

Protection by Ginseng Total Saponin of Rat Hepatocytotoxicity Induced by Toxic Chemicals

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ABSTRACT: The present experiment was performed to investigate the protective effects of ginseng total saponin (GTS) and possible mechanisms on the hepatocytotoxicity induced by *tert*-butylhydroperoxide (*t*-BuOOH), 4-Bromo-calcium ionophore A23187 (Br-A23187) and KCN. Hepatocytes were isolated by collagenase perfusion of livers from fasted male Sprague Dawley rats and cultured overnight. After various treatments in Krebs-Ringer-HEPES buffer at pH 7.4, cell viability was determined by propidium iodide using fluorocytometry. GTS (5-20 μ M) inhibited cell killing induced by *t*-BuOOH, and KCN, dose-dependently. However, GTS did not inhibit Br-A23187-induced cell killing. These findings support that GTS could protect the hepatocytotoxicity induced by some toxic chemicals. The mechanisms of these protective effects by GTS seem to be associated with antioxidant activity and increase of cellular ATP.

Key words: Hepatocytes, ginseng total saponin (GTS), *t*-BuOOH, Br-A23187, KCN, cytotoxicity.

INTRODUCTION

The liver is very often the target organ to the toxic chemicals. Toxicants can induce a variety of toxic effects on different organelles in the cells in liver, exhibiting different types of liver injury. The hepatoprotective effects of *Panax ginseng* have been published.¹⁾ Ginseng prevents 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced toxicity on liver.²⁾ The administration of ginseng total saponin (GTS) recovered the elevation of SGOT and SGPT activities induced by carbon tetrachloride.³⁾ *Panax ginseng* also protects galactosamine-induced cytotoxicity in primary cultured rat hepatocytes.⁴⁾ Saponin fraction increased the activity of hepatic antioxidative enzymes.^{5,6,7)} They mainly have focused on the antioxidant effects of ginseng, as providing protective effects against cellular toxicity of liver to the chemical toxicants.

However, many cell-killing models have been developed to understand the mechanisms of cytotoxicity using toxic chemicals.⁸⁾ In this study, three toxic compounds, *t*-BuOOH, Br-A23187 and KCN that are distinctive toxic chemicals themselves, respectively, were chosen to clarify the mechanism of protective effects by GTS on the cell

killing of those chemicals. The addition of *t*-BuOOH (an oxidant chemical) to hepatocytes produces oxidative stress and then necrotic/apoptotic cell death.⁹⁾ Br-A23187 (a Ca^{2+} ionophore) is frequently used as a model of Ca^{2+} -dependent cell killing.¹⁰⁾ KCN, an inhibitor of oxidative phosphorylation, mimics the ATP depletion occurring during anoxia¹¹⁾. This chemical hypoxia is used to investigate the role of the ATP in the cell death. Thus, the cellular event of cell death follows by reactive oxygen species, increase of intracellular Ca^{2+} and depletion of ATP. The present experiment was to investigate the protective effects and understand the possible mechanisms of protection by GTS on the cytotoxicity induced by *t*-BuOOH, Br-A23187 and KCN.

MATERIALS AND METHODS

1. Ginseng total saponin

Saponins mixture containing at least 10 glycosides known as ginsenosides from *Panax ginseng* C. A. Meyer, were extracted and purified by Namba et al.s method,¹²⁾ and were supplied by Korea Ginseng & Tobacco Research Institute.

2. Hepatocyte isolation and culture

Hepatocytes were isolated from overnight fasted male Sprague-Dawley rats (200-250 g) by collagenase perfusion of livers, as described previously.¹³⁾ Cell viability

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routinely exceeded 90%, as determined by trypan blue exclusion. Hepatocytes were then cultured in Waymouths MB-7521/1 medium containing 27 mM NaHCO₃, 2 mM L-glutamine, 10% fetal calf serum, 100 nM insulin, and 100 nM dexamethasone. For cell viability assay, hepatocytes were plated onto 24-well microtiter plates (Falcon, Lincoln Park, NJ) coated with 0.1% Type 1 rat-tail collagen at a density of 1.5×10^5 cells/well in 1 ml of medium. Hepatocytes were used after overnight (14-16 h) incubation in humidified 5% CO₂, 95% air at 37°C. Experiments exposing cells to *t*-BuOOH, Br-A23187 or KCN were then carried out in modified Krebs-Ringer-HEPES buffer (KRH) containing 25 mM Na-HEPES, 115 mM NaCl, 5 mM KCl, 1 mM KH₂PO₄, 1.2 mM MgSO₄, and 0.5 mM CaCl₂ at pH 7.4.

3. Cell viability assay

Viability of hepatocytes cultured on microtiter plates was monitored by propidium iodide fluorometry using a multi-well fluorescence scanner (FLUOstar 403, BMG LabTechnologies, Durham, NC).⁷⁾ Briefly, hepatocytes in 24-well microtiter plates were incubated in KRH containing 30 μM propidium iodide. Fluorescence from each well was measured using excitation and emission wavelengths of 544 nm (25 nm band pass) and 590 nm (35 nm band pass), respectively. For each experiment, an initial fluorescence measurement (A) was made 20 min after addition of propidium iodide and then at intervals thereafter. Individual experiments were terminated with 375 μM digitonin to permeabilize all cells, and a final fluorescence measurement (B) was obtained 20 min later. The percentage of viable cells (V) was calculated as $V = 100(B-X)/(B-A)$, where X is fluorescence at any given time. Cell killing in this assay corresponds to that assessed by trypan blue nuclear staining.

RESULTS

1. Inhibition of *t*-BuOOH-induced cell killing by GTS

When hepatocytes in 24-well microtiter plates were exposed to 20 μM *t*-BuOOH in KRH, loss of viability occurred progressively over 2 hr (data not shown). Close to half-maximal (53.0%) cell killing occurred after 50 min. 20 μg/ml GTS inhibited the cytotoxicity of 20 μM *t*-BuOOH from 13.7 to 38.9% after 40 min, 35.0 to 55.3% after 60 min and 50.0 to 69.2% after 80 min (Fig. 1). However, 20 μg/ml GTS by itself caused no loss of cell viability. These data with overnight cultured hepatocytes

are consistent with our earlier study with freshly isolated hepatocytes in suspension, which showed that 20 μM *t*-BuOOH caused over 50% cell killing within 90 min. We suggest that GTS provides temporary protection against cytotoxicity induced by oxidants.

2. Effects of Br-A23187-induced cell killing by GTS

Similarly, when hepatocytes in 24-well microtiter plates were exposed to 1 μM Br-A23187 in KRH, loss of viability occurred progressively over 2 hr (data not shown). Close to half-maximal (56.0%) cell killing occurred after 70 min. However, GTS did not inhibit cell killing after 1 μM *t*-Br-A23187 (Fig. 2).

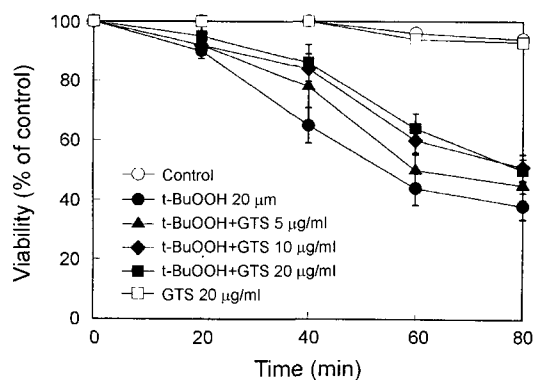


Fig. 1. Protection of *t*-BuOOH-induced cell killing by GTS. Hepatocytes cultured in 24-well plates were incubated for 20 min in KRH prior to adding 20 μM *t*-BuOOH and 5-20 μM GTS. Cell death was measured as described in Materials and Methods. Results are mean ± SEM from triplicated experiments with three different cell isolations.

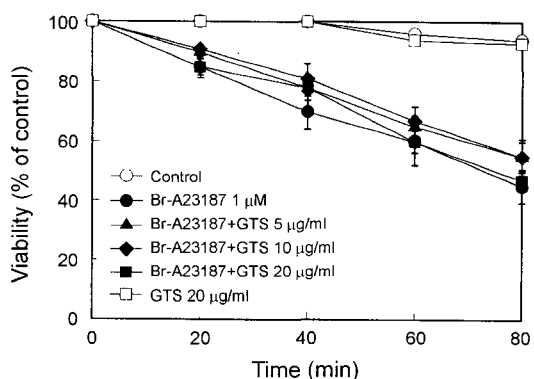


Fig. 2. Effects of Br-A23187-induced cytotoxicity by GTS. Hepatocytes cultured in 24-well plates were incubated for 20 min in KRH prior to adding 1 μM Br-A23187 and 5-20 μM GTS. Cell death was measured as described in Materials and Methods. Results are mean ± SEM from triplicated experiments with three different cell isolations.

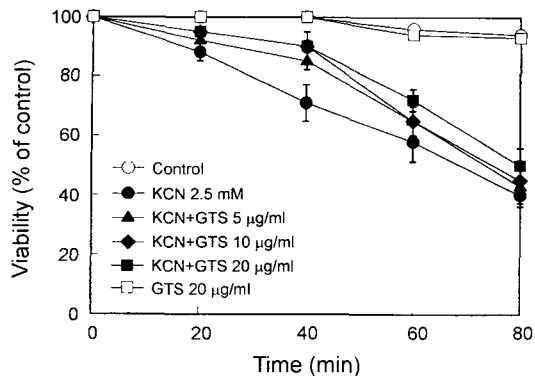


Fig. 3. Protection of KCN-induced cytotoxicity by GTS. Hepatocytes cultured in 24-well plates were incubated for 20 min in KRH prior to adding 2.5 mM KCN and 5-20 µM GTS. Neutralized KCN was prepared daily in a fume hood by addition of 100 ml of concentrated HCl to 1.15 ml of 1.087 M KCN yielding 1.0 M stock solution. Cell death was measured as described in Materials and Methods. Results are mean±SEM from triplicated experiments with three different cell isolations.

3. Inhibition of KCN-induced cell killing by GTS.

Half-maximal (55.0%) cell killing of 2.5 mM KCN occurred after 70 min. 5 µg/ml GTS inhibited the cytotoxicity of 2.5 mM KCN from 17.5 to 38.9% after 40 min. 20 µg/ml GTS inhibited the cytotoxicity of 2.5 mM KCN from 13.7 to 38.9% after 40 min, 35.0 to 55.3% after 60 min (Fig. 3). We suggest that GTS provides temporary protection against cytotoxicity through increase of ATP.

DISCUSSION

The goal of the experiments described here was to determine whether GTS would inhibit apoptotic and necrotic cell killing induced by some toxic chemicals. Oxidative stress, cellular calcium loading and depletion of ATP, strongly showed the toxic effect. We found that 20 µg/ml GTS alone affected neither necrotic nor apoptotic killing of cultured hepatocytes. The oxidant chemical, *t*-BuOOH, induces the mitochondrial permeability transition (MPT) in hepatocytes, leading to cell death. Mitochondrial reactive oxygen species (ROS) formation stimulated by *t*-BuOOH is the critical event promoting onset of the MPT and subsequent cell death (Niemenen et al., 1995). 20 µM *t*-BuOOH was employed, which caused a slower progression to necrotic cell death. GTS delayed cell killing after exposure of hepatocytes to 20 µM *t*-BuOOH. These observations are again consistent with the earlier results of acute cytotoxicity by 20 µM *t*-BuOOH.

On the other hand, it was reported that saponin fraction showed antioxidant effects, increasing the activity of hepatic antioxidant enzyme and decreasing lipid peroxidation.⁶⁾ Many related reports are focused on the antioxidant effects of ginseng (Lee *et al.*, 1995). Therefore, we may confirm that GTS protects hepatotoxicity by antioxidative activity.

Br-A23187 and related Ca^{2+} ionophore are widely used to study Ca^{2+} -dependent cell injury. Toxicity of Ca^{2+} is commonly attributed to activation of Ca^{2+} dependent degenerative enzymes.⁷⁾ Br-A23187 increases intracellular and intramitochondrial calcium and induces onset of the MPT with subsequent acute necrotic cell death.¹⁵⁾ Here, we employed 1 µM Br-A23187, which cause a slower rate of cell killing. However, GTS did not inhibit cell killing after addition of 1 µM Br-A23187. We presume that GTS could not decrease intracellular Ca^{2+} level in the hepatocytes.

Cell injury in many cells is characterized by ATP depletion. In hypoxia and ischemia, ATP formation from oxidative phosphorylation is blocked due to oxygen deprivation. KCN inhibits oxidative phosphorylation and stimulates the ATP depletion of anoxia.^{11,16)} For many of these injurious stresses, either necrotic or apoptotic cell death can result, depending upon other modifying factors such toxicant dose and ATP levels. Ginsenosides protected neurons against the neuronal damage induced by NaCN. Ginsenosides also inhibited NaCN induced elevation of intracellular [Ca^{2+}]. They demonstrated that protective effects of ginsenosides on cyanide-induced neurotoxicity in cultured rat granule cells were associated with ATP level in the hepatocytes.¹⁷⁾ During chemical hypoxia, hepatocytes are protective by adding fructose in the media.¹⁸⁾ Although we did not measure the ATP level in the cells, it is presumed that GTS protected hepatocyte toxicity against KCN because GTS has a lot of glucoses at the side chain of ginsenoside molecule.

Taken together, the data show that GTS protects hepatocytotoxicity caused by three different classes of inducers: reactive oxygen species and inhibitor of oxidative phosphorylation. Since GTS is a common and widely used household remedy for prevention of disease, the present work raises the possibility that GTS might subtly protect hepatotoxicity by various hepatotoxins. 5-20 µM GTS at the present study is not the high dosage concentration after regularly drinking of ginseng tea. GTS is administered orally, GTS may protect the liver and other organs.

In conclusion, GTS could inhibit cell death induced by toxic chemicals. Therefore, GTS has the potential to ben-

officially protect the hepatotoxicity by some toxicants.

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