Effect of Diallyl Disulfide on the Hepatic Glutathione S-Transferase Activity in Rat: Diallyl Disulfide Effect on the Glutathione S-Transferase

Keun Huh, Sang-Il Lee, Jong-Min Park and Seok-Hwan Kim*

Dept. of Pharmacology, College of Pharmacy, Yeungnam University, Gyongsan 632, Korea,

**Dept. of Food and Nutrition, Dong-A University, Pusan 600-02, Korea*

(Received October 5, 1986)

Abstract □ Glutathione s-transferase is thought to play a key role in initiating the detoxication of potential alkylating agents, including pharmacologically active compounds. It is widely accepted that garlic contains allin which is converted to allicin by alliinase. Allicin is easily degraded to diallyl disulfide and other components. This report attempted to observe the effect of diallyl disulfide on some biological activities.

It was observed that the activity of serum transaminase was not changed by the treatment of diallyl disulfide. The liver cytosolic glutathione s-transferase was significantly increased. whereas the microsomal glutathione s-transferase was not increased.

Keywords □ Diallyl Disulfide, Glutathione s-transferase

Glutathione s-transferase (EC 2.5.1.18) is present in various organs of many species and these enzymes catalyze the conjugation of glutathione with electrophilic groups of many hydrophobic molecules (1-3). Studies on the purification and characterization of glutathione s-transferases revealed that they consist of various isozymes referred to as A, B, C, *etc* (2, 4-6). Thus, glutathione s-transferase is a family of multifunctional detoxication proteins (7, 8).

Meanwhile, selenium-independent glutathione peroxidase activity expressed by glutathione s-transferase may provide protection against lipid peroxidation initiating free radicals and resultant lipid hydroperoxides (9-11). These proteins protect tissue against damages by electrophilic intermediates and several organic hydroperoxides (1-3, 9-11).

In the previous study, it was recognized that garlic components increased microsomal aniline hydroxylase, phase I enzyme (12), and glutathione s-transferase, phase II enzyme, and glutathione peroxidase activity (13).

However, the action mechanism of garlic constituents is poorly understood. Therefore, this work was undertaken to further investigate of garlic components on the hepatic glutathione s-transferase activity.

Address for correspondence: Keun Huh, Department of Pharmacology, College of Pharmacy, Yeungnam University, Gyongsan 632, Korea

EXPERIMENTAL METHODS

Materials

Bovine serum albumin was obtained from Sigma Chemical Co. and 1-chloro-2,4-dinitrobenzene from Junsei Chemical Co. and 5,5′-dithiobis (2-nitrobenzoic acid) from Nakarai Chemical Co. and diallyl disulfide from Tokyo Kasei Chemical Co. and reduced glutathione from Fluka A.G. The other reagents used were of reagent grade.

Animal treatment

Male Sprague-Dawley rats weighing 250 g were used for all studies. They were divided into 4 groups. One group, the control, received olive oil subcutaneously. The other groups received diallyl disulfide (80, 120, 160 mg/kg respectively) subcutaneously once daily for 3 days. All experimental animals were allowed free access to food and water but deprived of the 16 hr prior to sacrifice.

Preparation of microsomal and cytosolic fraction

The animals were killed by exsanguination from the abdominal aorta. The liver was exhaustively perfused with cold 0.15 M sodium chloride solution through the portal vein until uniformly pale and quickly removed. After micing, the pieces of liver was homogenized with 4 volumes of cold 0.25 M sucrose solution. Each homogenate was centifugated at 10,000×g for 20 min. The pellet was discarded and the postmitochondrial fraction was further centrifugated at 105,000×g for 1 hr. The supernatant fraction was used as the cytosolic fraction and pellet as the microsomal fraction.

Enzyme assay

Glutathione s-transferase activity was measured by the method of Habig *et al.* (2) with 1-chloro -2, 4-dinitrobenzene and glutathione as substrates. Enzyme activity defined as formed thioether n mole per mg protein per min at 25℃. Protein was determined by the method of Lowry *et al.* (14), using bovine serum albumin as standard.

Serum transaminase activity was estimated according to the procedure described by Reitman and Frankel (15). A unit of transaminase is expressed as the Karmen (16) unit per m*l* of serum. Reduced glutathione was determined by the methods of Owens *et al.* (17) and Mitchell *et al.* (18).

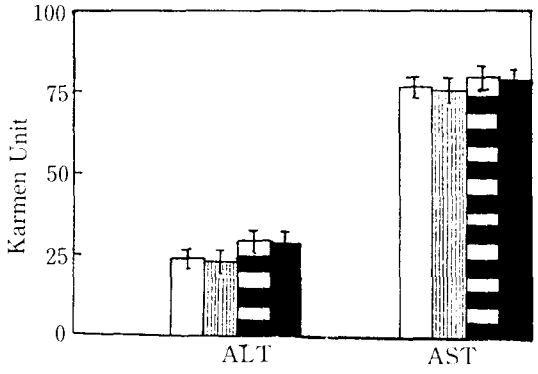

Fig.1. Effect of diallyl disulfide on the serum transaminase activity.

Diallyl disulfide (DADS; 80, 120, 160 mg/kg) were injected subcutaneously to rats for 3 days. The assay procedure was described in the text. Values are mean ±S.E. of 5 animals in each group.

- Control
- DADS 80 mg/kg
- DADS 120 mg/kg
- DADS 160 mg/kg

RESULTS

(1) ***Effect of diallyl disulfide on the serum transaminase activity***

Serum transaminase (ALT, AST) activities were not changed by the treatment of various concentrations of diallyl disulfide compared with control group (Fig.1).

(2) ***Effect of diallyl disulfide on the content of glutathione in liver***

Fig.2 shows the effect of diallyl disulfide on the glutathione level in liver. Glutathione contents were not changed by the treatment of various concentrations of diallyl disulfide for 3 days.

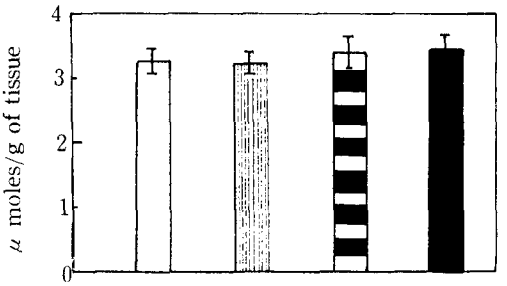

Fig.2. Effect of diallyl disulfide on the content of glutathione in rat liver.

The assay procedure was described in the text. Values are mean ±S.E. of 5 rats in each group. The other conditions are the same as described in Fig.1.

- Control
- DADS 80 mg/kg
- DADS 120 mg/kg
- DADS 160 mg/kg

(3) ***Effect of diallyl disulfide on the glutathione s-transferase activity***

In the liver cytosol of diallyl disulfide injected -rats, glutathione s-transferase activities were significantly increased by injection concentration of diallyl disulfide (Fig.3). However, the activities of microsomal glutathione s-transferase in rats were not significantly changed by the treatment of diallyl disulfide (Fig.4).

(4) ***Effect of diallyl disulfide on the enzymatic activities of liver cytosolic glutathione s-transferase in vitro***

The activities of rat liver cytosolic glutathione s -transferase in the presence of diallyl disulfide were not affected (Fig.5).

(5) ***Effect of diallyl disulfide on the kinetics of liver cytosolic glutathione s-transferase***

Km and V_{max} values of cytosolic glutathione

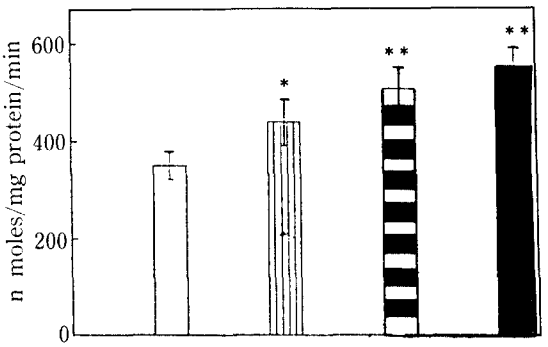

Fig.3. Effect of diallyl disulfide on the liver cytosolic glutathione s transferase activity in rat.

The assay procedure was described in the text. Values are mean ±S.E. of 5 rats in each group. The other conditions are the same as described in Fig.1. * ; $p<0.05$, ** ; $p<0.01$.

□ Control
▨ DADS 80 mg/kg
▬ DADS 120 mg/kg
■ DADS 160 mg/kg

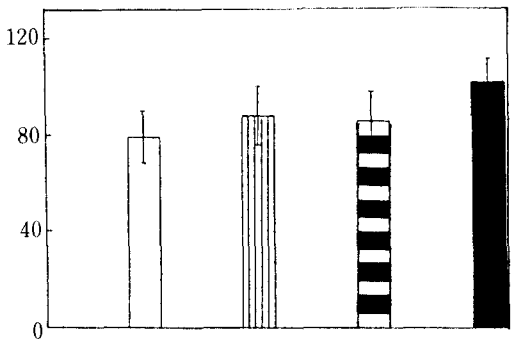

Fig.4. Effect of diallyl disulfide on the liver microsomal glutathione s-transferase activity in rat.

Values are mean ±S.E. of 5 rats in each group. The other conditions are the same as described in Fig.3.

□ Control
▨ DADS 80 mg/kg
▬ DADS 120 mg/kg
■ DADS 160 mg/kg

s-transferase were examined by the treatment of 160 mg/kg with diallyl disulfide for 3 days.

As shown in Figs.6 and 7, the V_{max} value (control; 322 n moles/mg/min, diallyl disulfide; 541 n moles/mg/min) without affecting the Km value (167 μM) for 1-chloro-2, 4-dinitrobenzene was increased by the treatment of diallyl disulfide (Fig.6). However, both the Km (control; 167 μM, diallyl disulfide; 204 μM) and the V_{max} (control; 322 n moles/mg/min, diallyl disulfide; 510 n moles/mg/min) values for glutathione were increased by the injection with diallyl disulfide for 3 days (Fig.7).

DISCUSSION

It was reported that garlic has various phar-

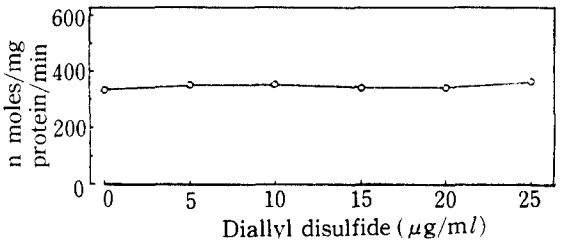

Fig.5. Effect of diallyl disulfide on the hepatic glutathione s-transferase activity *in vitro*.

The reaction mixture contained 0.1 M phosphate buffer, pH 6.5, various concentration of diallyl disulfide, 1 mM 1-chloro-2, 4-dinitrobenzene, 1 mM glutathione and cytosolic enzyme solution. Values are mean of 3 separate experiments.

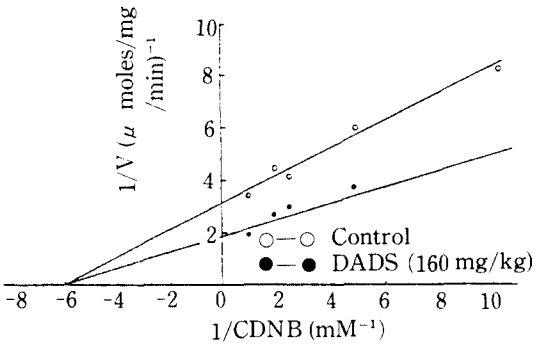

Fig.6. Double reciprcal plots of cytosolic glutathione s-transferase in rats treated with diallyl disulfide for 1-chloro-2, 4-dinitrobenzene.

Diallyl disulfide (160 mg/kg) was injected subcutaneously to rats for 3 days. The reaction mixture contained 0.1 M phosphate buffer, pH 6.5, various concentration of 1-chloro-2, 4-dinitrobenzene, 1 mM glutathione and control enzyme or diallyl disulfide treated-rat enzyme solution. Points are the mean of 3 separate experiments.

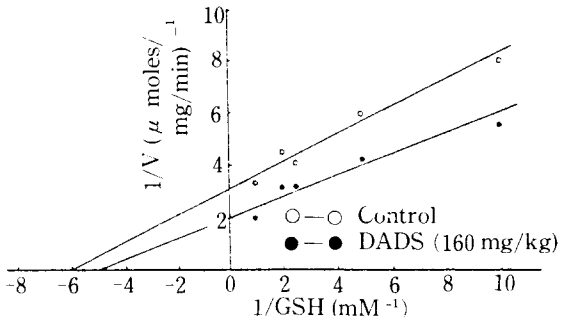

Fig.7. Double reciprocal plots of cytosolic glutathione s-transferase in rats treated with diallyl disulfide for glutathione.

The reaction mixture contained 0.1 M phosphate buffer, pH 6.5, various concentration of glutathione, 1 mM 1-chloro-2, 4-dinitrobenzene and the other conditions are the same as described in Fig.6.

macological effects (19-21). Recent studies have shown that garlic components regulate many metabolic diseases, such as atherosclerosis (22, 23), diabetes (24, 25), and gout (26, 27).

Therefore, we examined the action mechanism of diallyl disulfide, one of the breakdown product of allicin (28), on the glutathione s-transferase activity in experimental animals.

It was observed that serum transaminase activities were not changed by the treatment of various concentrations of diallyl disulfide. This result indicated that diallyl disulfide treatment to rats would not induce hepatocellular damage in this experimental conditions.

When diallyl disulfide was given for 3 days, liver cytosolic glutathione s-transferase activities were powerfully induced. Even though the data were not shown in this paper, it was also observed that cytosolic glutathione s-transferase activities in intestine and kidney were increased.

Glutathione s-transferase is regarded as detoxifying enzymes which catalyzes the first step in mercapturic acid formation (1-3). These results strongly suggested that diallyl disulfide may prevent the cell damage due to electrophilic compounds and lipid hydroperoxides.

Meanwhile, glutathione s-transferase has two types such as protein-bound and free form in tissues (29, 30). No change was observed in the free glutathione content of liver after administration of diallyl disulfide, indicating that the increase of glutathione s-transferase activities had no relation with the concentration of glutathione, co-substrate.

Moreover, glutathione s-transferase activity was not affected by the addition of diallyl disulfide *in vitro*. This result suggested that the increment of enzyme activity was unlikely to have arisen directly by diallyl disulfide. Furthermore, Vnii of glutathione s-transferase for 1-chloro-2, 4-dinitrobenzene was significantly increased by the treatment of diallyl disulfide for 3 days. Thus, as phenobarbital (31, 32), the characteristics of the increase in the enzyme activity by diallyl disulfide may include induction of enzyme proteins.

These findings led us to conclude that diallyl disulfide may regulate the hepatic glutathione s-transferase activity to prevent the toxic effect of xenobiotics and oxidative stress by the lipid peroxides.

In this study, it is indicated that the action mechanism of garlic may be associated with diallyl disulfide, one of the breakdown product of allicin, but further research in this field is neeed.

ACKNOWLEDGEMENT

We would like to thank Dr. Chong-Suk Kim, Dong Taegu Hospital, Taegu, Korea for his financial help and valuable advice.

LITERATURE CITED

1. Boyland, E. and Chasseaud, L.F.: The role of glutathione and glutathione s-transferase in mercapturic acid biosynthesis. *Adv. Enzymol.*, **32**, 173(1969).
2. Habig, W.H., Pabist, M.J. and Jakoby, W.B.: Glutathione s-transferase: The mercapturic acid formation. *J. Biol. Chem.*, **249**, 7130(1974).
3. Peter, M., Dean, P.J., Kari, O. and Sten, O.: Formation and metabolism of a glutathione conjugate in isolated rat liver and kidney cells. *Biochem. Biophys. Res. Commun.*, **83**, 195(1978).
4. Mannervik, B. and Jenssen, H.: Binary combination of four protein subunits with different catalytic specificities explain the relationship between six basic glutathione s-transferases in rat liver cytosol. *J. Biol. Chem.*, **257**, 9909(1982).
5. Boyer, T.D. and Kenney, W.C.: Acidic glutathione s-transferases of rat testis. *Biochem. J.*, **230**, 125(1985).
6. Singh, S.V., Dao, D.D., Partridge, C.A., Theodore, C., Srivastava, S.K. and Awasthi, Y.C.: Different forms of human liver glutathione s-transferases arise from dimeric combi-

nations of at least four immunologically and functionally distinct subunits. *Biochem. J.*, **232**, 781(1985).
7. Kaplowitz, N., Kuhlenkamp, J. and Clifton, G.: Drug induction of hepatic glutathione s-transferases in male and female rats. *Biochem. J.*, **146**, 351(1975).
8. Nichollas, F.A. and Ahokas, J.T.: Inhibition of glutathione s-transferases by indometacin. *Biochem. Biophys. Res. Commun.*, **119**, 1034(1984).
9. Prohaska, J.R. and Ganther, H.E.: Glutathione peroxidase activity of glutathione s-transferase purified from rat liver. *Biochem. Biophys. Res. Commun.*, **76**, 437(1977).
10. Burk, R.F., Nishiki, K., Lawrence, R.A. and Chance, B.: Peroxide removal by selenium-dependent and selenium-independent glutathione peroxidases in hemoglobin-free perfused rat liver. *J. Biol. Chem.*, **253**, 43(1978).
11. Reddy, C.C., Tu, C.P.D., Burgess, J.R., Ho, C.Y., Scholz, R.W. and Massaro, E.J.: Evidence for the occurence of selenium-independent glutathione peroxidase activity in rat liver microsome. *Biochem. Biophys. Res. Commun.*, **101**, 970(1981).
12. Huh, K., Nam, S.H., Park, J.M. and Chang, U.K.: Effect of garlic on liver microsomal aniline hydroxylase activity in mouse. *J. Resource Development*, Yeungnam University, **4**, 71(1985).
3. Huh, K., Park, J.M. and Lee, S.I.: Effect of garlic on the hepatic gluathione s-transferase and glutathione peroxidase activity in rat. *Arch. Pharm. Res.*, **8**, 197(1985).
4. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **16**, 530(1951).
5. Reitman, S. and Frankel, S.: A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *J. Clin. Pathol.*, **28**, 26(1957).
6. Karmen, A., Wroblewski, F. and La Due, J. S.: Transaminase activity in human blood. *J. Clin. Invest.*, **34**, 126(1955).
. Owens, C.W. and Belcher, R.V.: A colorimetric micromethod for the determination of glutathione. *Biochem. J.*, **94**, 705(1965).
. Mitchell, T.R., Jollow, D.R., Potter, W.Z., Gillette, J.R. and Brodie, B.B.: Acetaminophen-induced hepatic necrosis. IV. protective role of glutathione. *J. Pharmacol. Exp. Ther.*, **187**, 211(1973).
Cavallito, C.J., Bailey, J.H. and Buck, J.S.: Antibacterial principle of Allium sativum. III. Its precusor and essential oil of garlic. *J. Am. Chem. Soc.*, **67**, 1032(1945).
20. Barone, F.E. and Tansey, M.R.: Isolation, purification, synthesis and kinetics of activity of the anticandidal component of Allium sativum and a hypothesis for its mode of action. *Mycologia*, **69**, 793(1977).
21. Dipaolo, J.A. and Carruthers, C.: Effect of allicin from garlic on tumor growth. *Cancer Research*, **20**, 431(1960).
22. Jain, R.C.: Onion and garlic in experimental atherosclerosis. *Lancet*, **1**, 1240(1975).
23. Bordia, A., Verma, S.K., Vyas, A.K., Khabya, B.L., Rathore, A.S., Bhu, N. and Bedi, H.: Effect of essential oil of onion and garlic on experimental atherosclerosis in rabbits. *Atherosclerosis*, **26**, 379(1977).
24. Jain, R.C. and Vyas, C.R.: Garlic in alloxan-induced diabetic rabbits. *Am. J. Clin. Nutri.*, **28**, 684(1975).
25. Mathew, P.T. and Augusti, K.T.: Studies on the effect of allicin on alloxan-diabetes. I. Hypoglycaemic action and enhancement of serum insulin effect and glycogen synthesis. *Indian J. Biochem. Biophys.*, **10**, 209(1973).
26. Huh, K., Choi, C.W., Cho, S.Y. and Kim, S. H.: Effect of Allium sativum L. on the hepatic xanthine oxidase activity in rat. *J. Resource Development*, Yeungnam University, **2**, 111(1983).
27. Huh, K., Lee, S.I. and Park, J.M.: Effect of garlic on the hepatic xanthine oxidase activity in rats. *Korean Biochem. J.*, **18**, 209(1985).
28. Wills, E.D.: Enzyme inhibition by allicin, the active principle of garlic. *Biochem. J.*, **63**, 514(1956).
29. Kaplowitz, N., Kuhlenkamp, J., Goldstein, L. and Reeve, J.: Effect of salicylates and phenobarbital on hepatic glutathione in the rat. *J. Pharmacol. Exp. Ther.*, **212**, 240(1979).
30. Higashi, T., Furukawa, M., Hikita, K., Naruse, A., Tateishi, N. and Sakamoto, Y.: Re-evaluation of protein-bound glutathione in rat liver. *J. Biochem.*, **98**, 1661(1985).
31. Kaplowitz, N., Kuhlenkamp, J. and Clifton, G.: Drug induction of hepatic glutathione s-transferase in male and female rats. *Biochem. J.*, **146**, 351(1975).
32. Friedberg, T., Bentley, P., Stasiecki, P., Glatt, H.R., Raphael, D. and Oesch, F.: The identification, soubilization, and characterization of microsome-associated glutathione s-transferase. *J. Biol. Chem.*, **254**, 12028 (1979).