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Inhibition of Acrylamide Toxicity in Mice by Three Dietary Constituents

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The inhibitory effects of three dietary constituents, tea polyphenols, resveratrol, and diallyl trisulfide, on acrylamide−biomacromolecule (liver DNA, protamine, and hemoglobin) adduct formation at human exposure level were studied by accelerator mass spectrometry. The results demonstrated that the three dietary constituents all significantly inhibited the formation of acrylamide adducts with liver DNA, whereas tea polyphenols and diallyl trisulfide reduced protamine and hemoglobin adducts, respectively. Further biochemical studies showed that acrylamide could significantly inactivate creatine kinase and glutathione *S*-transferase and deplete glutathione. When the inhibitors were cotreated with acrylamide, all of them could effectively recover the activities of creatine kinase. In addition, tea polyphenols and diallyl trisulfide could increase glutathione *S*-transferase activity remarkably. On the basis of these results, mechanisms of the effects are discussed. This study might provide a beneficial guide to people's diet for the purpose of reducing the harmful effect of acrylamide.

KEYWORDS: Acrylamide−biomacromolecule adducts; tea polyphenol; resveratrol; diallyl trisulfide; inhibition; creatine kinase; glutathione *S*-transferase; accelerator mass spectrometry

INTRODUCTION

Acrylamide is an important industrial chemical primarily used in the production of polymers and copolymers. Previously, extensive studies have shown that acrylamide could induce neurotoxic, genotoxic, carcinogenic, developmental, and reproductive toxic effects in animals (*1*). In 1994, acrylamide was classified as a probable human carcinogen by the International Agency for Research on Cancer (*2*).

In rats 60−70% of acrylamide is conjugated with glutathione (GSH) and excreted within 24 h (*3*), whereas about 13% of it is metabolized by cytochrome P_{450} subtype CYP2E1 to form glycidamide (*4*), which has been reported to be responsible for many toxic effects of acrylamide (*5, 6*). Glycidamide subsequently conjugates with GSH or undergoes hydrolysis of the epoxide group, possibly catalyzed by epoxy hydrolase (*3*). Both acrylamide and glycidamide have ready access to most target tissues and exert toxic effects on many important proteins such as protamine, creatine kinase (CK), enolase, and phosphofructokinase (*7–9*). However, no in vivo acrylamide−DNA adducts have yet been reported. Nevertheless, glycidamide−DNA adducts have been identified both in vitro (*10*) and in vivo (*11*).

In 2002, acrylamide was reported to be generated from asparagine when starch-rich foods are cooked at high temperature (*12*). This has evoked great concern for the health effects of acrylamide to the public. Soon after, the World Health Organization published that the average daily intake of acrylamide for the general population was about 1 μg/kg of body weight (bw) (*13*). In fact, toxicological evaluation at such a low level is too difficult. However, our previous study by ultrasensitive accelerator mass spectrometry (AMS) has shown that acrylamide−biomacromolecule adducts were detectable even at such low dose levels (*14*). This suggests that daily exposure to a low dose of acrylamide may bring about a risk factor of reproductive toxicity as well as carcinogenicity in human beings.

The public exposure to acrylamide through food processing is unavoidable; it is therefore of particular importance to explore and establish approaches for reducing its toxic effects. Previously, scientists have reported that the pretreatment of rats with modifiers of mixed function oxidase, such as phenobarbital, dithiothreitol, $CoCl_2$, and SKF-525A, could delay or prevent the neuropathy of acrylamide (*15–17*). Saita et al. demonstrated that 4-methylcatechol could accelerate the recovery from acrylamide-induced neuropathy by stimulating nerve growth

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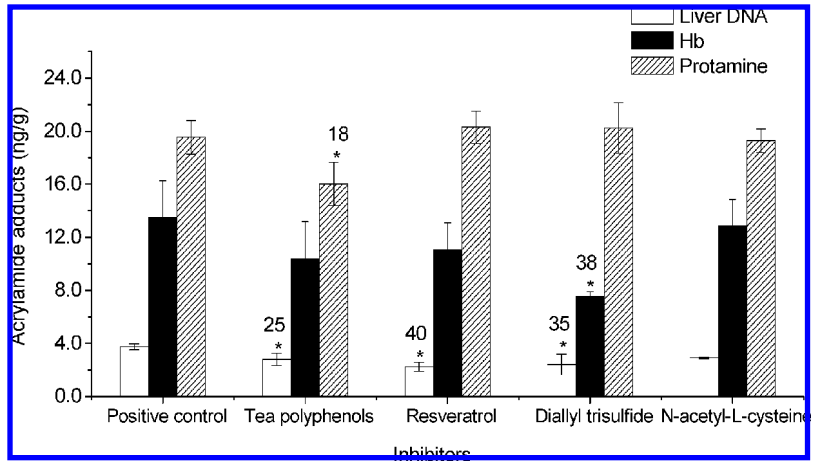

Figure 1. Inhibitory effects of pretreatment with tea polyphenols, resveratrol, diallyl trisulfide, and *N*-acetyl-L-cysteine on acrylamide-biomacromolecule adduct formation in mice. Values represent means ± SD ($n = 4$). The values at the top of each bar indicate the percent of inhibition, compared with the positive control. The background contribution of radiocarbon has been subtracted. *, significantly different from the positive control with $P < 0.05$, using ANOVA. Percent of inhibition (%) = [(acrylamide adduct number of the positive control − acrylamide adduct number with inhibitor pretreatment)/acrylamide-adduct number of the positive control] × 100%.

(*18*). Sabri et al. reported exogenous sodium pyruvate had a protective effect on acrylamide-induced neuropathy (*19*). 1-Aminobenzotriazole, a CYP2E1 inhibitor, prevented dominant lethal mutations (*20*) and reduced utero mortality induced by acrylamide (*21*). Moreover, *N*-acetyl-L-cysteine was reported to have a protective effect on acrylamide-induced cytotoxicity (*22, 23*). Nevertheless, most of these studies used synthetic chemicals, which are not of daily access, and all of the inhibition studies were based on the observation of macrotoxic end points induced by high doses of acrylamide. Also, no study on the prevention of acrylamide-derived biomacromolecule adducts has yet been reported. Therefore, it is highly desirable to find some inhibitors that are easily approachable for the inhibition of acrylamide toxic effects at human exposure dose levels.

In fact, many dietary constituents can act as chemopreventives or inhibitors against cancer (*24–26*). In this study, three widely existing dietary constituents, tea polyphenols, resveratrol, and diallyl trisulfide, are chosen as inhibitors. *N*-Acetyl-L-cysteine, a synthesized chemical, is also used for comparison. Their inhibitory effects on acrylamide adduct formation with biomacromolecules are studied in vivo by AMS at human exposure dose levels. Furthermore, their preventive effects on acrylamide inactivation of the muscle CK activity, which is one of the main symptoms of acrylamide neurotoxicity, are studied. Meanwhile, glutathione *S*-transferase (GST) activity and GSH level in liver are measured to gain further understanding of the inhibition mechanism.

MATERIALS AND METHODS

Chemicals. [2,3-^{14}C]Acrylamide (5 mCi/mmol) was purchased from American Radiolabeled Chemical Inc. (St. Louis, MO). Proteinase K (40 milliAnson units/mg) came from Merck (Darmstadt, Germany). RNase A (81 k units/mg) and *N*-acetyl-L-cysteine (>99%) were purchased from Sigma (St. Louis, MO). Resveratrol (98.66%) was obtained from Shanxi Saide Biological Technology Co., Ltd. (Shanxi, China). Diallyl trisulfide (30 mg/2 mL) was from Shanghai Harvest Pharmaceutical Co., Ltd. (Shanghai, China). Tea polyphenols (catechin, 91.2%; other polyphenols, 8.6%) were from Wuxi Lubao Biological Technology Co., Ltd. (Jiangsu, China). CK and GSH detection kits were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other chemicals were of analytical grade or better.

Animal Treatments. Adult male ICR mice, 8−10 weeks old (ca. 35 g), were obtained from Beijing Vitalriver Experimental Animal Technology Co. They were weighed and randomly grouped by their body weight. Each group of mice was housed in one cage with wooden chip bedding and was maintained on a 12 h light/dark cycle; they received food (Complete Granule Forage for Common Mouse, BLARC, China and Australia Cooperation Co.) and water ad libitum. Saline (0.9% NaCl, w/v) was used as the vehicle for the inhibitors. The inhibitors were administered by intraperitoneal injection. All of the animal studies were in compliance with the International Guiding Principles for Biomedical Research Involving Animals (1985), and the protocols were approved by the Animal Use and Care Committee of Peking University.

For the study of the inhibition of acrylamide adduct formation with biomacromolecules, after a 3-day acclimation period, animals (20 mice/group) were injected with tea polyphenols, resveratrol, diallyl trisulfide, and *N*-acetyl-L-cysteine of 1 mg/kg of bw for 6 consecutive days. The blank control and positive control groups were given only saline. On the sixth day, all mice except the blank control group were administered a single dose of [2,3-^{14}C]acrylamide (10 μg/kg of bw). This dose was chosen on the basis of our previous study (*14*), which was comparable to the human relevant dose, and induced significant acrylamide−biomacromolecule adducts for the inhibition experiment. Then animals were sacrificed 24 h post [2,3-^{14}C]acrylamide exposure, and mouse livers, blood, and epididymides were collected and pooled, 5 mice/sample.

For the biochemical study, mice (5 mice/group) were injected with 25, 10, 5, and 10 mg/kg of bw of tea polyphenols, resveratrol, diallyl trisulfide, and *N*-acetyl-L-cysteine, respectively, for 8 consecutive days. The doses were chosen according to their solubility in our preexperiment without observable toxicity. Mice administered with saline served as blank control. For the study of the inhibitor effects on acrylamide inactivation of CK and GST and depletion of GSH level, from the third day, acrylamide (100 mg/kg of bw) was injected 6 h after the administration of inhibitors through the next 6 days. Then animals were sacrificed 24 h after the last injection, and mouse livers and hind leg muscles were collected, 1 mouse/sample.

Isolation of Protamine, Hemoglobin (Hb), and Liver DNA. Mouse protamine was isolated according to the method of Balhorn (*14, 27*). Briefly, sperm was separated from the mouse epididymides by teasing the tissue on ice in 0.01 M Tris−saline (pH 8.0) and filtering the suspended sperm through 200 mesh nylon gauze. The sperm tails were removed by dithiothreitol and cetyltrimethylammonium bromide. The obtained sperm heads were dissolved in 5 M guanidine hydrochloride and sonicated to dissociate the proteins, and DNA was precipitated by 0.5 M HCl. Then, the supernatant was dialyzed and lyophilized to obtain the protamine powder.

Hb was isolated according to our previous paper (*14*). Red blood cells were separated from heparinized blood by centrifugation. After hemolyzation using ice-cold distilled deionized water, the hemolysate was dialyzed against deionized water. Hb was precipitated using ice-

cold ethanol. After being dehyrated with ethanol and ether, the sample was air-dried overnight to obtain the Hb powder.

Liver DNA was isolated from liver according to the method described previously (*28*). Briefly, the tissue was homogenized in a buffer containing 1% SDS, 1 mM EDTA, and 50 mM Tris-HCl. Then the tissue proteins were removed by proteinase K treatment and subsequent extraction with phenol, chloroform/phenol (1:1), and chloroform/isoamyl alcohol (24: 1) in turn. Coarse DNA was precipitated using NaCl and absolute ethanol (−20 °C). RNA was removed by RNase A treatment. Then, the pure DNA was precipitated again and stored in absolute ethanol.

AMS Sample Preparation and Measurement. Briefly, biomacromolecule samples, sealed under vacuum in a quartz tube, were oxidized to CO_2 in the presence of copper oxide. The CO_2 was then cryogenically transferred to a second glass tube and reduced to filamentous graphite in the presence of cobalt, titanium hydride, and zinc powder (*29*). The ^{14}C contents were measured by a 2×0.6 MV Tandem AMS facility (National Electrostatic Co.) at the Institute of Heavy Ion Physics, Peking University. Then, the $^{14}C/^{12}C$ ratio was normalized and converted to acrylamide equivalents (ng) per gram of the tissue (*14*).

Determination of CK, GST Activities, and GSH Content. Muscle was weighed, suspended in saline, and homogenized. Then, the obtained suspension (2%, w/v) was centrifuged for 10 min at 1470*g* and 0 °C. CK activity in the supernatant was determined colorimetrically at 660 nm with a commercially available kit based on the CK catalyzed reversible transfer of the phosphoryl group from MgATP to creatine. Then the phosphocreatine was further hydrolyzed and reacted with ammonium molybdate to form phosphomolybdic acid, which proceeded to further reduction and produced molybdic blue. CK activity was expressed as units per milligram of protein.

Liver was weighed, suspended in buffer (50 mM KH_2PO_4/K_2HPO_4, pH 7.2/150 mM KCl/1 mM EDTA), and homogenized. The obtained suspension (20%, w/v) was centrifuged for 15 min at 9000*g* and 0 °C. The GST activity in the supernatant was measured according to the method of Habig et al. (*30*) using 1-chloro-2,4-dinitrobenzene as the substrate. GST activity was expressed as nanomoles of substrate−GSH conjugate per minute per milligram of protein.

For GSH content determination, liver was weighed, suspended in saline (10%, w/v), and homogenized. Four volumes of saturated $(NH_4)_2SO_4$ was added to the homogenate, and then the obtained suspension was centrifuged for 10 min at 1380*g* and 0 °C. GSH content in the supernatant was measured with a commercially available kit, which measured the increase of absorbance at 420 nm by the reaction of sulfhydryl with 5,5-dithiobis(2-nitrobenzoic acid). GSH content was expressed as milligrams of GSH per gram of protein.

Protein content in the supernatant was determined according to the method of Lowry et al. (*31*), using bovine serum albumin as the reference standard.

Data Analysis. Because the AMS could not differentiate the parent compound from its metabolites, acrylamide−biomacromolecule adducts were expressed in terms of total nanogram acrylamide equivalents per gram of biomacromolecules. Four parallel samples were prepared for AMS measurements and five for determination of the CK and GST activities as well as the GSH level. Means of each treatment group were statistically compared using a one-way analysis of variance (ANOVA, SPSS 13.0 software). When the *F* test from ANOVA was significant, if equal variances were assumed, the least significant difference *t* test was used to compare means between the control and each of the experimental groups; if equal variances were not assumed, Dunnett's T3 was used. Statistical significance was considered at $P < 0.05$.

RESULTS AND DISCUSSION

Preventive Effects of the Inhibitors on Acrylamide−Biomacromolecule Adduct Formation. As shown in the positive control column in **Figure 1**, 10 μg/kg of bw [2,3-^{14}C]acrylamide induces remarkable biomacromolecule adduct formation, and the adduct levels are in the following sequence: liver DNA < Hb < protamine. On the one hand, this may be caused by the difference of the available acrylamide dose as well as its active metabolite glycidamide in various tissues. On the other hand, the adduct levels of protamine and Hb are much higher than that of liver DNA, which suggests that proteins are the major target molecules of acrylamide, probably by the reaction with −SH and/or $-NH_2$ groups (*32, 33*), even at human exposure level. Meanwhile, higher amounts of liver DNA adducts were observed than of sperm DNA, which are around the background level (data not shown). This could be attributed to the effective protection resulting from the compact structure of protamine−DNA complex in sperm chromatin.

It is well-known that the binding of highly reactive electrophilic compounds to biomacromolecules, such as proteins and nucleic acids in the target tissues, may lead to mutagenic and/or carcinogenic effects (*24*). According to the present study and our previous work (*14*), even at dietary exposure level, acrylamide might cause subtle quality changes of the biomacromolecules, although the harmful effects are not detectable by the macrotoxic end points. Therefore, exploring approaches that are of daily access to reduce or prevent acrylamide−biomacromolecule adduct formation at the human relevant low-dose level is of great significance.

Here, we selected the inhibitors on the basis of the following knowledge. Tea polyphenols, typical polyphenols in green tea, possess anticarcinogenic activity for tumors in various organs (*24*). Resveratrol, a phytoalexin in grapes and other food products, has shown chemopreventive activities in all three major stages of carcinogenesis (*25*). Diallyl trisulfide and diallyl sulfide are specific allyl sulfide compounds in garlic that have been reported to inhibit CYP2E1, thereby preventing the formation of genotoxic oxidative metabolites from xenobiotics (*26, 34, 35*). Besides, *N*-acetyl-L-cysteine, a GSH precursor, is capable of detoxifying electrophiles and free radicals and thus functions as an antimutagenic and anticarcinogenic agent (*36*).

The effects of the four inhibitors on acrylamide−biomacromolecule adduct formation are varied (**Figure 1**). For liver DNA adducts, the strongest inhibitory effect is observed with resveratrol of 40% reduction compared with the positive control, followed by diallyl trisulfide and tea polyphenols. Meanwhile, diallyl trisulfide shows significant inhibitory effect of 38% on the formation of Hb adducts, and tea polyphenols present significant inhibitory effect of 18% on the protamine adduct formation. Besides, *N*-acetyl-L-cysteine is ineffective on any of the adduct formation. The inconsistency of the inhibitory effects may be caused by the different influences of the inhibitors on acrylamide metabolism as discussed in later sections.

Effects of Inhibitors on Acrylamide Inactivation of CK. The primary health concerns associated with human exposure to acrylamide are its neurotoxic effects, involving both the central and the peripheral nervous systems (*37*). The toxic mechanism behind these effects is proposed by the thiol alkylation of CK with acrylamide (*8*). CK widely distributes in high energy requiring tissues, particularly in the nervous system and muscles. It is important in maintaining a normal level of adenosine triphosphate, which is crucial to provide energy for the functioning of the nervous system and muscle. Therefore, CK activity has been proposed as the most sensitive indicator of acrylamide intoxication (*38*).

Figure 2A shows that giving the mice only inhibitors (except *N*-acetyl-L-cysteine) does not influence the CK activity significantly, whereas the positive control column in **Figure 3A** shows that treatment with acrylamide (100 mg/kg of bw) could significantly inactivate CK. When the inhibitors are applied, the activity of CK can be effectively recovered by all of the inhibitors, with diallyl trisulfide having the strongest effect of

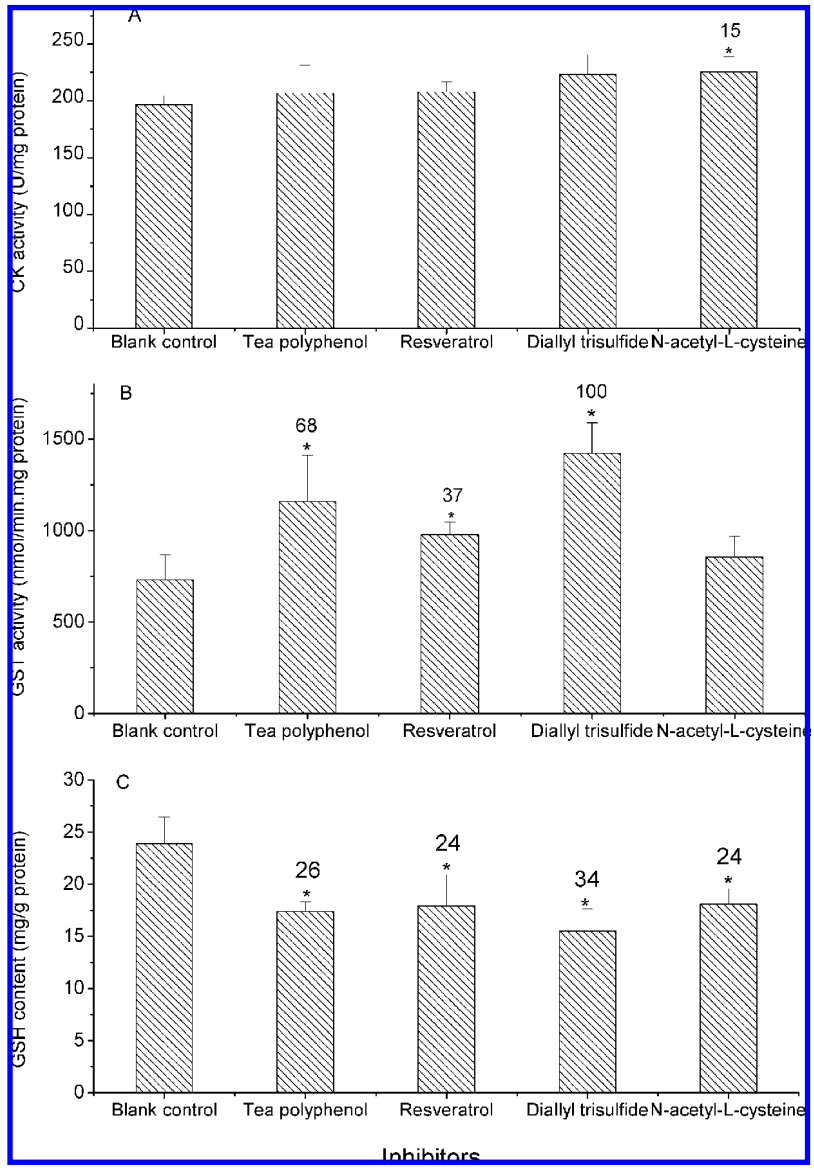

Figure 2. Effects of treatment with tea polyphenols, resveratrol, diallyl trisulfide, and *N*-acetyl-L-cysteine on the activities of CK (**A**) and GST (**B**) and on GSH level (**C**) in mice. Values represent means ± SD ($n = 5$). The value at the top of each bar indicates the percent of increase of the activities of CK and GST, or decrease of the GSH level, compared with the blank control. ∗, significantly different from blank control with $P < 0.05$, using ANOVA. Percent of increase (%) = [(enzyme activity with inhibitor treatment − enzyme activity of the blank control)/enzyme activity of the blank control] × 100%; percent of decrease (%) = [(GSH level of the blank control − GSH level with inhibitor treatment)/GSH level of the blank control] × 100%.

35% compared with the positive control. The results suggest that providing GSH is of limited influence on CK activity. There might be other factors, for instance, inducing GST activity that makes the main point for the protective effects of the three dietary constituents on CK activity.

Possible Mechanisms for the Preventive Effects of These Inhibitors. First, on the basis of the known evidence of the role of glycidamide in acrylamide-induced toxicity (*5, 6, 11*), modulation of phase I enzyme CYP2E1 is proposed as one important mechanism. For acrylamide−DNA adduct formation, acrylamide requires metabolic conversion to its reactive intermediate glycidamide by CYP2E1 (*11*). Inhibition of CYP2E1 by chemopreventive agents will effectively block the conversion of acrylamide to glycidamide and reduce the acrylamide−DNA adducts. When we chose the inhibitors, we noted the fact that all three dietary constituents could inhibit CYP450 and/or CYP2E1 (*26, 35, 39, 40*). Our results indicate that resveratrol, diallyl trisulfide, and tea polyphenols indeed significantly reduce the acrylamide−DNA adduct formation. But for acrylamide−protein adduct formation, acrylamide itself is the main reactive species that should be considered rather than glycidamide (*4, 11*). The inhibition of hepatic activation enzymes may reduce the conversion of acrylamide to glycidamide, and as a result protein adduct formation might even increase as seen in **Figure 1** for protamine adduct formation.

Second, induction of phase II enzyme GST is suggested to be another mechanism. GST can detoxify the carcinogens by catalyzing the reaction of potential carcinogenic electrophilic intermediates with the −SH group of GSH to render the products more water-soluble, less toxic, and easily excretable (*30*). It has been known that after absorption, acrylamide was rapidly metabolized primarily by GSH conjugation (*3*). Thus, GST or GSH inducers are considered to be the potential inhibitors of the acrylamide−biomacromolecule adduct formation. To test this hypothesis, we investigated the effects of the four inhibitors on the activity of GST and the GSH level in mouse liver.

Effects of Inhibitors per se on the Activity of GST and the GSH Level in Mouse Liver. Figure 2B shows that the

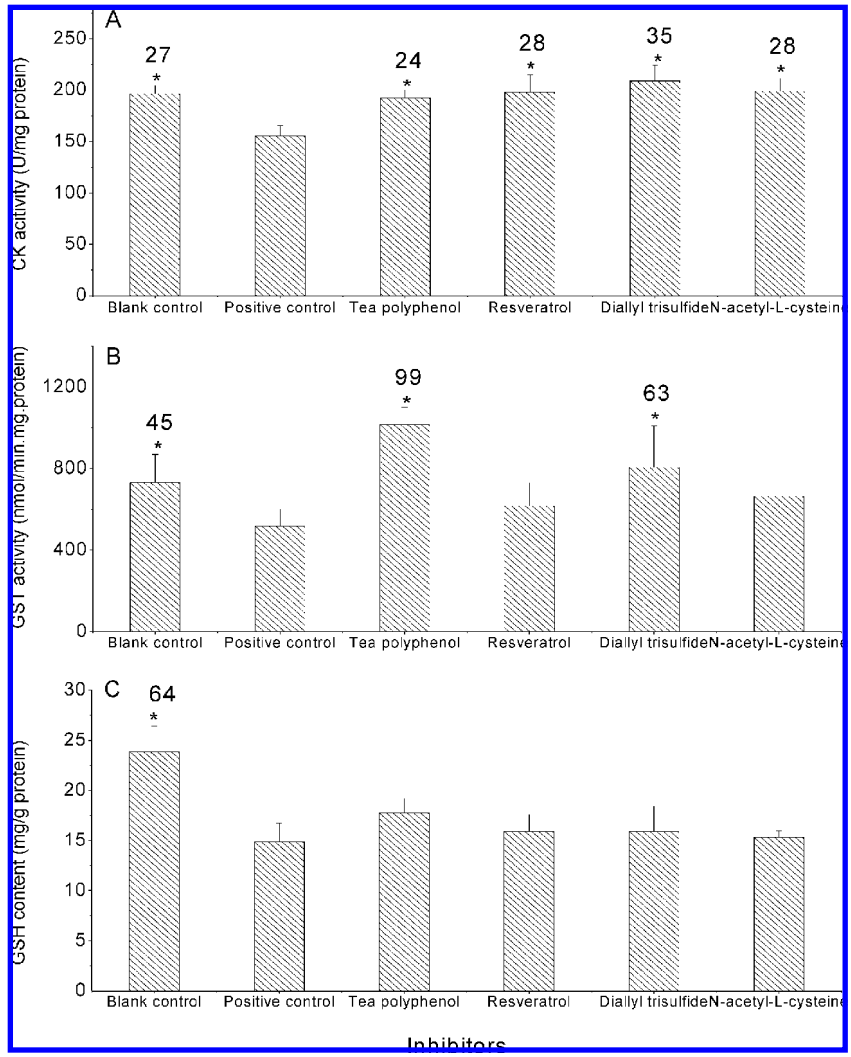

Figure 3. Inhibitory effects of pretreatment with tea polyphenols, resveratrol, diallyl trisulfide, and *N*-acetyl-L-cysteine on acrylamide inactivation of CK (**A**) and GST (**B**) and on depletion of GSH (**C**) in mice. Values represent means ± SD ($n = 5$). The value at the top of each bar indicates the percent of increase on the activities of CK and GST, or on the GSH level, compared with the positive control. ∗, significantly different from positive control with $P < 0.05$, using ANOVA. Percent of increase (%) = [(enzyme activity or GSH level of inhibitor − enzyme activity or GSH level of the positive control)/enzyme activity or GSH level of positive control] × 100%.

inhibitors (except *N*-acetyl-L-cysteine) can significantly increase the activity of GST, in which diallyl trisulfide is most effective at 100%, followed by tea polyphenols and resveratrol. This is consistent with the data (see **Figure 1**) that diallyl trisulfide and tea polyphenols significantly decreased Hb and protamine adduct formation, respectively. It is noteworthy that all inhibitors significantly decrease the GSH level, and diallyl trisulfide is a most effective one with an effect of 34% (**Figure 2C**), followed by tea polyphenols, resveratrol, and *N*-acetyl-L-cysteine. The data suggest a negative correlation between the GST activity and the GSH level. The possible explanation is that along with the increase of GST activity more GSH are depleted by binding with electrophiles. Thus, the GSH level is decreased.

Effects of Inhibitors on Acrylamide Inactivation of GST and Depletion of GSH. This study shows that acrylamide (100 mg/kg of bw) exposure to mice for 8 days can significantly decrease the activity of GST (**Figure 3B**) and deplete the GSH level in the liver (**Figure 3C**). Das et al. (*41*) reported that administration of acrylamide to rats at 50 mg/kg for 3 and 6 days produced no changes in hepatic GST activity. However, a significant decrease in the GST activity was seen at 10 days of acrylamide exposure. On the contrary, a recent study by Yousef et al. reported that acrylamide could increase the activity of rat liver GST in a dose-dependent manner (*42*). These paradoxical results might come from the significant difference of the exposure doses and time and animals used. Yousef's dose range is 0.5−500 μg/kg of bw, which is far less than Das's and ours, and Das et al. pointed out that the initial increase in hepatic GST activity by acrylamide may be a phenomenon of substrate induction of its biotransformation pathway (*41*). In our case when the dose of acrylamide increases, more GSH is depleted, which makes the −SH group in GST more vulnerable to acrylamide attack. Fortunately, tea polyphenols and diallyl trisulfide can significantly prevent the acrylamide inactivation of GST (**Figure 3B**) with 99 and 63% increases, respectively. This also supports the results in **Figure 1**, namely, that the inhibitor that induces GST activity has an inhibition effect on acrylamide−biomacromolecule adduct formation. Nevertheless, no inhibitor shows significant influence on acrylamide depletion of GSH (**Figure 3C**). This suggests that the three dietary constituents do not exert their inhibitory effects by inducing GSH.

Comparison of the Effect of *N*-Acetyl-L-cysteine with the Three Dietary Constituents. The effect of *N*-acetyl-L-cysteine on the recovery of acrylamide-induced CK intoxication is similar to the three dietary constituents (**Figure 3A**); however,

N-acetyl-L-cysteine does not effectively influence the acrylamide−biomacromolecule adduct formation (**Figure 1**). This may be partially ascribed to the fact that *N*-acetyl-L-cysteine can only serve as a sulfhydryl group donor, which does not exert significant increase on the activity of GST (**Figures 2B**and **3B**). As for the effect of *N*-acetyl-L-cysteine on the acrylamide-induced GSH depletion, our results are consistent with those of Park et al. (*22*), who found that *N*-acetyl-L-cysteine insignificantly increased the GSH level (**Figure 3C**). In addition, Wispriyono et al. also reported that *N*-acetyl-L-cysteine failed to protect rats from acrylamide neurotoxicity (*43*). Therefore, the results herein indicate that generally *N*-acetyl-L-cysteine is less effective than the three dietary ingredients in preventing acrylamide toxic effects, including acrylamide−biomacromolecule adduct formation and inactivation of CK and GST.

In conclusion, our results indicate that resveratrol, tea polyphenols, and diallyl trisulfide are effective inhibitors of acrylamide adduct formation at the human exposure level with liver DNA, protamine, and Hb, respectively. Moreover, these inhibitors can relieve the intoxication of acrylamide on the activities of CK and GST. Modulation of CYP2E1 as well as GST activity seems to be the key point of the inhibition mechanism. This study might offer a beneficial guide to people's diet against acrylamide toxicity, including productive toxicity, neurotoxicity, and potential carcinogenecity. In other words, commonly used foods, such as tea, red wine, and garlic, may play an important role in preventing the toxic effects caused by acrylamide. Nevertheless, it should be pointed out that this study employed intraperitoneal injection instead of oral feeding, which is how dietary ingredients are consumed by humans. It is quite likely that bioavailabilities of orally fed compounds might influence their activities against acrylamide toxicity. Therefore, oral feeding studies are necessary in the future.

ABBREVIATIONS USED

AMS, accelerator mass spectrometry; ANOVA, one-way analysis of variance; CK, creatine kinase; CYP2E1, cytochrome P450 2E1; GSH, glutathione; GST, glutathione *S*-transferase; Hb, hemoglobin.

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