J. Microbiol. Biotechnol. (2005), 15(4), 887–890

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Hepatoprotective Effect of Lactic Acid Bacteria

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Received: November 7, 2004
Accepted: February 11, 2005

Abstract To evaluate the hepatoprotective activity of lactic acid bacteria, their effects on *tert*-butylperoxide (*t*-BHP)-induced hepatotoxicity in mice were measured. When lactic acid bacteria at doses of 0.5 and 2 g (wet weight)/kg were orally administered to mice with *t*-BHP-induced liver injury, these bacteria significantly inhibited the increase of plasma alanine aminotransferase and aspartate aminotransferase activities by 17–57% and 57–66% of the *t*-BHP control group, respectively. However, these lactic acid bacteria did not protect cytotoxicity induced by *t*-BHP against HepG2 cells. The inhibitory effects of these lactic acid bacteria at a dose of 0.5 g/kg were comparable with that of diphenyl dimethyl bicarboxylate at a dose of 0.2 g/kg, which has been used as a commercial hepatoprotective agent. Among these lactic acid bacteria, *Bifidobacterium longum* HY8001 exhibited the most potent hepatoprotective effect. These orally administered lactic acid bacteria inhibited liver lipid peroxidation on *t*-BHP-induced hepatotoxicity of mice. We suggest that lactic acid bacteria may be an effective agent against liver injury.

Key words: Lactic acid bacteria, hepatoprotective, HepG2, *tert*-butyl hydroperoxide

Lactic acid bacteria are regarded as safe microorganisms, and some of them have been claimed to contribute to the health and fitness of the individual who consumes them [8, 25]. The main effects of probiotics reported inculde the improvement of disturbances of the indigenous microflora [5, 7, 20–22], amelioration of the development of microflora [8, 25], antidiabetic and antihyperlipidemic effects [10, 13, 18, 24, 27, 28], health enhancement through inhibition of carcinogenesis [1, 12], and non-specific activation of the host immune system [1, 9, 15, 17, 25]. The favorable image and biological effects of lactic acid bacteria led to the concept of probiotics. However, the hepatoprotective effects of probiotics, except for the effect of lactic acid bacteria on alcohol metabolism [16], have not been thoroughly studied. As part of our continuing search for hepatoprotective agents from lactic acid bacteria, we screened lactic acid bacteria as a hepatoprotective agent, and investigated its hepatoprotective activity on *tert*-butyl hydroperoxide (*t*-BHP)-induced hepatotoxified mice.

t-BHP and thiobarbituric acid (TBA) were purchased from Sigma Co. (U.S.A.). Diagnostic kits for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were purchased from Asan Pharmaceutical Co., Ltd. (Korea). Diphenyl dimethyl bicarboxylate (DDB) was kindly donated by Dr. N. J. Kim, Kyung Hee East-West Medical Research Institute, Kyung Hee University, Korea. Tryptic soy broth was purchased from Difco Co. (U.S.A.). Other chemicals used in this study were of analytical reagent grade.

Lactic acid bacteria [*Lactobacillus brevis* HY7401 (**7**), *Lactobacillus acidophilus* CSG (**C**) and *Bifidobacterium longum* HY8001 (**8**)] were cultured in tryptic soy broth, and collected at 10,000 ×*g* for 30 min. The precipitates were washed twice with saline and used in the experiment.

Mice (ICR, male, 20–22 g) were supplied from Orient Charles Liver Co., Ltd. (Korea), and were maintained on pellet food (Orient Co., Ltd., Korea) and tap water. Five mice in each group were used. All procedures relating to animals and their care conformed to the international guidelines *Principles of Laboratory Animals Care* (NIH publication no. 85–23, revised 1985).

To evaluate the protective effect of lactic acid bacteria on liver injury of mice induced by *t*-BHP, animals were orally administered with lactic acid bacteria (suspended in 1% CMC-Na) at doses of 0.5 g/kg and 2 g/kg, and DDB (suspended in 1% CMC-Na) at a dose of 0.2 mg/kg. Control group animals were given saline (0.2 ml/20 g) instead of the sample. The samples were given three times (once per day). Animals were intraperitoneally treated with 1.5 mmol

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of *t*-BHP/kg at 1 h after the final sample administration. Blood samples were collected for 18 h after *t*-BHP administration by cardiac puncture under ether anesthesia and the serum was obtained by centrifugation (2,000 ×*g*, 15 min).

Lipid peroxidation in the liver was estimated by measuring malondialdehyde (MDA) formation by the thiobarbituric acid method according to Ohkawa *et al.* [19]. The reaction mixture containing 100 μl of sample, 1.5 ml of acetate buffer (pH 3.5), 0.2 ml of 8.1% sodium dodecyl sulfate, and 1.5 ml of 0.8% thiobarbituric acid was heated at 95°C for 1 h; 5 ml of BuOH/pyridine (15:1, v/v) were then added, and the mixture was shaken thoroughly. The reaction mixture was centrifuged at 1,500 ×*g* for 10 min, and absorbance of the supernatant was then measured at 532 nm. 1,1,3,3-Tetraethoxypropane was used as a standard for lipid peroxide.

Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were analyzed according to the manufacturer's procedure (Asan diagnostic kits) [23].

HepG2 cells (hepatocellular carcinoma cell line) donated by the Korean Cell Bank (Seoul, Korea) were cultured in MEM containing 10% FBS, 1% antibiotic-antimycological solution, and 1.5 g/l sodium bicarbonate under 5% CO_2 at 37°C. The protective and cytoprotective effects of the lactic acid bacteria on HepG2 cells treated with and without *t*-BHP were measured, respectively, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay [4]. Briefly, HepG2 cells were dispensed into 96-well plates at the concentration of 1×10^4 cells per well. The test lactic acid bacteria lysates, the supernatant of sonicated lactic acid bacteria, were added into HepG2 cells and the mixture was preincubated for 2 h. Then, the cultured media were replaced with the media containing *t*-BHP (100 μM), incubated for 3 h, and rinsed with phosphate-buffered saline. MTT reagent (0.25 mg/ml) was added into the cells, incubated for 1 h, and 100 μl of dimethyl sulfoxide were then added. Absorbance at 540 nm was measured to estimate survived cells.

All the data were expressed as mean±standard deviation and statistical significance was determined using student *t*-test.

To evaluate the hepatoprotective effect of the lactic acid bacteria, their hepatoprotective effects on *t*-BHP-induced liver injury in mice were also investigated (Fig. 1). When *t*-BHP was intraperitoneally given to mice, serum ALT and AST levels were significantly increased, compared to those of the control group. The reference agent DDB (200 mg/kg), which has been used as a commercial hepatoprotective agent [26, 29], inhibited the increased serum ALT and AST levels to 28% and 39%, respectively, of the control group treated with *t*-BHP alone. All the orally administered lactic acid bacteria potently inhibited the increase of serum ALT and AST levels induced by *t*-BHP treatment: *L. brevis* HY7401, *L. acidophilus* CSG, and *B. longum* HY8001 at a dose of 0.5 g/kg inhibited serum ALT and AST levels by

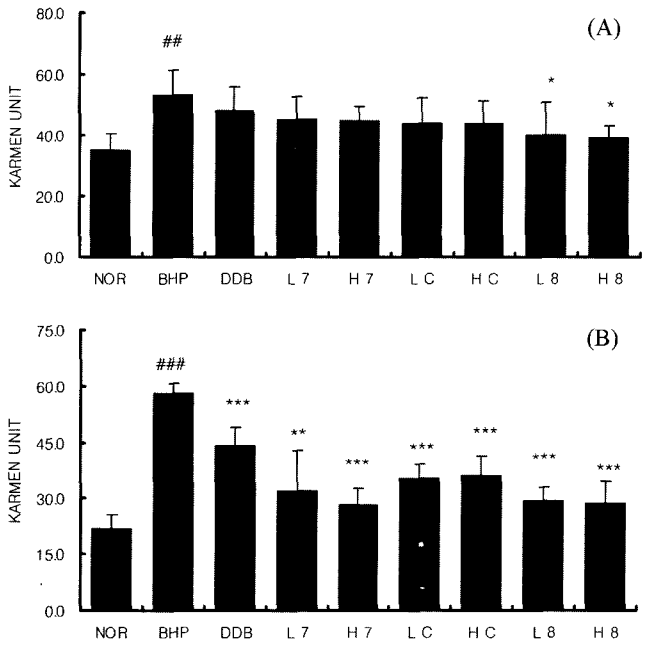

Fig. 1. The protective effect of lactic acid bacteria on *t*-BHP-induced liver injury in mice.
(A) ALT, (B) AST. L7, 0.5 g/kg *L. brevis* HY7401; H7, 2 g/kg *L. brevis* HY7401; LC, 0.5 g/kg *L. acidophilus* CSG; HC, 2 g/kg *L. acidophilus* CSG; L8, 0.5 g/kg *B. longum* HY8001; H8, 2 g/kg *B. longum* HY8001; DDB, diphenyl dimethyl bicarboxylate. #Significantly different from normal group (#p<0.05; ##p<0.01; ###p<0.001). *Significantly different from normal group (*p<0.05; **p<0.01; ***p<0.001).

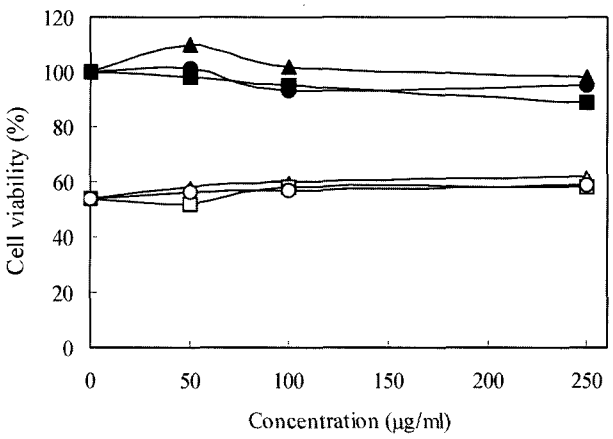

Fig. 2. The cytoprotective effect of lactic acid bacteria on HepG2 cells treated with or without *t*-BHP.
Open and closed symbols indicate the cytotoxicity of lactic acid bacteria lysates on HepG2 cells and their protectivities on *t*-BHP-treated HepG2 cells, respectively. The cultured lactic acid bacteria were sonicated, centrifuged at 10,000 ×*g* for 30 min, and sterilized with a millipore membrane (0.45 mm). The amount of applied lysate was based on protein, determined by the method of Bradford [2]. Triangle, kg *L. brevis* HY7401; square, *L. acidophilus* CSG; circle, *B. longum* HY8001.

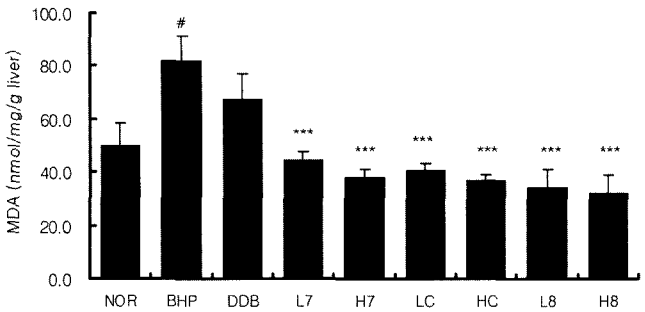

Fig. 3. The inhibitory effect of lactic acid bacteria on liver lipid peroxidation in *t*-BHP-injured mice.
L7, 0.5 g/kg *L. brevis* HY7401; H7, 2 g/kg *L. brevis* HY7401; LC, 0.5 g/kg *L. acidophilus* CSG; HC, 2 g/kg *L. acidophilus* CSG; L8, 0.5 g/kg *B. longum* HY8001; H8, 2 g/kg *B. longum* HY8001; DDB, diphenyl dimethyl bicarboxylate. #Significantly different from normal group (#p<0.05). *Significantly different from normal group (*p<0.05; **p<0.01).

40, 48, and 73% and 47, 62, and 79% of the *t*-BHP control group, respectively. The hepatoprotective activity of 0.5 g/kg *B. longum* HY8001was more potent than that of 0.2 g/kg DDB.

To investigate the toxicity of lactic acid bacteria, the cytotoxicity of lactic acid bacteria against HepG2 cells was measured (Fig. 2). These lactic acid bacteria did not exhibit toxicity against HepG2 cells. However, when HepG2 cells were also treated with 100 μM *t*-BHP, *t*-BHP showed potent cytotoxocity. The lactic acid bacteria could not protect the cytotoxicity induced by *t*-BHP.

To evaluate the hepatoprotective activity of lactic acid bacteria, the effect of lactic acid bacteria on liver lipid peroxidation of *t*-BHP-hepatotoxified mice were measured (Fig. 3). MDA levels in the liver of the *t*-BHP-treated group increased by 65%, compared to those of the normal control group. However, oral administration of lactic acid bacteria to the *t*-BHP-treated group significantly lowered MDA levels. The inhibitory activity of 0.5 g/kg lactic acid bacteria was more potent than that of 0.2 g/kg DDB.

t-BHP can be metabolized to free radical intermediates by cytochrome P450 (hepatocytes) or hemoglobin (erythrocytes), which can subsequently initiate lipid peroxidation [14], affect cell integrity, and form covalent bonds with cellular molecules, resulting in cell injury [3, 6]. We used *t*-BHP as a chemical inducer for the preparation of the liver-injured animal model. The treatment with *t*-BHP caused a significant increase in serum ALT and AST activities of mice. Oral pretreatment with lactic acid bacteria, however, protected the mice from *t*-BHP-induced liver injury. When the liver lipid peroxidation activity was measured to investigate the protective mechanism of lactic acid bacteria for liver injury, these lactic acid bacteria were found to lower the levels of liver MDA increased by *t*-BHP treatment. However, the lactic acid bacteria did not protect the cytoxicity induced by *t*-BHP. The hepatoprotective effects of lactic acid bacteria are comparable to that of DDB, which is a representative hepatoprotective agent used in clinic [29]. The DDB potently inhibited the levels of liver MDA increased by chemicals. Based on these findings, lactic acid bacteria can protect the formation of free radicals induced by *t*-BHP and may be an effective agent against liver injury.

Acknowledgement

This study was supported by a grant of Korea Yakult Co., Ltd. (2003).

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