Evaluation of the Toxicity of a Single Oral Dose of \(N\)-(\(\beta\)-Ribopyranosyl)taurine Sodium Salt in Mice

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Abbreviations

ANOVA  One-way analysis of variance
BW      Body weight
H&E     Hematoxylin and eosin
HFF     Health functional food
SEM     Standard error of the mean
T-Rib   \(N\)-(\(\beta\)-Ribopyranosyl)taurine sodium salt

1 Introduction

Taurine is a simple sulfur-containing compound and one of the most abundantly available free amino acids in human and animal tissues. Taurine has been reported to possess biological activities such as bile acid conjugation (Hardison 1978), physiological functions in the retina (Lombardini 1991), and both anti-oxidative (Keys and Zimmerman 1999) and anti-diabetic (Franconi et al. 1995) effects. However, taurine has some disadvantages such as poor absorption, high-dose requirement, and a fast renal extraction rate (Cho et al. 2014). To overcome these disadvantages, numerous taurine derivatives, such as thiotaurine (Budhram et al. 2013) taurine
chloramine (Kontny et al. 2007), and tauromustine (Gunnarsson et al. 1989) have been developed.

Recent studies reported that taurine-carbohydrate derivatives possess anti-adipogenic effects on human adipocyte differentiation (Cho et al. 2014) with T-Rib showing hypolipidemic, anti-obesity (Kim et al. 2014a), and antioxidative (Kim et al. 2014b) effects in Sprague Dawley rats. In addition, a patent application has been filed for T-Rib in Korea (Kim et al. 2013).

Because of recent interest in health and in improving standards of living, the need for various HFFs is rising, forming a receptive world market for HFF. Health functional food is defined as “A product intended for use to enhance and preserve human health with one or more functional ingredients or constituents (Ministry of Food and Drug Safety 2010)”. N-(α-Ribopyranosyl)taurine sodium salt with its various functions is likely to be developed as a HFF. In order for a compound to be developed as an ingredient of a HFF, the functionality and safety of the compound must be verified. Therefore, the objective of this study was to verify the safety of T-Rib by evaluating the toxicity of a single oral dose of T-Rib in ICR mice.

2 Methods

2.1 Animals and T-Rib

Male and female ICR mice were purchased from Koatech (Pyeongtaek, Korea). All mice were housed at the laboratory animal housing at Inha University according to the guidelines outlined by the Experimental Animal Ethics Committee and kept under a constant 12 h light and dark cycle (AM 09:00–PM 09:00) with controlled temperature (23 ± 1 °C) and humidity (55 ± 10%).

N-(α-Ribopyranosyl)taurine sodium salt is a newly synthesized taurine-carbohydrate derivative from taurine and ribose. The reaction of α-ribose with taurine was carried out as described previously (Cho et al. 2014). The chemical structure of T-Rib was identified by nuclear magnetic resonance spectroscopy (Fig. 1).

The toxicity of a single oral dose of T-Rib was evaluated according to modified OECD-420 guidelines (OECD 1987). Eight ICR mice/group (four males and four females) were used for the experiment. Following 1 week of acclimatization, male and female ICR mice were randomly divided into three groups and fed a pelleted commercial diet for a period of 14 days. Feed and water were provided ad libitum.

Distilled water was administered to the control groups; the T-Rib treatment groups received either 2,000 mg T-Rib/kg BW (according to the OECD guidelines) or 5,000 mg T-Rib/kg BW (according to the harmless material classification standard of the US Environmental Protecting Agency). The respective T-Rib dose or control was administered via oral gavage with a 2 ml syringe at a volume of 20 ml/kg using distilled water.
2.2 Cageside Observations

Cageside observations were performed every hour for 6 h and then every day for 14 days to evaluate the toxicity of T-Rib. Cageside observations included mortality and clinical signs such as changes in skin, fur, and eyes; respiratory and autonomic effects such as salivation, diarrhea, and urination; central nervous system effects including tremors, convulsions, relaxation, and coma (Demma et al. 2007). Body weights were measured on the day of administration (day 0) and both 7 and 14 days after T-Rib or control administration.

2.3 Sampling

At the end of the experimental period (14 days after T-Rib administration) and following 12 h of fasting, the animals were anesthetized with ether. A section of liver tissue was sampled for histological examination.

2.4 Histological Examination of Liver Tissue

Histological examination of liver tissue was performed using the paraffin method. Fresh tissues were fixed immediately with 10 % buffered formalin and paraffin-embedded sections were stained with hematoxylin and eosin (H&E). The stained sections were then examined by light microscopy (Axioskop 2, Zeiss, Jena, Germany).

2.5 Statistical Analysis

All analyses were performed using the SPSS 20.0 software program. Each value was expressed as the mean ± standard error of the mean (SEM). Changes in body and organ weights were analyzed by one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test at a p<0.05.
3 Results and Discussion

3.1 Cageside Observation of Mice

All mice appeared to be healthy and normal throughout the experimental period and no mortalities were found in the experimental and control groups. In addition, no abnormal clinical signs, such as changes in skin, fur, and eyes; respiratory and autonomic effects such as salivation, diarrhea, and urination; and central nervous system effects, including tremors, convulsions, relaxation, and coma were observed regardless of administration.

3.2 Body Weight Measurement of Mice

No significant differences in BW were found between the experimental groups from day 0 to day 14 (Table 1). It has previously been reported (Lee et al. 2007, 2012) that BW decreased transiently after acute toxicity test of 5,000 mg/kg BW of Leuconostoc eitreme GJ7 or Lactobacillus plantarum AF1 isolated from kimchi. However, BW returned to normal levels after a relatively short period. Based on our findings from this study that no difference in body weight was found 7 days after administration of T-Rib, a single oral dose of T-Rib may have no negative effect on the growth of mice in the experimental groups compared with the control group.

3.3 Histological Examination of Liver Tissue

Previous histological examinations have shown that in vivo administration of the toxic compounds, t-BHP and azathioprine, causes neutrophil infiltration, swelling of hepatocytes, and liver necrosis (Wang et al. 2000; Amin and Hamza 2005).

In our study, histological examination of liver tissue revealed no significant differences between the experimental groups and the control group (Figs. 2 and 3).

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<th>Table 1 Body weight of mice</th>
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*Values are expressed as mean±SEM; ns not significant
Fig. 2 Light micrography of hepatocytes in male mice. (a) 100× magnification; (b) 200× magnification. Representative pictures are H&E-stained liver sections. No significant differences were found in liver histology between the different treatment groups.

Fig. 3 Light micrography of hepatocytes in female mice. (a) 100× magnification; (b) 200× magnification. Representative pictures are H&E-stained liver sections. No significant differences were found in liver histology between the different treatment groups.

4 Conclusions

In this study, we evaluated the toxicity of a single oral dose of T-Rib in male and female ICR mice to verify its safety as a potential ingredient of a HFF. Our results showed no mortality, abnormal clinical signs, and differences in body weight in all treatment groups. In addition, no abnormal differences were observed in the
histological examination of liver tissue of T-Rib-treated and control mice. We therefore suggest that a single oral dose of T-Rib as high as 5,000 mg/kg BW exerts no toxic response in mice. However, further studies assessing the toxicity of repetitive oral dosing are required to define the safety profile of T-Rib for future HFF use.

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References

Kim SY, Lee YJ, You JS, Kim SH, Chang KJ (2014b) Hepatic protective and antioxidant effects of taurine-ribose supplementation in rats fed a high-fat diet. In: Taurine meeting, Krakow, p 5.8