Effects of L-Arabinose on Drug Metabolizing and Antioxidant Enzymes in Rat

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Keywords: L-arabinose, Antioxidant enzymes, Cytochrome P450, Glutathione-S-transferases, UDP-glucuronosyltransferase, Rat

Abstract: This study was conducted to assess the effects of L-arabinose on the antioxidant and drug metabolizing enzymes in rats. Forty-eight rats were equally divided into six groups. The rats in three groups were respectively administered with 500, 1 000 and 2 000 mg/kg L-arabinose for 38 days. In blank control, L-arabinose was replaced by an equal volume of distilled water. In two positive control groups, the rats were intraperitoneally injected with 80mg/kg sodium barbital or dexamethasone for three consecutive days since the 36th day. Twenty-four hours after the last dose, blood was sampled from their femoral artery. And the activities of antioxidant enzymes and the concentration of free radicals in serum were measured. Rat liver tissues were collected and homogenized, centrifuged at 4 000 r/min and 4 ºC. Then, a portion of the supernatant was used for the measurement of the activities of antioxidant enzymes and the concentration of free radicals. Liver microsomes and cytoplasmic fluid were obtained by differential centrifugation. The concentration of cytochrome b_5, the activities of three phase-I metabolic enzymes NADPH-cytochrome C reductase, CYP3A, CYP2E1 and two phase-II metabolic enzymes glutathione-S-transferase (GST) and UDP-glucuronyl-transferases (UGT) were measured using double-beam UV/Vis spectrophotometry. The treatment with 2 000 mg/kg L-arabinose significantly reduced the SOD and GSH-PX activities in rat serum, the NOS activity, MDA concentration, NO concentration in liver tissue, greatly increased the GST activity in liver tissue, microsomes and cytoplasmic fluid, and greatly reduced the UGT activity in microsomes and cytoplasmic fluid. The treatment with 1 000 mg/kg L-arabinose significantly reduced the NO concentration in rat serum, and the UGT activity in microsomes and cytoplasmic fluid. The treatment with 500 mg/kg L-arabinose significantly reduced the GSH-PX activity, the NO concentration in rat serum and the NOS activity in liver tissue. L-arabinose can decrease the antioxidant capacity in rat blood and the concentration of free radicals in rat liver. L-arabinose has no adverse effect on the major drug-metabolizing enzymes in rat, but it reduces the activities of phase-II drug-metabolic enzymes GST and UGT to a certain degree, suggesting that no or slight metabolic drug-drug interactions in liver can be induced by the combined use of L-arabinose and drugs.

Introduction

Antioxidant enzymes are an important class of specific and closely related enzymes that can scavenge free radicals and protect organisms from the injury of reactive oxygen species [1-2]. A free radical is a highly active molecule generated during life activities in human bodies. Under normal circumstances, a dynamic equilibrium exists between the generation and scavenging of free radicals.

However, diseases or some exogenous substances (such as radiation) will result in the abnormal metabolism of free radical and then excessive free radicals will be accumulated. The oxidative stress response caused by excessive free radicals will cause various diseases [1, 3]. Phase-I and phase-II drug metabolizing enzymes are respectively responsible for the metabolism of exogenous and endogenous substances. Cytochrome P450 is a major phase-I enzyme responsible for drug metabolism, and phase-I enzymes like UDP-glucuronosyl transferase (UGT) and glutathione-S-transferase (GST) can accelerate the metabolism of environmental pollutes, carcinogens, poisons and son on [4-5]. Metabolic drug interactions mean two or more types of drugs have interferences to each other during metabolism after they are taken, which may enhance or weaken drug effect or produce toxics to human bodies. Forty percent of metabolic drug interactions occur during the four stages of pharmacokinetics, which are mainly caused by the inhibition or induction of CYP450 [6-10].

L-arabinose is a monosaccharide containing five carbon atoms and an aldehyde (CHO) functional group. It is the second most abundant sugar in plants after D- xylose. In nature, most monosaccharides exist as the “D”- form, but L- arabinose is an exception. However, L- arabinose is rarely present in the form of monosaccharides, but mainly in the form of heteropolysaccharide in hemicellulose, pectic acid and some glycosides [11]. In addition, L- arabinose is a low-calorie sweetener, with good physical activity and functions. It is classified as an anti-obesity nutritional supplement and non-prescription drug by U.S. Food and Drug Administration (FDA), a special health food additive used for regulating blood sugar level by Japanese Ministry of Health, Labour and Welfare. It was approved as a new resource food by Chinese Ministry of Health in 2008. Therefore, this study was conducted to detect the effects of L-arabinose on drug-metabolizing enzymes and antioxidant enzymes. The results may provide information to evaluate the potential drug interactions and to optimize dosing regimen when L-arabinose is used in combination with other drugs.

Materials and Methods

Reagents and kits

L-arabinose was purchased from Thomson Biotech (Xiamen) Co., Ltd. BCA protein assay kit was bought from Beyotime Institute of Biotechnology; $NADP^+$, glucose-6-phosphate, glycerol, glucose-6-phosphate dehydrogenase, glutathione, tritonx-100 and NADPH were purchased from Beijing Solarbio Science & Technology Co., Ltd. Aminopyrine, 2- aminophenol and 4-aminophenol were bought from Sinopharm Chemical Reagent Co., Ltd. Dexamethasone was a product of Zhengzhou LingRui Pharmaceutical Co., Ltd. Phenobarbital was purchased from Shanghai New Asiatic Pharmaceuticals Co., Ltd. Cytochrome C and UDP- glucuronic acid were purchased from Sigma Corporation. 1-chloro -2, 4-nitrobenzene was bought from Amresco. All other reagents were of analytical grade.

Instruments

The low speed benchtop centrifuge was purchased from Zhuhai Hema Medical Instrument Co, Ltd. The high-speed centrifuge MIKRO 200 was purchased from Heraeus Group and the ultracentrifuge Beck-Coulter Optima XL-100K from Beckman. TU-1901 UV-visible spectrophotometer was purchased from Beijing Pulaixi Technology Co., Ltd. The digital water bath HH-8 was purchased from Shenhua Guohua Power Co., Ltd. The electronic balance was purchased from Mettler-Toledo Instruments (Shanghai) Co., Ltd. The microplate reader was purchased from BioTek Instruments, Inc.

Experimental animals

SD male rats were provided by Hunan SJA Laboratory Animal Co., Ltd., with the number of manufacturing licence: SCXK (Xiang) 2009-0004.

Methods

Forty-eight rats were equally divided into six groups. The rats in three groups were orally administered with 500, 1 000 and 2 000 mg/kg L- arabinose for 38 consecutive days. In negative control group, L-arabinose solution was replaced by an equal volume of distilled water. In two positive control groups (administered with sodium barbital or dexamethasone), the rats were intraperitoneally injected with 80 mg/kg sodium barbital or dexamethasone for three consecutive days since the 36th day.

Measurement items

After the last dose of was given, all the rats were fasted for 24 h with free access to water, and then blood was sampled from their femoral artery. Serum was separated by low speed centrifugation and stored at -20 ºC for later analysis. Subsequently, the rats were sacrificed and their livers immediately excised, thoroughly washed with ice-cold 0.9% saline till the remaining blood was white clay-like. The liver samples were surface-dried with filter-paper, weighed, and immediately cut into pieces with a pair of scissors, soaked in ice-cold (4 ºC) 0.05 mol/L Tris- 0.15 mol/L KCl (pH7.4) buffer, homogenized, centrifuged at 4 000 r/min, 4 ºC for 15 min to remove the precipitate. A portion of the supernatant was stored, and the remaining was centrifuged at 10 000 g, 4 ºC for 20 min to remove the precipitate. Then, the supernatant was centrifuged again at 10 000 g, 4 ºC for 60 min. The resulting precipitate was microsomes, and the supernatant was cytoplasmic fluid. The assays for alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutathione S-transferase (GST), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), malondialdehyde (MDA), nitric oxide (NO), creatinine (Cr), urea nitrogen (Ur), total antioxidant capacity (T-AOC), hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), nitric oxide synthase (NOS), catalase (CAT), glutathione reductase (GR) and glutathione (GSH) were performed according to the manufacturer's instructions. Aminopyrine-N-demethylase activity (CYP3A), aniline hydroxylase activity (CYP2E1), GST, the content of cytochrome b_5, NADPH- cytochrome C reductase (P-450 reductase) activity and UDP- glucuronyl -transferases (UGT) activity were measured with the methods for pharmacological experiments.

Statistical analysis

All the data were expressed in terms of $\bar{x} \pm s$, and *t* test was performed to compare the data between groups. Microsoft Excel was adopted for data processing.

Results and Analysis

Effects of L- arabinose on serum ALT, AST and GST in rat

As shown in Table 1, the serum ALT, AST and GST in the rats administered with 500, 1 000 and 2 000 mg/kg of L-arabinose for 38 days did not change greatly, compared with those of negative control.

Table 1 Effects of L- arabinose on serum ALT, AST and GST ($\bar{x} \pm s$, n = 8)

Treatment	ALT (U/L)	AST (U/L)	GST (U/ml)
Negative control	5.52±1.66	20.14±1.91	24.98±11.03
500 mg/kg L-arabinose	4.79±2.40	20.64±2.82	25.31±6.25
1 000 mg/kg L-arabinose	4.26±2.04	20.32±1.98	23.81±3.58
2 000 mg/kg L-arabinose	5.70±2.27	19.63±1.88	30.22±11.74

Effects of L- arabinose on serum Cr and Ur in rat

It could be concluded from Table 2 the serum Cr and Ur in the rats administered with 500, 1 000 and 2 000 mg/kg of L-arabinose for 38 days had no significant difference from those of negative control.

Table 2 Effects of L- arabinose on serum Cr and Ur ($\bar{x} \pm s$, n = 8)

Treatment	Cr (μmol/L)	Ur (μmol/L)
Negative control	122.74±12.66	8.31±0.77
500 mg/kg L-arabinose	125.83±6.36	8.33±0.89
1 000 mg/kg L-arabinose	124.21±15.78	8.42±1.64
2 000 mg/kg L-arabinose	150.90±5.92	8.12±1.91

Effects of L- arabinose on serum T-AOC, SOD, GSH-PX, MDA and NO

As it could be seen from Table 3, serum NO in the rats administered with 500 mg/kg of L-arabinose for 38 days was significantly lower than that of negative control (at P<0.05 level); serum GSH-PX in the rats administered with 500 mg/kg of L-arabinose for 38 days showed an extremely significant reduction, compared with that of negative control (at P<0.01 level); serum NO in the rats administered with 1 000 mg/kg of L-arabinose for 38 days showed an extremely significant decrease (at P<0.01 level) and that in the rats administered with 2 000 mg/kg of L-arabinose for 38 days showed a significant decrease (at P<0.05 level), compared with that of negative control. Serum SOD in the rats administered with 2 000 mg/kg of L-arabinose for 38 days showed a significant reduction, compared with that of negative control (at P<0.05 level), and serum GSH-PX in the rats administered with 2 000 mg/kg of L-arabinose for 38 days showed an extremely significant reduction, compared with that of negative control (at P<0.01 level).

Table 3 Effects of L- arabinose on serum T-AOC, SOD, GSH-PX, MDA and NO ($\bar{x}\pm s$, n = 8)

Treatment	SOD (U/ml)	GSH-PX [μmol/(min•ml)]	MDA (nmol/ml）	NO (μmol/L)	T-AOC (unit)
Negative control	184.88±10.26	339.49±37.15	4.88±0.88	19.86±3.41	2.99±1.61
500 mg/kg L-arabinose	180.11±13.12	288.02±19.44**	5.05±1.18	14.63±4.13*	4.63±3.00
1 000 mg/kg L-arabinose	178.19±21.78	315.02±40.79	4.90±0.68	9.65±4.12**	3.94±2.21
2 000 mg/kg L-arabinose	169.48±15.71*	275.09±32.97**	5.00±0.78	13.24±3.63*	4.05±0.75

*represents significant differences at P<0.05 level and **represents extremely significant differences at P<0.01 level, compared with the negative control.

Effects of L- arabinose on SOD, MDA, NOS, NO, CAT and H_2O_2 in liver tissue

As could be seen from Table 4, compared with that of the negative control, the NOS activity and NO content in liver tissue of the rats administered with 500 mg/kg of L-arabinose for 38 days showed an extremely significant reduction, (P <0.01); and the NOS activity of the rats administered with 2 000 mg/kg of L-arabinose also showed an extremely significant reduction (P <0.01); and the MDA content of the rats administered with 2 000 mg/kg of L-arabinose showed a significant decrease (P<0.05)

Table 4 Effects of L- arabinose on SOD, MDA, NOS, NO, CAT and H_2O_2 in liver tissue of rat ($\bar{x}\pm s$, n = 8)

Treatment	SOD （U/mg）	MDA （nmol/mg）	NOS （U/mg）	NO （μmol/L）	CAT (U/g)	H_2O_2 (U/g)
Negative control	487.18±61.09	0.46±0.21	1.53±0.17	0.22±0.04	80.95±9.17	3.62±0.30
500 mg/kg L-arabinose	474.38±44.87	0.39±0.12	1.25±0.06**	0.16±0.02**	74.30±12.85	3.59±0.37
1 000 mg/kg L-arabinose	541.59±62.42	0.32±0.14	1.39±0.40	0.24±0.14	75.25±24.24	4.12±0.82
2 000 mg/kg L-arabinose	501.51±29.06	0.20±0.08*	1.14±0.21**	0.21±0.05	72.47±8.79	3.74±0.81

*represents significant differences at P<0.05 level and **represents extremely significant differences at P<0.01 level, compared with the negative control.

Effects of L- arabinose on GSH, T-AOC, GST, GSH-PX, GR and ·OH in liver tissue of rat

It could be concluded from Table 5 that the GST activity in liver tissue of rat administered with 2 000 mg/kg of L-arabinose for 38 days was significantly increased, compared with that of the negative control (P<0.05).

Table 5 Effects of L- arabinose T-AOC, GST, GSH-PX, GR, · OH and GSH in liver tissue of rat ($\bar{x}\pm s$, n = 8)

Treatment	T-AOC （unit）	GST （U/mg）	GSH-PX [μmol/(min•mg)]	GSH （mg GSH•G）	GR (U/g)	·OH (U/mg)
Negative control	1.56±0.12	130.89±23.33	436.65±87.26	2.39±0.39	2.14±0.44	1054.45±87.79
500 mg/kg L-arabinose	1.64±0.31	134.33±12.53	388.25±77.52	2.15±0.25	2.12±0.53	1035.43±105.47
1 000 mg/kg L-arabinose	1.76±0.37	122.08±14.48	434.77±77.87	2.74±0.98	2.24±0.45	1033.98±92.24
2 000 mg/kg L-arabinose	1.51±0.26	161.54±130.80*	419.24±93.29	2.13±0.83	1.96±0.31	1026.99±128.79

*represents significant differences at P<0.05 level , compared with the negative control.

Effects of L- arabinose on GST and UGT activity in liver tissue of rat

It could be concluded from Table 6 that UGT activity in liver tissue of the rats administered with 1 000 mg/kg of L-arabinose for 38 days exhibited an extremely significant decrease (P<0.01), compared with that of the negative control. Compared with the negative control, the treatment with 2 000 mg/kg L-arabinose significantly improved GST activity (P<0.05), and extremely and significantly reduced UGT activity (P<0.01); the treatment with sodium barbital extremely and significantly increased the GST and UGT activities in liver tissue (P<0.01); the treatment with dexamethasone also significantly increased UGT activity (P<0.05).

Table 6 Effects of L-arabinose on GST and UGT in liver tissue of rat ($\bar{x}\pm s$, n = 8)

Treatment	GST (U/mg)	UGT [nmol/(min•mg)]
Negative control	621.49±97.90	0.27±0.04
500 mg/kg L-arabinose	715.13±100.98	0.23±0.04
1 000 mg/kg L-arabinose	616.72±125.32	0.14±0.04**
2 000 mg/kg L-arabinose	783.91±104.26*	0.10±0.05**
80 mg/kg Sodium barbital	997.42±91.48**	0.42±0.12**
80 mg/kg Dexamethasone	--	0.33±0.05*

*represents significant differences at P<0.05 level and **represents extremely significant differences at P<0.01 level, compared with the negative control.

Effects of L-arabinose on CYP3A, CYP2E1, cytochrome b5, NADPH- cytochrome C reductase activities in liver tissues of rat

As shown in Table 7, compared with the negative control, the treatment with sodium barbital significantly improved CYP3A activity (P<0.05); the treatment with dexamethasone extremely and significantly increased CYP3A activity and NADPH- cytochrome C reductase activity (P <0.01).

Table 7 Effects of L-arabinose on CYP450 in liver tissue of rat ($\bar{x} \pm s$, n = 8)

Treatment	CYP3A [nmol/(min•mg)]	CYP2E1 [nmol/(min•mg)]	Cytochrome b_5 (nmol/mg)	P-450 reductase [nmol cytochrome C /(min•mg)]
Negative control	0.64±0.55	0.061±0.022	0.65±0.09	2.21±0.61
500 mg/kg L-arabinose	0.52±0.31	0.057±0.028	0.63±0.10	2.18±0.31
1 000 mg/kg L-arabinose	0.78±0.67	0.055±0.036	0.57±0.10	2.25±0.41
2 000 mg/kg L-arabinose	1.13±0.92	0.066±0.026	0.74±0.16	2.53±0.68
80 mg/kg Sodium barbital	1.48±0.71*	--	0.59±0.12	2.71±1.53
80 mg/kg Dexamethasone	1.54±0.44**	--	0.71±0.24	4.57±1.92**

*represents significant differences at P<0.05 level and **represents extremely significant differences at P<0.01 level, compared with the negative control.

Conclusions

ALT and AST are both sensitive indicators of liver injury. GST is a phase-Ⅱ drug-metabolizing enzymes abundantly found in liver cells, and serum GST activity is positively correlated with the degree of liver injury [12]. This study found that the liver ALT, AST and GST activities of the rats administered with L- arabinose for a long period of time were not increased, indicating that L-arabinose was not toxic to the liver. The generation, metabolism and discharge of Cr and Ur were little affected by extra-renal factors, and thus can be used as ideal indices of kidney function [13]. This study found that the Cr and Ur levels in rats administered with L-arabinose were not significantly increased, suggesting that L-arabinose was not toxic to kidney. SOD, an important intracellular oxygen radical-scavenging enzyme, catalyzes the dismutation of superoxide anion into HO and oxygen molecules, and thus protects cells from the damage caused by free radicals [14]. GSH-PX is an important enzyme that catalyzes the decomposition of hydrogen peroxide, and it also catalyzes the reduction of alkyl hydroperoxide and phospholipid hydroperoxide, protecting extracellular fluid and cell surface from the damage caused by peroxides [15]. It is found that oxidative stress is a major contributor to kidney injury. A dynamic equilibrium exists between oxidation and antioxidation under normal circumstances, and it will be broken when the antioxidant defense system is damaged, and the free radicals will be greatly increased. And the increased free radicals may cause injury to kidney. Our results revealed that L- arabinose can suppress the activities of serum SOD and GSH-PX. NO is an extremely unstable biological radical, and widely distributed in various tissues, and in particular nervous tissues. It is a new biological messenger molecule, generated by the catalysis of nitrogen oxide synthase (NOS), and plays an important role in cardiovascular, cerebrovascular, nervous and immune regulation. However, we found that L-arabinose at all doses reduced the NO level in rat serum; 2 000 and 5 000 mg/kg L-arabinose greatly reduced the NOS activity in liver tissue, and 2 000 mg/kg L-arabinose also greatly reduced the NO concentration in liver tissue, indicating that L- arabinose possibly change cardiovascular, cerebrovascular, nervous and immune regulation in human bodies.

Cytochrome P450 enzyme system consists of three parts: hemoglobin, including cytochrome P450 and cytochrome b5; flavin proteins, including NADPH- cytochrome C reductase and NADPH-cytochrome b5 reductase; lipids, mainly including phospholipids choline. All the three

parts together constitute the electron transfer system. Cytochrome b5 is a part of electronic transfer of cytochrome P450, which is involved in drug oxidation. NADPH- cytochrome C reductase transfers the electrons from NADPH to cytochrome P450 during the metabolism of certain drugs, and thus converts the oxidized form of cytochrome P450 to its reduced form. Among all the members of cytochrome P450 superfamily, CYP3A is the most important. According to previous studies, about 50% of commercially available drugs are metabolized by CYP3A [6-8]. CYP2E1 converts the procarcinogens into carcinogens by catalyzing dealkylation and denitration. Its activity varies slightly in different organisms. So far it has been proven that the activity of CYP2E1 is the same in human and animals, so the information of CYP2E1 from animals has important reference significance to human [16]. Induced phase-Ⅱ metabolic enzymes such as uridine diphosphate -glucuronyl transferase and glutathione transferase can speed up the metabolism of environmental pollutes, carcinogens, poisons and etc. Glutathione -S- transferase (GST), a phase-Ⅱ metabolic enzyme with multiple functions, catalyzes the reactions between GSH and various electrophiles, free radicals in living bodies, so that GSH can be excreted with bile or urine. UGT is the most important microsomal enzyme for phase II biotransformation of chemical substances in living bodies. About 35% of the drugs metabolized *via* phase-II enzymes were metabolized through UGT. After entering into liver cells, free bilirubin is combined to glucuronide at first under the catalysis of UGT-1 to form water-soluble bilirubin glucuronide and thus can be excreted [17]. Pentobarbital sodium is a classical inducer to CYP3A, GST and UGT; dexamethasone is also a classical inducer to CYP3A and UGT. Our results found that pentobarbital sodium significantly improved the activities of CYP3A, GST and UGT; dexamethasone significantly increased the activities of CYP3A and UGT, suggesting that the results of this study were reliable. L-arabinose had little effects on cytochrome b5, NADPH- cytochrome C reductase, CYP3A and CYP2E1 levels in rat liver tissue. 2 000 mg/kg L-arabinose improved GST activity in liver tissue; 2 000 and 1 000 mg/kg L-arabinose increased UGT activity in liver tissue, indicating that L-arabinose had no effects on drug metabolizing enzymes. The results proved L-arabinose can be used safely. As it can reduce UGT activity, L-arabinose should be used cautiously for the treatment of pathological jaundice.

Acknowledgements

This work was funded by the Natural Science Foundation of Guangxi (Nos. 2015GXNSFBA139120); Bagui Scholar Program of Guangxi（Guangxi Institute of Botany）.

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