Effect of Potassium Sorbate on Cellular GSH Level and Lipid Peroxidation in Cultured Rat Hepatocytes

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Change in cellular reduced glutathione (GSH) level was examined after the addition of 1—10 mM potassium sorbate (SA-K) to cultured rat hepatocytes. The cellular GSH content was decreased to the lowest level at 6 h after the addition of SA-K, and then gradually returned to the normal level except for hepatocytes exposed to 10 mM SA-K. Although the decrease in GSH level was not associated with lactate dehydrogenase (LDH) leakage in hepatocytes exposed to SA-K up to the concentration of 5 mM, cell injury was caused in cells exposed to 10 mM SA-K. When eicosapentaenoic acid was added in conjunction with various concentrations of SA-K to hepatocytes, peroxidation of the fatty acid was accelerated in parallel with the decrease in cellular GSH level. The enhanced lipid peroxidation in the hepatocytes co-exposed to SA-K and eicosapentaenoic acid (EPA) induced the development of cell injury. These results suggest that hepatocytes exposed to SA-K become susceptible to oxidative stress such as lipid peroxidation.

Key words potassium sorbate; cellular GSH; cultured rat hepatocyte; eicosapentaenoic acid; lipid peroxidation

Sorbic and sorbate are antimicrobial agents which are used worldwide in a wide range of foodstuffs. Sorbic acid is a doubly unsaturated aliphatic straight-chain monocarboxylic acid. In the body, this chemical is largely metabolized by the same pathway of oxidation as the natural 6-carbon saturated fatty acid, caproic acid, for the production of energy.12 Both sorbic acid and sorbates are far less toxic than other preservatives known to date. It has been reported that the acute toxicity of sorbic acid is about 10 g/kg body weight, and in a long-term feeding test there was no increase in mortality or the incidence of histological lesions in rats fed diets containing 10% sorbic acid for 80 weeks.2,3 However, genotoxic effects of sodium sorbate were shown in cultured Chinese hamster V79 cells,4-5 and there are also a few reports in which 88 week feeding on a 15% sorbic acid-containing diet caused hepatoma in mice.6,7 It has been proposed that the carcinogenic mechanism of sorbic acid might involve the depletive of the cellular reduced glutathione (GSH) level in the liver when mice are fed a high level dose of the additive.8 In this study, the harmful effect of potassium sorbate (SA-K) on cellular GSH which plays a crucial role in the cellular defense system against oxidative stress was examined in vitro using cultured rat hepatocytes. The addition of SA-K to hepatocytes caused a marked loss of cellular GSH and enhanced the peroxidation of eicosapentaenoic acid within the cells.

MATERIALS AND METHODS

Materials The materials and chemical reagents used in the present study were obtained from the following companies: plastic culture dishes from Falcon, Becton Dickinson Co., Japan; Williams' medium E from GibCO BRL, Life Technologies, Inc., U.S.A.; SA-K and eicosapentaenoic acid (EPA) from Nacalai Tesque Inc., Japan. All other chemicals used were of the highest quality available.

Preparation of Cultured Hepatocytes Hepatocytes were isolated from male Wistar rats weighing 180 to 220 g according to the collagenase perfusion method.9 The isolated cells were subjected to fractionation on Percoll density gradi-
RESULTS

The addition of SA-K to cultured hepatocytes produced a decrease in cellular GSH level dose-dependently from 1 to 10 mM, and time-dependently up to 6 h. At 6 h after the addition of SA-K, the cellular GSH level was decreased to 82.1, 60.4, 46.7 and 36.5% of that in normal cells at 1.0, 2.5, 5.0 and 10 mM, respectively. The GSH level in SA-K-treated hepatocytes was then returned to normal value within 24 h for 1 or 2.5 mM, and within 36 h for 5 mM, but remained depleted for up to 48 h at 10 mM. Although the decrease in GSH level was not associated with cell injury in SA-K-treated hepatocytes up to a concentration of 5 mM, LDH leakage occurred at 10 mM, beginning at 24 h and increased to 25.7% of total activity at 48 h.

Peroxidative susceptibility of EPA was examined in cultured hepatocytes exposed to various concentrations of SA-K. Lipid peroxidation, assessed in terms of the production of MDA, was not observed during a 10 h incubation in hepatocytes exposed to SA-K alone up to the concentration of 10 mM. Upon the addition of 0.2 mM EPA to non-treated hepatocytes, MDA was accumulated in the medium at the rate of 0.88 nmole/10 h/mg protein. This rate of accumulation was accelerated in SA-K-treated hepatocytes above 2.5 mM, being increased by approximately three fold at 10 mM. In parallel with the increase in peroxidation of EPA in SA-K treated hepatocytes, the leakage of LDH into the medium occurred above 2.5 mM, reaching 52.6% at 10 mM. Lipid peroxidation in hepatocytes co-exposed to SA-K and EPA was almost completely prevented by the addition of the antioxidants DPPD or V.E. The inhibition of lipid peroxidation by antioxidants protected hepatocytes from the cell injury caused by SA-K and EPA. These results suggest that the enhanced lipid peroxidation was responsible for cell injury caused in hepatocytes co-exposed to SA-K and EPA.

DISCUSSION

A decrease of cellular GSH level at various concentrations of SA-K was examined using cultured rat hepatocytes. The harmful activity of sorbic acid to decrease cellular GSH in the liver was previously shown in an animal experiment where mice were fed on a diet containing 15% sorbic acid for 3 months. One possible mechanism underlying such action of sorbic acid was via its conversion to metabolites conjugating with GSH. It was also reported that a long-term feeding of sorbic acid caused a selective induction of 2,4-dienoyl-CoA hydratase which could dramatically increase the conversion of sorbic acid to 3-keto-4-hexenoyl-CoA which is reactive with SH-compounds. The present in vitro study showed that cellular GSH was promptly decreased in cultured rat hepatocytes upon the exposure to SA-K at concentrations of mM order. SA-K may exert its GSH-consuming activity at lower concentrations in the liver cells in which the

Fig. 1. Cellular GSH Levels (A) and LDH Leakage (B) after the Addition of Potassium Sorbate to Cultured Rat Hepatocytes

Cultured rat hepatocytes were incubated with 0 (○), 1.0 (□), 2.5 (△), 5.0 (▲), 10 (●) mM potassium sorbate (SA-K). After incubation for the indicated times, the cells and the medium were taken to assay cellular GSH and LDH activity, respectively. * Significantly different from the cells incubated without SA-K.

Fig. 2. MDA Accumulation(A) and LDH Leakage (B) after the Addition of Potassium Sorbate with and without Eicosapentaenoic Acid to Cultured Rat Hepatocytes

Hepatocytes were incubated with (●) and without (○) 0.2 mM eicosapentaenoic acid (EPA) in the presence of various concentrations of potassium sorbate (SA-K). After incubation for 10 h, the medium was taken to assay MDA and LDH activity. * Significantly different from the cells incubated without SA-K.
Fig. 3. Effects of Antioxidants on Lipid Peroxidation (A) and Cell Injury (B) Induced by Potassium Sorbate and Eicosapentaenoic Acid in Cultured Rat Hepatocytes

Hepatocytes were incubated with potassium sorbate (SA-K, 10 mM) and eicosapentaenoic acid (EPA, 0.2 mM) in the presence of DPPD (5 μM) or VE (100 μM). Other experimental conditions were the same as in Fig. 2. * Significantly different from the cells incubated with SA-K and EPA without an antioxidant (None).

hydrate was induced by a long-term feeding of animals with SA-K.

There is a question about the relevance of the concentrations of SA-K at mM order in relation to those encountered in tissues in vivo. SA-K is permitted as a food preservative at the concentration of up to 2 g/kg, which corresponds to about 20 mM in foodstuffs. Hence, SA-K may achieve a concentration of mM order at least in a local area in gastrointestional tissues by dietary intake of foods containing a high concentration level of SA-K. Furthermore, hepatocytes can afford to replenish their GSH level to a greater extent than other tissues. The reduction of cellular GSH by SA-K addition may be exerted at lower concentrations in other tissues than in hepatocytes.

GSH provides the cell an efficient means of detoxification of toxic oxygen species and reactive electrophilic agents. Thus, a marked reduction of intracellular GSH level would make the cell susceptible to oxidative stress including lipid peroxidation or arylation of cellular macromolecules by reactive intermediates which are generated during the metabolism of many xenobiotics. In fact, there are many reports in which depletion of cellular GSH by the addition of diethylmaleate or 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) sensitized hepatocytes to the cytotoxicity of various drugs such as acetaminophen, adriamycin and bipyrindyl herbicides. In the present study, susceptibility of SA-K treated hepatocytes to lipid peroxidation was increased probably because of the reduction of cellular GSH level. Thus, SA-K may exert cytotoxic potential in the body by deteriorating the cellular GSH-dependent defense system.

REFERENCES