differences in hepatic metabolism of these two compounds between the two races.

Keywords: conjugate, glucuronide, metabolite, pharmacokinetics, races.

‘

*Corresponding Author Email: anammahime@gmail.com; diadora832003@yahoo.fr
Received 10 September 2015, Accepted 22 September 2015

Please cite this article as: Alolga RN *et al*., Pharmacokinetic Differences of the Glucuronide-Conjugated Metabolites of Magnoflorine, and Jatrorrhizine between Healthy Chinese and African Volunteers. American Journal of Pharmacy & Health Research 2015.

INTRODUCTION

Magnoflorine and jatrorrhizine were found to be present in high concentrations in human plasma upon the ingestion of a Chinese multicomponent herbal formulation, K-601from our previous study[1]. Both were identified to be constituents of Phellodendron Chinense. However, magnoflorine is an aporphine alkaloid while jatrorrhizine belongs to the class of benzylisoquinoline alkaloids. Magnoflorine has been found to possess immunomodulatory effects[2], acetylcholinesterase inhibitory effect[3], anti-hemolytic effect[4], sedative and anxiolytic properties[5], and hypoglycemic properties[6] etc. Jatrorrhizine is also known to possess anti-oxidation and anti-inflammation effects, neuroprotective effects[7], antihypercholesterolemic effect[7], antimetastatic effects (against melanoma)[8] etc. Pharmacokinetic profiles of magnoflorine[9,10] and jatrorrhizine [8,11] have been done in rats but no report has been made of their glucuronide-conjugated metabolites in Africans and Chinese. The aim of this study was to assess the pharmacokinetic differences between Africans and Chinese, with the focus on the glucuronide-conjugated metabolites of these two compounds. Knowledge of the pharmacokinetic differences implicitly indicates the metabolic differences between the two races.

MATERIALS AND METHOD

Study subjects

A total of six healthy male volunteers, ages ranging from 22-47 years took part in this study, three (3) Africans and three (3) Chinese.

Study design

All volunteers avoided the intake of alcohol/alcoholic beverages, for at least 12 hours prior to the study. None was also on any medication. Standard meals were taken by all volunteers within this period. Blood samples were withdrawn from subjects at the following time intervals, 0 hours (before taking medication and breakfast), 2, 4, 6, 8, 10 and 12 hours after taking the medication. These blood samples were taken by a qualified phlebotomist. Each volunteer took 40mL of same batch of the medication as a single dose. The study was approved by the Ethics committee of the First Affiliated Hospital of Nanjing Medical University and conducted under the guidelines of the Helsinki Declaration and the International Conference on Harmonization-Good Clinical Practices (ICH-GCP).

Treatment of plasma samples

All blood samples taken at each time were immediately centrifuged at 13000rpm for 10 minutes, and the plasma separated and stored at -80^{o}C until analysis. Plasma samples were thawed at

37^{o}C before solid-phase extraction (SPE) treatment for UPLC-QTOF-MS analysis. An aliquot of methanol (5mL) was slowly run through SPE column, C18 bonded stationary phase, to activate it. Methanol: water (5:95$^{v}/_{v}$, 5 mL), was then run through under the same conditions to equilibrate the column. The plasma samples, 100µL was prepared in 2 mL of 5:95$^{v}/_{v}$ methanol: water. The plasma samples were loaded onto the column and collected slowly. An additional solution of methanol: water (5:95$^{v}/_{v}$, 3mL) was then used to wash the column, and added to the first extract. The column was finally eluted with methanol (4mL) into the combined extracts. The extract was dried using nitrogen gas. The residue was then dissolved in methanol, vortexed for 2min and centrifuged at 13000rpm for 10min and the supernatant used for the UPLC-QTOF-MS analysis. Analysis was done in the positive ion mode. UPLC conditions were the same as earlier reported [1].

Preparation of stock solutions, calibration standards and quality control samples

Stock solutions of magnoflorine and jatrorrhizine as well as internal standard, artemisinic acid were prepared in methanol at concentration of 1mg/mL. A series of working standard analyte mixtures containing 0.04,0.2,1,2, 4 and 10µg/mL of these two analytes were prepared, by dilution from the stock solutions with methanol. Internal standard (artemisinic acid) to final concentration of 2µg/mL was prepared. Low, medium and high quality control working stock solutions (0.3, 5 and 8µg/mL), were prepared in methanol using separately weighed stock solutions of the two analytes. Seven calibration standard solutions at 0.002, 0.005, 0.02, 0.05, 0.2, 0.5 and 2µg/mL were prepared by spiking blank plasma with appropriate amounts of working standards. QC plasma samples at 0.01, 0.25 and 1µg/mL were prepared, the same way as the calibration standard. Blank plasma samples with analytes and internal standard and zero plasma samples with internal standard but no analytes were prepared. All standard solutions were stored at 4^{o}C until analysis

Method Validation

The analytical procedure was validated for linearity, accuracy, precision, specificity and repeatability using the QC samples.

Analyses of metabolic conjugates

Conjugated metabolites in plasma were determined through hydrolysis with glucuronidase. Plasma (100µL) was mixed with 100µL of β-glucuronidase (1037 units/mL in pH 5, in sodium phosphate buffer) and incubated for 1 hour. After hydrolysis, 50µL of 0.1N HCl was added, partitioned with 400µL of ethyl acetate (containing internal standard), and centrifuged at

13000rpm for 10min. The ethyl acetate layer was evaporated under nitrogen gas and reconstituted with mobile phase for UPLC-QTOF analysis.

Pharmacokinetic Analyses

Pharmacokinetic analyses were conducted using PK Solver, a menu-driven add-in program for Microsoft Excel written in Visual Basic for Applications (VBA)[12].

Statistical Analysis

Pharmacokinetic data were analyzed using standard descriptive statistics. Data are presented as mean ±standard deviation (SD). The Student's t-test was used to compare calculated pharmacokinetic parameters between the two groups. P-values less than 0.05 were considered to indicate statistical significance.

RESULTS AND DISCUSSION

Table 1: Pharmacokinetic parameters of glucuronide-conjugated metabolites of magnoflorine in Chinese and African volunteers

Parameters	Unit	Chinese	Africans	P value
$t_{1/2}$	h	1.41±0.73	4.02±1.02	0.0458
T_{max}	h	4.0±1.15	6.0±0.1	0.1161
C_{max}	$ng.mL^{-1}$	1.97±0.14	0.99±0.01	<0.0001
AUC	$ng.h.mL^{-1}$	14.48±0.03	6.92±0.16	<0.0001
Vd	mL	5.45±2.61	25.94±5.18	0.0057
CL	$mL.h^{-1}$	2.68±0.06	4.47±0.31	0.0004

Table 2: Pharmacokinetic parameters of glucuronide-conjugated metabolites of jatrorrhizine in Chinese and African volunteers

Parameters	Unit	Chinese	Africans	P value
$t_{1/2}$	h	2.90±0.22	2.32±0.30	0.5736
T_{max}	h	6.0±0.1	4.0±0.1	-
C_{max}	$ng.mL^{-1}$	0.06±0.01	0.160±0.01	0.0012
AUC	$ng.h.mL^{-1}$	0.41±0.03	1.07±0.06	<0.0001
Vd	mL	353.42±42.35	114.24±12.30	0.0011
CL	$mL.h^{-1}$	84.36±4.35	34.06±1.85	<0.0001

Table 1 and 2 present the PK details of the glucuronide-conjugated metabolites of magnoflorine and jatrorrhizine in the Chinese and African volunteers. From our previous study, we reported higher concentrations of prototype jatrorrhizine in the African volunteers and the Chinese. Magnoflorine was also higher in concentration in the Chinese than the Africans. In this study, we traced the glucuronide-conjugated metabolites of these two compounds for 12 hours after the ingestion of K-601 formulation. We found this necessary since certain compounds have been found to be active in their metabolic forms and considering the fact that glucuronidation was

found to be a major metabolic pathway. By examining the glucuronide-conjugated metabolites of these compounds, it is also possible to determine partly the hepatic metabolic differences between the two races.

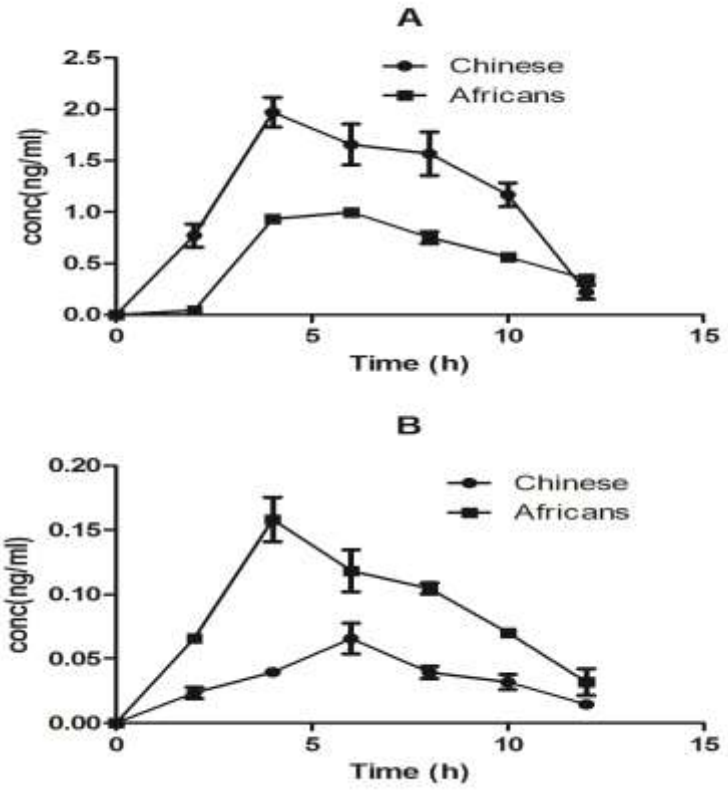

Figure 1 PK profiles of the glucuronide-conjugated metabolites of magnoflorine (A) and jatrorrhizine (B) in healthy male Chinese and African volunteers

As shown in figure 1A, the plasma concentration of glucuronide-conjugated metabolite of magnoflorine washigher in the Chinese than the Africans (i.e. AUC). The PK parameters were all statistically significant for the two groups except for T_{max}. These results indicate that magnoflorine is metabolized faster in the Chinese subjects than the Africans. These metabolites are however less distributed in the cellular volumes, and cleared at slower rates in the Chinese than the Africans. These metabolites reached maximum plasma concentration, T_{max} at time 4 h whereas it took 6 h for the Africans to attain maximum concentration (a value less than that of the Chinese). The converse was observed for glucuronide-conjugated metabolites of jatrorrhizine (figure 1B). Higher concentrations were observed in terms of C_{max} and AUC in the Africans. These metabolites were however cleared at a slower rate and less distributed than in the Chinese. The T_{max} was 6 h and 4 h for Chinese and Africans respectively. These PK differences could be due to the differences in the rates of absorption of these two compounds which are different in

chemical structure as well as the metabolic capabilities of the two races. Genetic difference plays a crucial role in the metabolism of drugs.

CONCLUSION

Further studies is needed to confirm or otherwise this study with more participants using single formulations of these compounds. Other forms of hepatic metabolism could be studied for instance sulphated metabolites to see if the trend is similar to or different from glucuronidation.

ACKNOWLEDGEMENT

The authors hereby wish to thank all the volunteers who participated in the study. We also wish to thank the phlebotomist for her expert assistance.

REFERENCES

1. Alolga RN., et al. Pharmacokinetics of a multicomponent herbal preparation in healthy Chinese and African volunteers. Sci Rep. 2015; 5: 12961.
2. Ahmad, W, Jantan, I, Kumolosasi, E, Bukhari, SN. Immunostimulatory effects of the standardized extract of Tinospora crispa on innate immune responses in Wistar Kyoto rats. Drug Des Devel Ther. 2015;9:2961-2973.
3. Kukula-Koch W, Mroczek T. Application of hydrostatic CCC-TLC-HPLC-ESI-TOF-MS for the bioguided fractionation of anticholinesterase alkaloids from Argemone mexicana L. roots. Anal Bioanal Chem. 2015;407: 2581-2589.
4. Sakumoto, H., et al. Sinomenine and magnoflorine, major constituents of Sinomeni caulis et rhizoma, show potent protective effects against membrane damage induced by lysophosphatidylcholine in rat erythrocytes. J Nat Med. 2015;69: 441-448 .
5. De la Pena JB., et al.The involvement of magnoflorine in the sedative and anxiolytic effects of Sinomeni Caulis et Rhizoma in mice. J Nat Med. 2013;67:814-821.
6. Patel MB, Mishra S. Hypoglycemic activity of alkaloidal fraction of Tinospora cordifolia. Phytomedicine. 2011;18:1045-1052.
7. Jiang W. et al. Jatrorrhizine protects against Okadaic acid induced oxidative toxicity through inhibiting the mitogen-activated protein kinases pathways in HT22 hippocampal neurons. CNS Neurol Disord Drug Targets 2015.
8. Liao C, Chang S, Yin S, Wang Z, Meng Y. A HPLC-MS/MS method for the simultaneous quantitation of six alkaloids of Rhizoma Corydalis Decumbentis in rat plasma and its application to a pharmacokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci. 2014;944: 101-106.

9. Tian, X., et al. Study on the PK profiles of magnoflorine and its potential interaction in Cortex phellodendri decoction by LC-MS/MS. Anal Bioanal Chem. 2014;406: 841-849.
10. Zhou J, et al. Orthogonal array design for optimization of hollow-fiber-based liquid-phase microextraction combined with high-performance liquid chromatography for study of the pharmacokinetics of magnoflorine in rat plasma. Anal Bioanal Chem. 2012;403: 1951-1960.
11. Zhang Q, Ma, YM, Wang ZT, Wang CH. Pharmacokinetics difference of multiple active constituents from decoction and maceration of Fuzi Xiexin Tang after oral administration in rat by UPLC-MS/MS. J Pharm Biomed Anal. 2014;92: 35-46.
12. Zhang Y, Huo M, Zhou J, Xie S. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. Comput Methods Programs Biomed.2010;99:306-314.