Communications to the Editor

[Chem. Pharm. Bull. 32(2) 795—796 (1984)]

HEPATOTOXICITY OF HYDRAZINE IN ISOLATED RAT HEPATOCYTES

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Cytotoxicity of hydrazine (Hz) and acetylhydrazine (AcHz) was investigated using isolated rat hepatocytes. 10^{-4}, 10^{-3} and 10^{-2}M of Hz decreased hepatocellular reduced glutathione (GSH) in a strongly dose-dependent manner, while AcHz showed little effect on the GSH levels at any concentration examined. The results reveal that Hz is more hepatotoxic than AcHz.

KEYWORDS —— hydrazine; acetylhydrazine; isolated rat hepatocyte system; reduced glutathione

Hydrazine (Hz) is a toxic, hazardous metabolite of isoniazid (INH), causing fatty liver and liver necrosis, and is a mutagen and a carcinogen.[1,2] It has been detected by gas chromatograph-mass spectrometer in the urine of INH-dosed patients with tuberculosis.[3] It is well-known that liver injury is frequently caused in patients on INH treatment. However, the precise metabolite responsible for INH-induced hepatitis has never been identified, although Timbrell *et al.* postulated that INH-induced liver necrosis may be caused by the chemically reactive metabolite of INH, monoacetylhydrazine (AcHz).[4] In 1981, Bahri and Timbrell *et al.* described that AcHz did not produce necrosis in nonpretreated rats but only in phenobarbital pretreated groups.[5] On the other hand, we found that Hz induced more marked hepatic injury than AcHz, and analogous but more intensive necrosis took place in rabbits pretreated with rifampicin. This suggested that Hz could be a key intermediate which induced hepatic injury during INH therapy.[6] These facts prompted us to compare the extent of cytotoxicity of Hz and AcHz using the isolated rat hepatocyte system.

Isolated hepatocytes were obtained from male Wistar rats (300-350 g) by the collagenase perfusion method according to Moldéus *et al.*[7] One ml of the cell suspension which contains 4 X 10^6 cells/ml was incubated for 10, 20 or 30 min with 10^{-4}, 10^{-3} or 10^{-2}M of a substrate under O_2/CO_2 (95:5, v/v%) at 37°C in a rotating round bottomed flask. The control experiment was performed with vehicle only.

Incubation of Hz or AcHz in isolated hepatocytes for 30 min induced markedly dose-dependent cell-death as indicated by a trypan blue test. This test, however, did not show a distinct difference between Hz and AcHz in the extent of cell-injury. For instance, 37.4% or 36.0% increase of cell-damage occurred, compared to the control experient, when 10^{-2}M of Hz or AcHz was employed, respectively. We determined, therefore, the content of reduced glutathione (GSH)[8] in the hepatocytes incubated with Hz or AcHz.

The amount of reduced GSH in the control hepatocyte system (7.0 ± 1.0 $\mu g/10^{-6}$ cells) decreased slightly during the incubation at 37°C for 30 min. A significant time- and dose-dependent depletion of hepatocellular reduced GSH levels was induced by Hz-treatment, and a dramatic decrease of GSH was observed by treatment with 10^{-2}M Hz (Fig. 1, left). However, AcHz caused very little GSH depletion (Fig. 1, right). Bahri *et al.* also reported that GSH was not depleted significantly by a hepatotoxic dose of AcHz in the liver homogenates obtained from phenobarbital-pretreated rats.[5]

The present results on reduced GSH depletion and our previous histological data,[6] with careful consideration of Bahris' data,[5] suggest that Hz is the more important hepatotoxin and that it plays a role in INH-induced hepatic injury greater than AcHz. It is evident that GSH depletion is closely related to the cytochrome P-450 dependent formation of the reactive electrophile(s) from Hz. Interestingly this seems not to be the case for AcHz.

Further work is in progress to confirm the role of Hz as well as AcHz in INH-induced hepatitis.

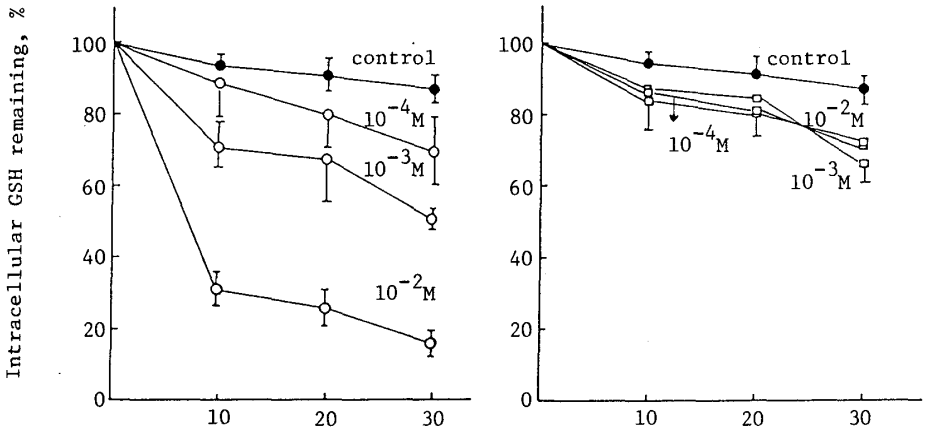

Fig. 1. Effects of Hydrazine (Left Figure) and Acetylhydrazine (Right Figure) on Intracellular GSH in Isolated Rat Hepatocytes (n=3)

ACKNOWLEDGEMENT The authors wish to thank Prof. S. Iguchi, Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University for his encouragement of our study. We are also greatful to Dr. M. Hirata, Shionogi Research Laboratories, Shionogi and Company, Ltd. for his helpful comments on the experiments, and to Ms. R. Deya for her technical assistance.

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(Received November 17, 1983)