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Effect of Betaine Administration on Metabolism of Hepatic Glutathione in Rats

Sang Kyum Kim, Sung Yeon Kim and Young Chul Kim*

College of Pharmacy, Seoul National University, San 56-1 Shinrim-Dong, Kwanak-Ku, Seoul 151-742, Korea

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Betaine is one of major components in fruits of *Lycium chinesis* which has long been used as tea and also in traditional medicine for hepatic disorders. Betaine, an oxidative metabolite of choline, is needed for the synthesis of methionine, which is mediated by betaine homocysteine methyltransferase (Fig. 1). This reaction is important in maintaining the hepatic methionine level, particularly when the intake of sulfur-containing amino acids is limited (Finkelstein *et al.*, 1971, 1982). Also the role of betaine as an osmotically active substance in renal medullary cells as well as in hepatic Kupffer cells has been noted (Garcia-Perez and Burg, 1991; Zhang *et al.*, 1996).

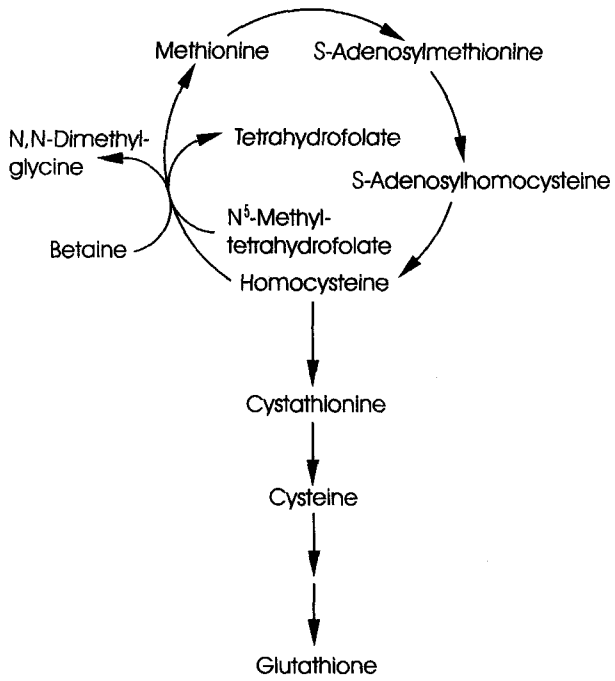

Fig. 1. Metabolism of sulfur-containing amino acids.

Correspondence to: Young Chul Kim, College of Pharmacy, Seoul National University, San 56-1 Shinrim-Dong, Kwanak-Ku, Seoul 151-742, Korea

In a recent study conducted in this laboratory, it was demonstrated that betaine induced time-dependent responses to a hepatotoxic dose of chloroform ($CHCl_3$) in mice (Kim *et al.*, 1998). Betaine administered 1 to 7 hr prior to injection of $CHCl_3$ potentiated the hepatotoxicity, but when mice were pretreated with betaine 24 hr before, almost complete protection against the $CHCl_3$-induced hepatotoxicity resulted. Also corresponding changes in the hepatic glutathione (GSH) level were observed, whereas the enzyme activities mediating the activation and/or detoxification of the solvent were not altered by betaine. Thus, the time-dependent responses to $CHCl_3$ appeared to be associated with the changes in availability of the hepatic levels of GSH. In this study the effect of betaine on the hepatic GSH metabolism was examined in female rats. We determined several important intermediates and precursors needed for its biosynthesis in the liver.

Fifty adult female SD rats were divided into 12 groups. Animals were housed in temperature (22±2°C) and humidity (55±5%) controlled rooms under artificial lighting (Light: 0800-2000). Laboratory chow and purified tap water were provided *ad libitum*. Rats were treated with betaine (1 g/kg, ip) and sacrificed 1, 2, 4, 8, and 24 hr thereafter. The 10,000 g fraction of the liver was prepared and used for the assays. Total GSH level was determined by an enzymatic recycling method of Griffith (1980). Cysteine level was determined spectrophotometrically (Gaitonde, 1967). *S*-Adenosylmethionine (SAM) and *S*-adenosylhomocysteine (SAH) were measured by using the method of She *et al.* (1994). A Jasco Model PU-980 pump (Tokyo, Japan) equipped with a Model 7725 injector (Rheodyne Co., Cotati, CA, U.S.A.), a Jasco Model UV-975 detector operating at 254 nm, and a TSKgel ODS-80Tm column (25 cm×4.6 mm id, Tosoh Co., Tokyo, Japan) was used. The mobile phase consisted of 40 mM $NH_4H_2PO_4$, 8 mM 1-heptanesulfonic acid sodium salt and 18 % (v/v) methanol at pH 3.0. The isocratic elution was carried out using a flow rate of 1.05 ml/min at 35°C.

Betaine administration increased SAM and SAH concentrations in the liver (Fig. 2, 3). Both SAM and SAH were slowly increased after the treatment, reaching a plateau after 4 to 8 hr, which returned to normal in 24 hr. The immediate precursor of SAM/SAH in the transsufuration pathway is methionine, which is synthesized from homocysteine and betaine. Therefore, it is suggested that betaine elevates SAM and SAH by

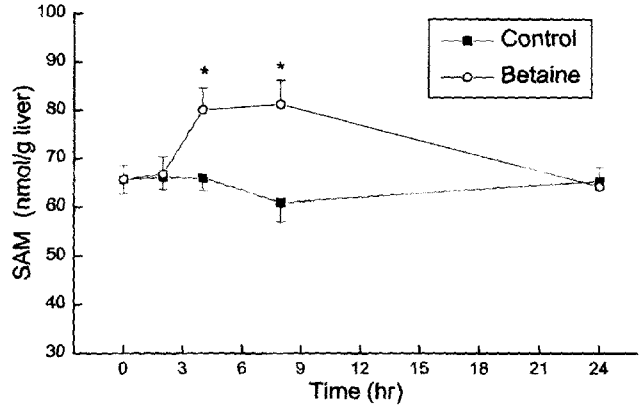

Fig. 2. Effect of betaine on hepatic SAM level. Rats were sacrificed at different time intervals after betaine (1 g/kg, ip) administration. Control rats were injected with isoosmotic NaCl solution. Each value represents mean±S.E. for 4 rats. An asterisk indicates a significant difference from control rats (Student's *t*-test, $P<0.05$).

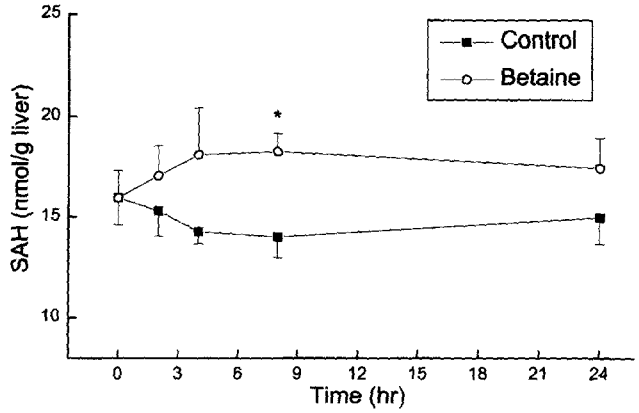

Fig. 3. Effect of betaine on hepatic SAH level. See the legend in Fig. 2 for details.

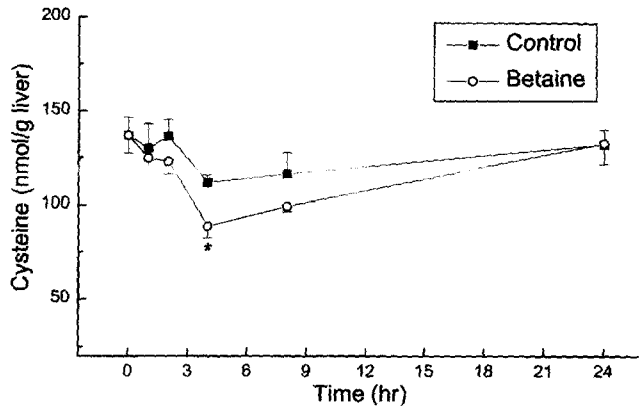

Fig. 4. Effect of betaine on hepatic cysteine level. See the legend in Fig. 2 for details.

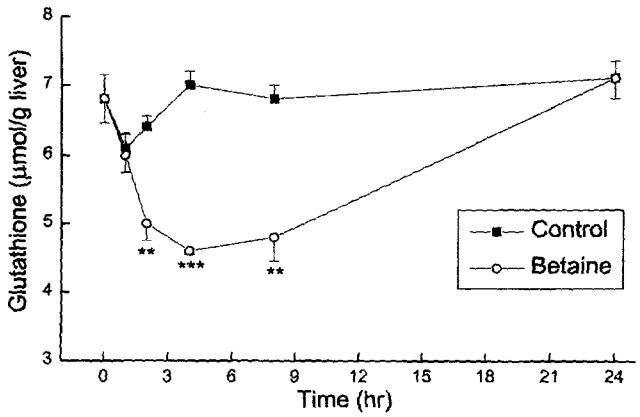

Fig. 5. Effect of betaine on hepatic GSH level. See the legend in Fig. 2 for details. Asterisks indicate significant differences from control rats (Student's *t*-test, *; $P<0.05$, **; $P<0.01$).

increasing the supply of this sulfur-containing amino acid. On the contrary, the hepatic cysteine level was reduced following the betaine treatment (Fig. 4). A statistically significant decrease in cysteine (ca. 75% of control) was observed at 4 hr after the treatment. The hepatic GSH level was more rapidly declining, and also the decrease was more pronounced and prolonged (Fig. 5). Both cysteine and GSH levels were recovered to normal in 24 hr after the betaine treatment. The fluctuation in hepatic GSH concentration seemed to be accounted by the change in cysteine level.

In the preceding study we observed time-dependent alterations in the effect of betaine on the hepatotoxicity of $CHCl_3$, which appeared to be associated with changes in availability of hepatic GSH (Kim *et al.*, 1998). It has been postulated that phosgene, the reactive metabolite generated from $CHCl_3$, is responsible for the induction of the hepatotoxicity (Pohl, 1979). The major catalyst for this metabolic reaction is known to be cytochrome P450 2E1 (Brady *et al.*, 1989). Hepatic GSH, the most abundant sulfhydryl compound in the hepatic tissue, has a critical role in the detoxification of the reactive metabolite of $CHCl_3$ (Pohl *et al.*, 1981). In the aforementioned study, the 2E1 activities and the enzyme activities associated with regeneration and consumption of GSH, such as GSSG reductase, GSH peroxidase, and GSH *S*-transferases, were all unaffected by the betaine treatment whereas the GSH level changed in a manner corresponding to its effect on the $CHCl_3$ hepatotoxicity. The hepatic GSH levels were rapidly decreased immediately following the betaine treatment, but rather elevated above the normal value when determined 24 hr later. In this study the changes in the hepatic GSH levels of rats induced by betaine appeared to be comparable to those of mice. But the GSH level was not rebounded at 24 hr after the treatment, which seems to be due to either species differences or discrepancy in feeding schedules for the animals used in each study.

Betaine serves as one of the substrates needed for biosynthesis of methionine, which is an immediate precursor of SAM/SAH. The results of the present

study showed that betaine increased the hepatic SAM and SAH levels effectively. However, increases in SAM and SAH were not reflected in the hepatic level of cysteine which is an essential building block for GSH. The reason for the failure of increased SAM/SAH to affect the cysteine/GSH levels is not known. Increased SAM and SAH levels would result in corresponding elevation in homocysteine production. But supply of homocysteine for cysteine synthesis could be depressed by exogenously added betaine which consumes homocysteine for generation of methionine. It is also well known that liver takes up methionine and cysteine while releasing GSH (Garcia and Stipanuk, 1992). Therefore, another possibility is inhibition of cysteine uptake into the liver by increased betaine in the liver and blood. Further study is being undertaken to clarify the mechanism of its action.

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