Effects of Potassium Sorbate on the Decrease of Cellular GSH Levels in Cultured Rat Hepatocytes

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Introduction

Sorbic acid and sorbates are used worldwide as food preservatives because of their inhibitory effect on the growth of yeasts, fungi and bacteria. Sorbic acid is a doubly unsaturated aliphatic straight-chain monocarboxylic acid. In the body, this chemical is metabolized in the same pathway of oxidation as the natural saturated 6-carbon fatty acid, caproic acid, for the production of energy. Both sorbic acid and sorbates are far less toxic than other preservatives known to date. Their acute toxicity 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; in contrast, genotoxic effects were revealed in cultured Chinese hamster V79 cells exposed to high concentrations of sodium sorbate. In this study, cytotoxicity of potassium sorbate (SA-K) *in vitro* was examined using of cultured rat hepatocytes.

Methods

Hepatocytes were isolated from male Wistar rats and suspended in Williams E medium in 35 mm plastic dishes. Monolayers of hepatocytes cultured for 9 h were used in the experiment. Eicosapentaenoic acid (EPA) complexed with bovine serum albumin was added to a cultured medium.¹⁾

Results and Discussion

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. The GSH level in the treated hepatocytes was then returned to normal value within 24 h using 2.5 mM, and within 36 h using 5

mM, whereas the GSH level in cells exposed to 10 mM SA-K remained depleted for up to 48 h. Although the decrease in GSH level was not associated with cell injury in hepatocytes up to the concentration of 5 mM, lactate dehydrogenase (LDH) leakage occurred, in cells exposed to 10 mM SA-K, beginning from 24 h and increasing to 25% of total activity. The involvement of lipid peroxidation in the mechanism of SA-K-induced cell injury was investigated since di-unsaturated bonds are contained in the structure of sorbic acid. Accumulation of malondialdehyde during incubation of hepatocytes with SA-K was not observed with the detectable limitation of 0.1 nmoles/24 h/mg protein. Antioxidants such as DPPD and Vitamin E had a small reduction effect on the SA-K toxicity but Fe ion chelater deferoxamine did not. Peroxidation of EPA added to SA-K-treated hepatocytes was accelerated in parallel with decrease in GSH level. The enhanced lipid peroxidation in the cells exposed to SA-K in combination with EPA caused cell injury to develop. These results suggest that hepatocytes exposed to SA-K become susceptible to chemically-induced oxidative stress. The loss of GSH content in hepatocytes by SA-K treatment was suppressed by the inhibition of P 450 following treatment with SKF-525A or metyrapone. SA-K has a system of conjugated double bonds at which molecular oxygen can attack. Therefore, it appears that cellular GSH is consumed by the oxidation product of SA-K in cultured rat hepatocytes.

Reference

- 1) Sugihara N., Tsuruta Y., Date Y., Furuno K., Kohashi K., *Toxicol. Appl. Pharmacol.*, **126**, 124–128 (1994).