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Protective Effect of 2, 3-Dimercapto-1-Propanol on Bis (Tributyltin) Oxide-Induced Cytotoxicity in Isolated Rat Hepatocytes

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Summary

The effects of 2, 3-dimercapto-1-propanol (BAL) on bis (tributyltin) oxide (TBTO)-mediated cell injury were studied using isolated rat hepatocytes. The cells (1×10^6 cells/ml) were incubated with or without TBTO alone, or with both TBTO and BAL at 37°C for 60 min. Simultaneous application of 100 μM BAL and 100 μM TBTO reduced the rate of cell injury as evaluated by lactate dehydrogenase leakage and cellular tin content in comparison to TBTO alone. BAL also prevented the stimulation of lipid peroxidation as measured by the production of malonedialdehyde and protected against the depletion of levels of antioxidants such as glutathione (GSH), vitamin C, and vitamin E, and antioxidant enzyme levels such as glutathione reductase and superoxide dismutase induced by TBTO. The results obtained in the present study revealed that BAL is able to prevent TBTO-induced cytotoxicity and suggest that this protective effect may be related to a reduction of the cellular tin content.

Key words: Isolated rat hepatocytes, 2, 3-Dimercapto-1-propanol, BAL, Tributyltin, Lipid peroxidation.

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Introduction

A few reports have been published with regard to the toxic effects of tributyltin compounds on the liver[14,23]. Wiebkin et al.[21] used isolated rat hepatocytes as an *in vitro* model system for to study metabolism and toxicity of the ethyl-substituted organometallic derivatives of tin. The reaction of dibutyltin chloride with the dithiol group seems to be of importance for the molecular mechanism of these toxic effects[17]. Recent observations have shown that sulfide ions, dithiothreitol and BAL decrease the ability of trialkyl and triaryltins to inhibit activity of mitochondrial ATPase[7], to cause hemolysis[8], and to stimulate the swelling of rat liver mitochondria[22]. These findings suggest that thiol compounds may be useful in the prevention and treatment of organotin poisoning.

In the present study, we used isolated rat hepatocytes to observe the effect of BAL on tributyltin-mediated cell injury. Furthermore, the contents of tin, lipid peroxide, and nonenzymatic antioxidants including glutathione (GSH), vitamin C and vitamin E, and the activities of enzymatic antioxidants including glutathione reductase (GR), glutathione peroxidase (GPx), catalase and superoxide dismutase (SOD) were also measured.

Materials and Methods

Chemicals

The chemicals tested were bis (tributyltin) oxide (TBTO; E. Merck Co.) and 2, 3-dimercapto-1-propanol (BAL; Nakarai Tesque, Inc. Kyoto, Japan), and all were of commercial reagent-grade quality.

Preparation of isolated rat hepatocytes

Hepatocytes were isolated from male Wistar rats (250 to 300g body weight) by the collagenase perfusion technique of Stacey and Klaassen[19] with some modifications as described previously[20]. The animals were allowed food (rat chow MF: Oriental Yeast Co., Ltd.) and water *ad libitum*. Anesthesia was induced with sodium pentbarbital (80 mg/kg, ip) and surgery was routinely performed between 9:00 and 10:00 a.m. Following isolation, the hepatocytes were counted and diluted to a concentration of 1×10^6 viable cells/ml in Tris-buffered balanced salt solution (131 mM NaCl, 5.2 mM KCl, 0.9 mM $MgSO_4$, 1 mM $CaCl_2$, 3 mM Na_2HPO_4, and 10 mM tris [hydroxymethyl] aminomethane, pH 7.4)[19]. Initial viability was assessed by the ability of the hepatocytes to exclude trypan blue and ranged from 90 to 95% for all preparations.

Incubation

Ten-ml aliquots of the cell suspension were pipetted into 50ml Erlenmeyer flasks. TBTO and BAL were dissolved in absolute ethanol and dimethyl sulfoxide, respectively, and added to the hepatocyte suspension. The final concentration of each soluvent was 0.1%, which did not affect any of the test systems. Incubation was continued aerobically at 37°C for 60 min in a water bath with shaking at 100 cycles/min.

Cytotoxicity assays

After chemical treatment, the media were withdrawn by centrifugation at 1,000 g for 5 min. The cells in suspension were lysed by repeated freezing and thawing, and centrifuged at 10,000 g for 20 min. Lactate dehydrogenase (LDH) in the media and cell supernatant were measured by the method of Mitchell et al.[16]. The LDH leakage (%) was then calculated as the ratio of that in the media to LDH in the cell lysate, and was used to evaluate the severity of cell injury due to TBTO toxicity.

Analytical methods

Estimation of tin in the cells was carried out with an atomic absorption spectrophotometer (Shimadzu AA-650) equipped with a graphite furnace atomizer (Shimadzu GFA-2) following wet ashing of the sample[9]. Under the same conditions, samples were also removed for estimation of thiobarbituric acid-reacting substances (TBARS) in the whole incubation mixture[6], the intracellular antioxidants GSH[5], vitamin C[13], and vitamin E[1], and for assaying of the activities of antioxidant enzymes GR[18], GPx[11], catalase[2], and SOD. Activity of SOD was assayed using a test kit (Wako Pure Chemical Industries, Ltd., Tokyo). Protein was measured by the method of Lowry et al.[15], using bovine serum albumin as the standard.

All the results are expressed as means and standard errors of 4 experiments. The differences between mean values for the data were evaluated by Student's *t*-test, and a p value less than 0.05 was considered to be statistically significant.

Results

The cytotoxicity of TBTO against isolated rat hepatocytes is shown in Fig. 1. After treatment with TBTO (10–100 μM), the leakage of LDH as an index of cytotoxicity showed time- and concentration-dependency. Under these conditions, TBTO did not affect LDH activity in the cell lysate after the 60-min treatment period (data is not shown).

When the cells were incubated with both of 100 μM BAL and 100 μM TBTO for 60 min,

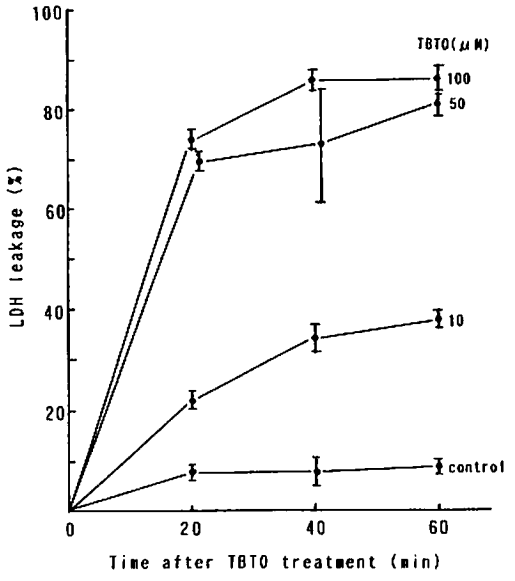

Fig. 1. Toxicity of TBTO against isolated rat hepatocytes. Hepatocytes (1×10^6 cells/ml) were incubated with 10, 50, and 100 μM TBTO for 60 min, and cell injury was evaluated as ratio of lactate dehydrogenase (LDH) in the media to that in the cell lysate as described in Materials and Methods. The values are the means±SE for 4 experiments.

there were significant decreases in rate of LDH leakage and content of cellular tin as compared to the TBTO group (Table 1).

After the 60-min incubation, TBTO at 100 μM produced a significant increase in production of TBARS (expressed as nmol malondialdehyde/mg protein) compared to the negative control. Simultaneous application of 100 μM BAL and 100 μM TBTO diminished TBARS in comparison to the TBTO group. Under the same conditions, contents of nonezymatic antioxidants such as GSH, vitamin C, and vitamin E were decresed markedly compared to the negative control. In hepatocytes treated with both BAL and TBTO, the levels of GSH, vitamin C, and vitamin E content were higher than in hepatocytes treated only with TBTO. These results are summarized in Table 2.

Activities of enzymatic antioxidants such as GR, GPx, catalase, and SOD were measured in the hepatocytes. After treatment with TBTO (100 μM), GR and SOD were decreased markedly compared to the negative control. On the other hand, GPx and catalase activities were elevated markedly by treatment with TBTO. BAL elevated GR and SOD activities suppressed by TBTO, and resulted in decreases in the activities of GPx and catalase elevated by TBTO. These results are summarized in Table 3.

Table 1. Effects of BAL on TBTO-induced cell injury

Treatment	LDH leakage (%)	Intracellular tin content (μg/mg protein)
Control	24.80±0.16	ND
TBTO	83.00±2.68[a]	1.51±0.06
TBTO+BAL	38.48±1.57[a,b]	0.78±0.05[b]
BAL	27.65±0.73	ND

Hepatocytes were incubated with each chemical at 100 μM for 60 min. Data are expressed as means±SE for 4 experiments. ND: Not detectable.
[a]and[b]: Significantly different ($p<0.05$) from control and TBTO alone, respectively.

Table 2. Effects of BAL on TBARS- and antioxidant-levels of TBTO-treated hepatocytes

Treatment	TBARS content (nmol MDA/mg protein)	Contents of antioxidants (μg/mg protein)		
		GSH	Vitamin C	Vitamin E
Control	0.97±0.11	6.59±0.16	0.95±0.03	0.26±0.04
TBTO	1.87±0.17[a]	2.26±0.24[a]	0.04±0.02[a]	0.06±0.01[a]
TBTO+BAL	1.27±0.07[b]	4.87±0.30[a,b]	0.77±0.08[b]	0.24±0.02[b]
BAL	0.95±0.05	6.16±0.14	0.78±0.03[a]	0.25±0.01

Hepatocytes were incubated with each chemical at 100 μM for 60 min. Data are expressed as means±SE for 4 experiments.
[a] and [b]: Significantly different ($p<0.05$) from control and TBTO alone, respectively.

Table 3. Effects of BAL on antioxidant-enzyme levels of TBTO-treated hepatocytes

Treatment	Activities of antioxidant enzymes			
	GR (nmole NADPH oxidized/min/ mg protein)	GPx (enzyme units/ mg protein)	Catalase (nmole H_2O_2 reduced/min/ mg protein)	SOD (unit/mg protein)
Control	42.14±1.02	9.91±0.65	0.52±0.03	14.04±1.96
TBTO	38.63±0.64[a]	14.01±1.42[a]	0.89±0.05[a]	8.92±0.91[a]
TBTO+BAL	45.28±0.91[b]	10.61±0.83[b]	0.71±0.03[a,b]	15.90±3.81[b]
BAL	44.26±1.02	11.07±1.08	0.43±0.04	11.75±0.82

Hepatocytes were incubated with each chemical at 100 μM for 60 min. Data are expressed as means±SE for 4 experiments.
[a] and [b]: Significantly different ($p<0.05$) from control and TBTO alone, respectively.

Discussion

In the present study, the protective effect of BAL on TBTO-mediate cell injury was confirmed to be accompanied by a decrease in cellular tin content. Furthermore, BAL was also able to prevent TBTO-induced stimulation of lipid peroxidation and decreases in levels of nonenzymatic and enzymatic antioxidants.

Aldridge and Cremer[3] suggested that tributyltin sulfate did not form stable compounds with GSH or BAL in experiments with a chemical model system. It has been shown that BAL combines with dialkyltin and prevents its biochemical effects. However, BAL had no effect on the response to trialkyltin, because it has very little affinity to this compound *in vitro*[4]. Byington et al.[8] reported that the addition of BAL to tributyltin-treated erythrocytes reduces the rate of hemolysis and that this decrease is attributable to the removal of tributyltin from cells. Gray et al.[10] also demonstrated that BAL prevented tri-n-butyltin (TBT)-mediated hemolysis, reduced the numbers of TBT aggregates per cell, and doubled the diameter of TBT aggregates formed, and suggested that BAL in particular may form a chelate complex with TBT rendering this material more soluble in water than either reactant alone. From these results, it is considered that the protective effect of BAL against TBTO-induced cell injury is probably due to reduction of Sn uptake by the cells as a result of the chelate complexation of BAL with TBTO in the incubation medium.

Iwai et al.[12] observed a transient increase in the rate of tributyltin accumulation within 24 h in the livers of rats exposed to tributyltin fluoride. This initial increase was followed by a rapid decrease in the hepatic tributyltin level and by corresponding increases in the contents of dibutyltin, monobutyltin, and inorganic tin. They suggested that tributyltin, once transported to the liver, is rapidly dealkylated there. It may be assumed from this that the reduction of Sn content is attributable to the removal of tin from the cells as a result of the complexation of BAL to dibutyltin derivatives produced by the cells. Since the preventive mechanism of BAL against TBTO-induced cytotoxicity is not clear from the results of this experiment, further investigation is necessary to determine whether BAL forms a chelate complex with TBTO and/or its metabolites.

In conclusion, BAL is useful for preventing TBTO-induced cytotoxicity when given stimultaneously with TBTO, and this protective effect may be at least partly due to the reduction of cellular tin content resulting from the chemical interaction of BAL with TBTO.

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