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Antioxidant activity of Qizhu Tang

WANG Xue-Jiang[1], FENG Ping
(*Beijing Union University, Traditional Chinese Medicine and Pharmacology College, Beijing 100013, China*)

KEY WORDS Radix Astragali; Rhizoma Atractylodis; *Poria cocos*; *Panax notoginseng*; Qizhu Tang; antioxidants

ABSTRAC

AIM: To study the antioxidant activity of Qizhu Tang (QZT) both *in vivo* and *in vitro*. **METHODS**: QZT consists of 4 herbal constituents (Rhizoma Atractylodis Macrocephalae, *Poria cocos*, Radix Notoginseng, and Radix Astragali), each of the components and their combinations were examined *in vitro* for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radical scavenging activities, and for the inhibition of thiobarbituric acid-reactive substances (TBARS) formation in rat liver homogenate. At the same time, their *in vivo* protective effect on cerebral ischemia-areperfusion injury was determined in rats. **RESULTS**: Only the preparations having a higher antioxidant activity comparable to QZT in all three *in vitro* assays were relatively active *in vivo* both for TBARS inhibition and glutathione peroxidase preservation, although the activities were much lower than that of QZT as a whole. **CONCLUSION**: QZT formula is a good natural antioxidant having an effective preventive effect against cerebral ischemia reperfusion damgge.

INTRODUCTION

Oxidative stress attracts much attention as a causative factor in numerous diseases[1]. Lipid peroxidation initiated by reactive oxygen and nitrogen species is implicated in aging, carcinogenesis and many other pathophysiological conditions[2-3]. The oxy free radicals also play a crucial role in cerebral ischemia-reperfusion injury[4]. The brain, however, furnishes relatively poor defense mechanisms against free radical injury such that the levels of antioxidant molecules, and also of radical or peroxide scavenging enzymes are low[5]. Brain ischemia following reperfusion leads to free radical production. The free radical chain reaction is thus the central pathological link of brain ischemic injury. Therefore, the antioxidant therapy attracts much attention to prevent or ameliorate the brain pathophysiological conditions induced by oxidative stress[6]. In the present study, we examined the antioxidant activities of Qizhu Tang (QZT) and its components both *in vivo* and *in vitro*, to see if QZT was able to effectively prevent the cerebral oxidative damage in rat produced by ischemia-reperfusion.

MATERIALS AND METHODS

Chemicals and herbal materials 2,2′-Azobis (2-amidino-propane) dihydrochloride (AAPH), glutathione reduced form (GSH), 2-thiobarbituric acid (TBA) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Wako Co, Ltd, Japan. Disodium edetic acid was obtained from Kanoto Chemical Co, Ltd, Japan. Sodium dodecy sulfate (SDS) was from Nakarai Co, Ltd, Japan. β-reduced nicotinamide adenine dinucleotide phosphate (β-NADPH) and GSH reductase were from Sigma Co, Ltd, USA, and dimethylpyrroline oxide (DMPO) were purchased from Labtec Co, Ltd, Tokyo. All other chemicals were of analytical grade.

Dried herbal materials, including Rhizoma Atractylodis Macrocephalae (B), *Poria cocos* (F), Radix Notoginseng (S), and Radix Astragali seu Hedysari (H) were obtained from Magiya Pharmacy Co, Ltd, Niigata, Japan.

QZT and related preparations QZT was prepared by mildly boiling a mixture of B (24 g), F (18 g), S (36 g) and H (36 g) in 250 mL of distilled water for 30 min after soaking at room temperature for 1 h. The decoction was then filtrerted with delipidated gauze and stored at 40 ℃ until use. The concentration of the decoction was 1.14 g dried herb mixture/mL. Similarly, the preparations containing each QZT component alone or the combinations lacking one or two component

[1] Correspondence to Assoc Prof WANG Xue-Jiang.
Phn 86-10-6422-9801. Fax 86-10-6423-7091.
E-mail Wang-xj@netease.com
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herbs from the complete QZT formula were decocted in the same way as above using the same amount of component herb(s) as in the QZT formula.

A rat model of cerebral ischemia-reperfusion Male Wistar rats (6 weeks old and 160 – 182 g body weight) purchased from the SLC Inc, Japan were allowed access to pelletted diet and water before experiment.

A small incision was given at the abdomen to expose duodenum under anesthesia, then QZT and its related preparations were administered directly into the lumen of the duodenum with a syringe at a dose of 4 mL/rat 2 h before the cerebral ischemia operation. For saline control, rats were administered saline instead of test preparations. The normal control rats were not given any treatment.

For induction of ischemia, a middle ventral incision was made in the neck. Both right and left common carotid arteries were exposed and occluded using nontraumatic aneurysm clips. After 85 min of ischemia, the aneurysm clips were removed to restore blood flow (reperfusion).

Brain sample collection and biochemical assaying After reperfusion for 45 min, the rats were decapitated under anesthesia, and the whole brain was removed quickly, rinsed with saline, and then frozen in a freezer (−80 ℃) until use. The tissue was suspended in cold 0.05 mol/L phosphate buffer containing 1.15 % (w/v) KCl in a ratio of 1 g wet tissue/9 mL, then homogenized using a glass homogenizer at 0 ℃.

Thiobarbituric acid-reactive substances (TBARS) were determined according to the method by Ohkawa, *et al*[7]. GPX activity was determined according to the method of Albrecht[8].

Determination of *in vitro* radical scavenging activity The radical scavenging activity of QZT and its related preparations was determined for DPPH radical according to the method by Yoshida[9]. AAPH-initiated lipid peroxidation was determined by TBARS formation in normal rat liver homogenate[10]. The effects of QZT and its related preparations on hydroxyl radical produced by Fenton reaction were studied by the spin trapping ESR method[16].

Statistical treatment of data Triplicate determinations were carried out for each sample and the data are given as $\bar{x} \pm s$ of 9 to 10 rats in *in vivo* and *in vitro* experiment. Data were evaluated by t-test and a P value <0.05 was accepted as statistically significant.

RESULTS

Effect of QZT and its related preparations on DPPH quenching, and DMPO-OH and TBARS formations *in vitro* Since QZT formula consists of 4 herbal components, the antioxidant activities of each component and the combinations were examined separately to know how each component of QZT contributes towards the antioxidant activity of the complete QZT formula.

First, DPPH radical quenching was examined. Both QZT formula and HBS combination showed a marked quenching activity for DPPH radical. Any other combination and the components alone did not show any significant quenching. Hydroxyl radical scavenging activity was determined for QZT and the component herbs using ESR spin trapping method in rat liver homogenate. The activity of HBS combination was comparable to that of QZT and was the highest among the preparations tested, followed by HBF, HS, and HB combinations. F, in contrast, behaved as a potent prooxidant rather than scavenger under the experimental conditions, likewise BF combination showed a weak prooxidant activity.

The antioxidant activities of QZT and the component herbs were examined for TBARS formation induced by Fenton reaction in rat liver homogenate. QZT formula showed the highest activity, followed by HBS combination (approximately 79 % of QZT). Considerable inhibition was also achieved by B, S, and HB, and moderate inhibition by HS, BS combinations, and H. It was notable that F and all the F containing preparations except complete QZT showed a very low inhibitory activity toward TBARS formation. Both HBF and HF combinations even behaved as prooxidants (Tab 1).

Comparison of *in vivo* and *in vitro* antioxidant activities of QZT and the related preparations To determine which *in vitro* biochemical parameter determined above was more reliably related to *in vivo* antioxidant activity, preventive effect on cerebral ischemia-reperfusion damage was studied in rat for several QZT-related preparations. The QZT formula showed the highest prevention for the TBARS formation in the damaged brain, followed by HBS combination. Although HBS combination showed rather stronger DPPH and hydroxyl radical quenching activities that QZT *in vitro* (approx 101 and 109 % of QZT, respectively), the *in vivo* activity was only 46 % of QZT. Further more, HBF and HS combinations which also showed potent hydroxyl radical scavenging activity comparable to QZT did not

significantly inhibit the TBARS formation *in vivo*. F was not effective at all. The same trend was observed in their action on GPX activity in the damaged brain such that only HBS showed a moderate protective effect. The superiority of the QZT formula was more clearly demonstrated in this case as the protective activity of HBS combination was only 33 % of QZT (cf 46 % in TBARS inhibition) (Tab 2).

Tab 1. *In vitro* radical quenching and antioxidant activity of QZT and related preperations. $n = 3$ determinations. $\bar{x} \pm s$.

Composite	Relative inhibitory activity/%		
	DPPH	TBARS	DMPO-OH
QZT	100	100	100
H+B+F	17.1±0.2	12.9±0.6	95.4±0.6
H+B+S	100.5±0.2	79.3±0.5	108.9±0.1
H+F+S	15.8±0.2	1.9±0.3	65.2±0.3
B+S+F	5.5±0.3	19.5±0.3	37.0±0.3
H+B	11.0±0.3	49.1±0.3	71.0±0.6
H+F	24.6±0.3	26.3±0.6	47.6±0.6
H+S	17.3±0.2	35.9±0.5	90.7±0.6
B+F	8.3±0.3	7.4±0.5	5.8±0.6
B+S	18.0±0.2	32.4±0.5	55.8±0.6
S+F	7.8±0.2	1.9±0.3	21.0±0.6
H	15.7±0.4	29.6±0.5	63.6±0.6
B	15.8±0.3	58.2±0.6	58.4±0.6
F	0.4±0.3	0.0±0.4	64.9±0.6
S	21.7±0.3	58.2±0.5	27.2±0.6

QZT: Qizhu Tang; B: Rhizoma Atracylodis Macrocephalae; S: *Radix Notoginseng*; H: *Radix Astragali seu Hedysari*; F: *Poria cocos*.

DISCUSSION

In the present study, we demonstrated that a Chinese traditional medicine, QZT that showed a marked antioxidant activity *in vitro* also had a high potential to prevent the oxidative damage in the rat brain after ischemia-reperfusion.

Since hydroxyl radical is the most reactive and toxic species among the reactive oxygen species[11], the *in vivo* protection of cerebral oxidative damage after ischemia-reperfusion was studied in rats for the preparations which showed high hydroxyl radical scavenging activity comparable to QZT formula. The results revealed that only complete QZT was effective to prevent the cerebral oxidative damage in rats indicating that the hydroxyl radical scavenging activity *in vitro* could not be a sole indication of *in vivo* antioxidant potential. No other preparations gave rise to significant protective activity except the HBS combination which showed strong antioxidant activities comparable to QZT in all three antioxidant assays. Thus any single antioxidant assay *in vitro* may not be sufficient to estimate the *in vivo* effectiveness.

The present study revealed, however, an important and interesting role of F in the combination formula. F behaved as a prooxidant rather than an antioxidant in the hydroxyl radical scavenging activity, and also contributed negatively to the TBARS inhibitory action of the preparations. However, when F was combined with HBS to complete the QZT formula, F remarkably enhanced the *in vivo* protective activity toward the cerebral oxidative damage in rats. Although further study is needed, it! is suggested that F plays a key role in modulating the mixed formula to be active as an antioxidant in the brain.

Since lipid peroxidation is a major step involved in the progression of brain damage after ischemia-reperfusion[4], several trials have been carried out using small molecular antioxidants such as lipoic acid[12] to intervene the damage progression in the brain. However, the effect was limited probably because several other steps besides lipid peroxidation are contributing towards the cerebral

Tab 2. Prevention of cerebral ischemia-reperfusion damage in rat by Qizhu Tang and the related preperations. $n = 10$ rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* saline control.

Composite combination	TBARS (nmol/mg protein)	Relative inhhibitory activity/%	GPX activity (NADPH oxidized nmol/mg protein·min^{-1})	Relative prevention activity/%
Normal control	16.93+0.23		0.098+0.002	
Saline control	23.52+0.18		0.049+0.001	
QZT	17.67+0.14^c	100	0.091+0.002^c	100
H+B+S	20.84+0.29^c	45.8	0.063+0.002^b	33.3
H+B+F	21.70+0.26	31.1	0.056+0.001	16.7
H+S	21.98+0.29	26.1	0.056+0.002	16.7
F	23.53+0.39	0	0.056+0.001	16.7

QZT: Qizhu Tang; B: Rhizoma Atracylodis Macrocephalae; S: Radix Notoginseng; H: Radix Astragali seu Hedysari; F: *Poria cocos*.

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oxidative damage after ischemia-reperfusion such as the release of excitable amines[13], Ca^{2+}[14], cytokines[15], and inducible NO[16]. Therefore, it is reasonable to consider an antioxidant based combination therapy in which antioxidant(s) are formulated with additional factors affecting on other target steps involved in damage progression or repair process. In this sense, Chinese traditional medicine is interesting because it usually consists of several different herbal components. Although the functional contribution of each herbal component is not yet fully verified in the complete formula, propriety of above idea becomes clear in the present study when the *in vivo* effectiveness of QZT and HBS was compared. The considerable difference in the activity between these two preparations clearly indicates that the antioxidant potency is not the sole factor determining the *in vivo* effectiveness of QZT.

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芪术汤的抗氧化作用

王学江，丰 平
（北京联合大学中医药学院，北京 100013，中国）

关键词 黄芪；白术；茯苓；三七；芪术汤；抗氧化剂

目的：探讨芪术汤的抗氧化作用. **方法**：芪术汤由黄芪、白术、三七和茯苓四种中药组成. 对芪术汤全方研究的同时，进行该方的拆方研究及单味药研究，以 DPPH、氧自由基清除活性以及药物对鼠肝组织匀浆中 TBARS 形成的抑制等项实验作为检测指标，进行体外抗氧化活性观察. 同时，复制大鼠脑缺血/再灌注损伤的动物模型，观察芪术汤在预防和治疗脑缺血/再灌注损伤中的作用. **结果**：芪术汤及其三种不同形式的组方、部分单味药都具有较好的体、内外抑制 TBARS 的形成，提高 GPX 酶活性的功能，但芪术汤全方比其他形式的组方及单味药具有更高的抗氧化活性. **结论**：芪术汤是良好的抗氧化损伤天然药物，对脑缺血/再灌注损伤具有有效的预防和治疗作用.