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• BASIC RESEARCH •

Effects of long-term tea polyphenols consumption on hepatic microsomal drug-metabolizing enzymes and liver function in Wistar rats

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Abstract

AIM: To investigate the effects of long-term tea polyphenols (TPs) consumption on hepatic microsomal drug-metabolizing enzymes and liver function in rats.

METHODS: TPs were administered intragastrically to rats at the doses of 833 mg· kg^{-1}· d^{-1} (n=20) and 83.3 mg· kg^{-1}· d^{-1} (n=20) respectively for six months. Controlled group (n=20) was given same volume of saline solution. Then the contents of cytochrome P450, b5, enzyme activities of aminopyrine N-demethylase (ADM), glutathione S-trasferase (GST) and the biochemical liver function of serum were determined.

RESULTS: The contents of cytochrome P450 and b_5 in the livers of male rats in high dose groups (respectively 2.66±0.55, 10.43±2.78 nmol· mg MS pro^{-1}) were significantly increased compared with the control group (1.08±1.04, 5.51±2.98 nmol· mg MS pro^{-1}; P<0.01, respectively). The enzymatic activities of ADM in the livers of female rats in high dose groups (0.91±0.08 mmol· mg MS $pro^{-1}min^{-1}$) were increased compared with the control group (0.82±0.08 mmol· mg MS pro^{-1}· min^{-1}; P<0.05). The GST activity was unchanged in all treated groups, and the function of liver was not obviously changed.

CONCLUSION: The antidotal capability of rats' livers can be significantly improved after long-term consumption of TPs. There are differences in changes of drug-metabolizing enzymes between the sexes induced by TPs and normal condition.

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INTRODUCTION

Tea polyphenols (TPs) are a large and diverse class of compounds extracted from tea. These polyphenolic compounds, specifically catechins epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), and epicatechin-3-gallate (ECG), account for 30-40 % of the extractable solids in green tea leaves[1]. Many health benefits are associated with consumption of tea and such effects are mainly attributed to the polyphenolic constitutes of tea[2-8]. We are more concerned about the beneficial effect of TPs on cancer[9-13].

Primary liver cancer (PLC) is a very prevalent form of cancer in the world. The incidence of PLC in China is high. Guangxi Zhuang Autonomous Region is a high mortality and morbidity region of PLC. Unfortunately, its curative effect is disappointed no matter what therapy is used. How to improve the prevention and treatment of PLC is a long-term goal of our research work. There are some evidences indicating that TPs may play a positive role in PLC prevention and treatment[14-16]. However, the mechanism of the action is not fully understood. It is known that the risk factors of PLC are intake of AFB_1, pollution of drinking water and HBV infection[17-20]. The factors are closely related to the activity of hepatic microsomal drug metabolizing enzymes and function of the liver. Because bioactivation of precarcinorgens and detoxification of ultimate carcinogens are mainly carried out by drug metabolizing enzymes in the liver, to explore the effects of TPs on these enzymes and the liver function will be helpful to understanding the mechanism of TPs in prevention and treatment of PLC, and the safety of TPs. Further more, this work will provide some useful information for the application of TPs in PLC chemoprevention and chemotherapy.

MATERIALS AND METHODS

Chemicals and reagents

TPs were purchased from Shili Natural Food Co. Ltd (Guilin, China), NADPH from Lizhudongfang Biological Technology Co. (Shanghai, China), aminopyrine from Shanghai Chemical Co., glutathione S-transferase (GST) and protein assay kits from Jiancheng Biological Technology Institude (Nanjing, China), serum biochemical tests of liver function kits from Shenneng Co. (Shanghai, China).

Animals

Five-week-old Wistar rats weighing from 80 to 130 g provided by Experimental Animal Center of Guangxi Medical University were used in the study. The rats were randomly divided into high dose, low dose, and control groups, 20 each group. The animals in high dose and low dose groups were administered intragastrically with TPs at doses of 833 mg· kg^{-1}· d^{-1} and 83.3 mg· kg^{-1}· d^{-1} respectively six times each week for six months. Same procedures were also performed in the control rats except feeding equal amount of normal saline (1.0 ml· 100 g^{-1}· day^{-1}) instead of TPs. The animals were housed in a temperature-controlled room at 22 ℃-24 ℃ and fed with standard rat chow. At the end of six months experimental period, all the rats were anesthetized with intramuscular injection of sodium pentobarbital (30 mg/kg) before sacrificed. Blood was collected

from the heart and serum obtained through centrifugation to measure liver function. The livers were removed immediately, perfused with cold 0.15M KCl and homogenized in 4 volumes of 0.15M KCl solution containing 10 mM EDTA using a Potter-type Teflon glass homogenizer. The homogenate was centrifuged, 10 000×g for 15 min at 4 ℃ in a refrigerated centrifuge (OM 3593 IEC Co. Ltd.USA). The supernatant was then centrifuged 105 000×g for 60 min at 4 ℃ in a preparative ultracentrifuge (20PR-52D; Hitachi, Tokyo). The pellet of microsomes was suspended in the homogenization solution in the homogenizer and centrifuged again as described above. The resulting pellet was suspended in 20 mM potassium phosphate buffer (PH7.4) containing 15 % glycerol until analysis.

Microsomal enzyme assays

The content of cytochrome P450 was determined by the method of Omura and Sato[21,22]. The content of cytochrome b5 was assayed as described by Omura and Takesue[23]. The activities of ADM were determined as described by Imai *et al*[24]. The content of liver microsomal protein and the activities of GST were measured as described in the booklet of kits. All the microsomal enzymes were assayed by using a spectrophotometer (DU-64; Beckman, Fullerton, CA, USA).

Biochemical liver function tests

Biochemical liver function tests (ALT, AST, TP, and ALB) were performed by using an automatic biochemical analyzer (7170A, Hitachi, Tokyo).

Statistical analyses

Data were counted separately in male and female rats and expressed as $\bar{x}\pm s$. Statistical significances were analyzed by *t*-test. The difference was considered significant in case of a two-tailed *P* value less than 0.05, and $P<0.01$ as very significant.

RESULTS

Effects of TPs on contents of P450, b5 and activities of ADM and GST

In high dose group, the contents of P450 and b5 were significantly increased in male rats (respectively 2.66±0.55, 10.43±2.78 nmol· mg MS pro^{-1}) compared with those in the control group (1.08±1.04, 5.51±2.98 nmol· mg MS pro^{-1}; $P<0.01$, respectively). The enzymatic activities of ADM in female rats (0.91±0.08 mmol· mg MS $pro^{-1}min^{-1}$) were higher than those in the control group (0.82±0.08 mmol· mg MS $pro^{-1}\cdot min^{-1}$; $P<0.05$). But the activities of GST were unchanged in all treated groups. In control group, the contents of b5 and the activities of ADM in male and female rats were significantly different (5.51±2.98, 13.42±1.85 nmol· mg MS pro^{-1}; 0.92±0.11, 0.82±0.08 mmol· mg MS $pro^{-1}min^{-1}$, respectively, $P<0.05$). The results indicated that there was a difference of hepatic microsomal drug-metabolizing enzymes under normal conditions in different sex rats (Table 1).

Effects of TPs on biochemical liver functions

TPs did not damage rat liver function after used for a long-term, and it indicated that TPs were a quite safe agent, even at a high dose of 833.3 mg· $kg^{-1}\cdot d^{-1}$, for six months (Table 2).

DISCUSSION

Hepatic drug metabolizing enzyme is called mixed-function oxidase or monooxygenase containing many enzymes including phase I enzymes such as cytochrome P450, cytochrome b5 and NADPH-cytochrome P450 reductase and phase II enzymes such as GST, sulfatase and UDP-glucuronyl transferase[25]. AFB1, one of the risk factors of PLC, damages DNA after conversion to the reactive compound AFB1-epoxide, by the action of cytochrome P450-dependent enzymes[26]. Sufficient evidences have shown that tea and TPs possessed anticarcinogenic effects[27-32]. Some works have been done in the field of TPs modulated or interacted with drug metabolizing enzymes. Maliakal *et al* reported that treating with green tea from different sources could markedly increase cytochrome P450 1A2 activity in rats, and green tea from certain sources could increase cytochrome P450 1A1 and cytosolic GST activities[33]. However, *in vitro* experiment, Mukhtar *et al* and Wang *et al* reported that TPs had an inhibitory effect on microsomal cytochrome P450 enzyme system[34,35]. Until now, no one could give a clear explanation of the different results. We tried to make clear what would happen in these enzyme activities in rats treated with TPs. Considering PLC chemopreventive and chemotherapic effects could not be achieved in a short term of TPs administration, and a long-term experiment has not been carried out in this aspect, so the rats were treated for 6 months. At the end of treatment, we determined the contents of cytochrome P450 and b5, the activities of ADM and GST, and the liver function in the rats. The results showed that the contents and activities

Table 1 Effects of long-term TPs consumption on microsomal enzymes

	Group	P450 nmol / mg MS pro	b5 nmol / mg MS pro	ADM mmol / mg MS pro / min	GST U / mgpro
♂	High dose (n=10)	2.66±0.55[a]	10.43±2.78[a]	0.90±0.12	24.66±4.06
	Low dose (n=10)	1.94±0.90	7.82±1.66	0.94±0.11	27.05±4.59
	Control (n=10)	1.08±1.04	5.51±2.98[c]	0.92±0.11[c]	25.88±4.02
♀	High dose (n=10)	0.66±0.42	11.74±2.31	0.91±0.08[b]	29.48±4.16
	Low dose (n=10)	0.66±0.38	11.34±3.17	0.73±0.09	26.44±4.54
	Control (n=10)	0.36±0.18	13.42±1.85	0.82±0.08	29.40±4.19

[a]$P<0.01$ *vs* ♂ control, [b]$P<0.05$ *vs* ♀ control, [c]$P<0.05$ *vs* ♀ control.

Table 2 Effects of long-term TPs consumption on major biochemical parameters of rat liver

	Group	ALT U / L	AST U / L	TP g / L	ALB g / L
♂	High dose (n=10)	76.31±32.0	294.69±68.8	75.26±3.44	32.96±1.39
	Low dose (n=10)	74.75±11.62	285.4±54.95	78.75±1.83	32.71±1.34
	Control (n=10)	65.5±9.89	271.5±37.32	80.97±3.43	32.42±1.90
♀	High dose (n=10)	59.15±9.14	247.3±61.03	81.43±3.87	34.64±1.11
	Low dose (n=10)	50.31±22.32	213.15±75.92	78.76±5.31	34.86±2.30
	Control (n=10)	56.92±7.62	236.08±51.94	79.56±2.35	35.63±1.06

ALT: serum alanine transaminase, AST: serum asparate transaminase, TP: total protein, ALB: albumin.

of drug metabolizing enzymes and the antidotal capability of liver were significantly improved in the high dose group. It shortened the time of carcinogen staying in the body and reduced DNA damages. Therefore, TPs could protect human against the risk of chemically induced PLC and other cancers.

Gender differences in drug metabolism in rats have been known for more than 60 years since it was reported that the much shorter duration of drug action in the male was due to the effects of testicular androgens[36]. The activities of hepatic drug-metabolizing enzymes, especially cytochrome P450 and sulfotransferase, were regulated through the sex-related secretion pattern of growth hormone[37]. Some studies reported the sex-related effect on drug -metabolizing enzymes[38,39]. In our study, a marked sex difference in the effects of long-term treatment with TPs on hepatic drug-metabolizing enzymes in rats was observed. In control groups, there were differences between male and female rats. The results indicated that there was a sex difference in activities of hepatic drug-metabolizing enzymes and ability of liver detoxification in normal rats. Epidemiological studies of PLC showed that there was a sex difference in human (male>female). But it is not known whether this difference is related to the difference of hepatic drug-metabolizing enzymes.

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