Effect of Sizofilan, an Immunomodulator, on Hepatic Microsomal Mixed-Function Oxidase Activities in Rats

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Abstract—Mixed-function oxidase activities of hepatic microsomal preparations from rats were examined after intraperitoneal administration of sizofilan (SPG), an immunomodulator. Repeated doses of SPG (3 mg/kg/12 hr, 4 times) depressed the hepatic cytochrome P-450 content and the activities of aminopyrine N-demethylase and aniline hydroxylase.

A recent trend in cancer treatment is the use of immunotherapy alone or in combination with chemotherapy and/or other methods (1, 2). Sizofilan (SPG) is a glucan extracted from the culture filtrate of Schizophyllum commune Fries. SPG shows antitumor activity in syngeneic and autochthonous tumors of rodents and in patients with malignant pleurisy and lung cancer (3-5). SPG has been developed as an immunotherapeutic agent for clinical use.

Numerous compounds are known to alter the hepatic microsomal mixed-function oxidase (MFO) activities. One of the most recently discovered classes of substances which inhibit P-450-dependent metabolism is the immunomodulators. Accordingly, it is possible that the pharmacokinetics of drugs will be altered in animals treated with an immunomodulator. These results might be important in the chemotherapeutic treatment of cancer because the chemotherapeutic agents are metabolized to active or inert metabolites by the microsomal MFO system.

In this communication, we have investigated the possibility that SPG affects the microsomal MFO system.

The animals used were 5-week-old Wistar rats obtained from the Shizuoka Laboratory Animal Center (Hamamatsu, Japan). They were allowed free access to water and food (CE-2, Clea Japan, Co. Ltd., Tokyo, Japan) and maintained in climate and light controlled rooms (23±1°C; 12/12 hr dark/light cycle, with lights on at 7:00) for at least 3 days prior to the experiments. SPG (pyrogen-free; Mr, 4-5x10^5) was kindly provided by Kaken Chemical Co., Tokyo, Japan, and dissolved in pyrogen-free saline. All the solutions were prepared immediately before use so that 0.1 g/10 g body weight provided the doses desired for rats. Unless otherwise stated, groups of 6 rats were used in the experiments. Animals were injected intraperitoneally with SPG (0.6, 1, 3, 6 and 10 mg/kg/12 hr, 4 times), and the test was done 12 hr after the last injection of SPG. This dose of SPG (approximately 5 mg/kg) was found to give the maximal effectiveness in immunotherapy and caused no obvious toxicity in the experiments. Control animals received saline. Aminopyrine N-demethylase, aniline hydroxylase, NADPH-cytochrome c reductase, NADH-ferricyanide reductase, cytochrome P-450 and cytochrome b5 were determined by the methods described in previous papers (6, 7). The dose-response of loss of the hepatic cytochrome P-450 contents after administration of SPG is shown in Fig. 1. The fact that dosages higher than 10 mg/kg body weight had no effect on the antitumor activity, as already demonstrated on enhancing immunity in the host by many investigators, led us to investigate just the dose-response curve after intraperitoneal injection of SPG. A parabolic dose-response curve was obtained, indicating that SPG depressed the hepatic microsomal MFO activities in a dose-dependent manner.
Fig. 1. Dose-response of loss of hepatic cytochrome P-450 contents after administration of sizofilan in rats. Sizofilan (SPG) was given intraperitoneally to rats twice a day for two days. Control rats received saline similarly. Animals were killed at 12 hr after the final injection of SPG, and microsomes were prepared from their livers. Cytochrome P-450 contents are expressed as a decrease percentage ± S.E. of 4–6 animals compared with the control value. Data were analyzed by Student’s t-test: *P < 0.05, with respect to the control group. The cytochrome P-450 content was 0.64 ± 0.07 nmol/mg protein in the control rats.

The curve of depression with a rather narrow optimum at 3 mg/kg in the hepatic microsomal content of cytochrome P-450 was observed when the rats were administered with SPG at a dose of 0.6, 1, 3, 6 or 10 mg/kg, respectively, twice a day for two days. In these cases, the depression ratio was 5.1%, 12.5%, 32.1%, 18.5% and 6.8%, respectively, when compared to the control value.

In all microsomal preparations, cytochrome P-420 could not be detected. Significant reductions of cytochrome P-450 content and aminopyrine N-demethylase and aniline hydroxylase activities were observed by the intraperitoneal administration of 3 mg/kg of SPG twice a day for two days (Fig. 2). However, if the activities of enzymes are expressed in nmol of metabolites formed per nmol of cytochrome P-450 per 20 min, there were no significant differences between the activities of SPG-treated rats and those of control rats. In addition, since no cytochrome P-420, an inactive form of cytochrome P-450, was found during the experiments, these results lead to the conclusion that the inhibition of the hepatic MFO activities by the administration of SPG was mainly based on the decrease in cytochrome P-450 content.

Recently, some investigators have reported that immunomodulators such as poly I:C, tilorone, bacterial endotoxin, OK-432 (a Streptococcus preparation, Picibanil) and PS-K (a glycoprotein extracted from Coriolus versicolor, Krestin) inhibited the activity of c reductase and NADH ferricyanide reductase did not change significantly (data not shown). Experiments were also carried out to determine whether SPG had a direct inhibitory effect on the metabolism of aminopyrine and aniline by hepatic microsomes.

The formation of formaldehyde or p-aminophenol from aminopyrine or aniline in incubation mixtures containing SPG (1 mg/ml incubation mixture) was identical to that in the control incubations.

From the data collected in these studies, it is clear that the administration of SPG in repeated doses can modify the activity of the drug-metabolizing enzymes in rat liver. These effects of SPG were demonstrated in vitro by measurement of decreases in microsomal aminopyrine N-demethylase and aniline hydroxylase activities and cytochrome P-450 content. The mechanism of this effect is not clear. It is apparently not due to the systemic toxicity of the SPG, because treated rats appeared healthy and gained body weight at the same rate as controls. It is unlikely that reduced metabolism is due to the binding of SPG or its degradation products to the endoplasmic reticulum, because direct addition of the SPG to microsomal preparations caused no change in enzyme activities. The depressions of aminopyrine N-demethylase and aniline hydroxylase activities were observed by the administration of SPG, when the activities were expressed in nmol of metabolite formed per 20 min per mg of protein. However, if the activities were expressed as nmol of metabolite formed per 20 min per nmol of cytochrome P-450, there were no significant differences between the activities of SPG-treated rats and those of control rats. In addition, since no cytochrome P-420, an inactive form of cytochrome P-450, was found during the experiments, these results lead to the conclusion that the inhibition of the hepatic MFO activities by the administration of SPG was mainly based on the decrease in cytochrome P-450 content.

Recently, some investigators have reported that immunomodulators such as poly I:C, tilorone, bacterial endotoxin, OK-432 (a Streptococcus preparation, Picibanil) and PS-K (a glycoprotein extracted from Coriolus versicolor, Krestin) inhibited the activity of
8-aminolevulinic acid synthetase, a rate-limiting enzyme of heme biosynthesis, and increased the activity of hepatic heme oxygenase, a heme-degrading enzyme (8–10). These findings suggest that the decrease in the microsomal MFO activity and the content of cytochrome P-450 result from the inhibition of 8-aminolevulinic acid synthetase and the increase in heme oxygenase activity.

Endotoxin also has differential effects on components of the cytochrome P-450 monooxygenase system (11), and poly I:C decreases the content of proteins of the hepatic endoplasmic reticulum, including certain cytochrome P-450 isozymes, by decreasing rates of protein synthesis and apoprotein of enzymes and increasing rates of protein and apoprotein degradation (12, 13). The results indicate that after treatment with SPG, the same phenomena may occur in the hepatocytes of rats. The mechanism by which SPG treatment produces a parabolic dose-response curve of depression of microsomal MFO activity is unclear. Further studies are needed to clarify the mechanism of suppression of microsomal MFO activity induced by SPG.

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