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Short report

Effects of oil-soluble organosulfur compounds from garlic on doxorubicin-induced lipid peroxidation

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Clinical efficacy of doxorubicin is compromised due to free radical generation leading to cardiac toxicity. Oil-soluble organosulfur compounds, diallyl sulfide (DAS), diallyl disulfide (DADS), dipropyl sulfide (DPS) and dipropyl disulfide (DPDS), present in garlic were examined for their antiperoxidant effects. DADS inhibited liver microsomal lipid peroxidation induced by NADPH, ascorbate and doxorubicin. DAS, DPS and DPDS were ineffective inhibitors of liver microsomal lipid peroxidation. DADS could be used in combination with doxorubicin to protect oxidative injuries to improve the clinical efficacy of doxorubicin. [© 1998 Rapid Science Ltd.]

Key words: Doxorubicin, diallyl disulfide, diallyl sulfide, dipropyl disulfide, dipropyl sulfide, garlic, lipid peroxidation.

Introduction

Doxorubicin, the anthracyclin antibiotic, is part of the chemotherapeutic regimen for the treatment of a variety of solid tumors and hematopoietic malignancies.[1] The effectiveness of doxorubicin is limited due to dose-dependent and potentially lethal cardiac toxicity.[2,3] The etiology of doxorubicin-induced cardiac toxicity is related to the free radical-mediated lipid peroxidation.[4–9] Myers *et al.*[9] have demonstrated reduced doxorubicin-induced cardiac toxicity and lipid peroxide formation in mice pretreated with the antioxidant and free radical scavenger α-tocopherol. Garlic has been evaluated and shown to have free radical scavenging and antioxidant effects.[10–12] The oil-soluble organosulfur compounds present in garlic have been shown to inhibit chemical carcinogenesis.[13,14] The purpose of this investigation is to study the effects of oil-soluble organosulfur compounds diallyl sulfide (DAS), diallyl disulfide (DADS), dipropyl sulfide (DPS) and dipropyl disulfide (DPDS) on enzymatically, non-enzymatically and doxorubicin-induced microsomal lipid peroxidation.

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Materials and methods

Doxorubicin, nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate (G-6-P), glucose-6-phosphate dehydrogenase (G-6-PD), sodium ascorbate and thiobarbituric acid were purchased from Sigma (St Louis, MO). DAS, DADS, DPS and DPDS were purchased from Aldrich (Milwaukee, WI). All other routine chemicals were obtained from Curtin Matheson Scientific (Eden Prairie, MN).

Preparation of microsomes

Male Sprague-Dawley rats (SASCO, Omaha, NE) weighing 190–220 g were fasted overnight and sacrificed by decapitation. The livers were removed, rinsed and homogenized in ice-cold 1.15% KCl. Microsomes were prepared by differential centrifugation as described earlier.[15] The microsomal pellet was washed twice and resuspended in potassium phosphate buffer (15 mM, pH 7.4). Microsomal protein was determined using a BioRad Protein Assay Kit (BioRad, Richmond, CA).

Incubation systems

Liver microsomes (0.5 mg protein/ml) were incubated with a freshly prepared NADPH-generating system in potassium phosphate buffer (15 mM, pH 7.4) in the presence or absence of doxorubicin (20 μM). Reactions were started by adding the NADPH generating system which contained G-6-P (5 mM), NADP

(0.3 mM) and 0.5 units of G-6-PD per ml. Control incubations without the NADPH-generating system were included to determine the baseline levels of peroxidation. For induction of non-enzymatic lipid peroxidation, the NADPH-generating system was replaced by ascorbate (0.5 mM) and ADP-iron complex (0.012 mM $FeCl_3$, 0.04 mM ADP). The incubations were carried out aerobically at 37 °C for 20 min in a Dubnoff metabolic water bath shaker. Total incubation volume was 2 ml per tube. DAS, DADS, DPS and DPDS were dissolved in dimethyl sulfoxide (DMSO) in appropriate concentrations, and were added to the incubation mixture. A blank was run with DMSO alone.

Lipid peroxidation assay

The thiobarbituric acid assay for malonaldehyde was used to measure lipid peroxidation.[16] Reactions were stopped after 20 min of incubation by adding 1 ml of 35% trichloric acid followed by the addition of 1 ml of thiobarbituric acid solution (1.5%). The reaction mixture was allowed to cool and then filtered. The absorbance of the filtrate was read at 532 nm using a Beckman DU-64 spectrophotometer. Malonaldehyde values were calculated using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ at 532 nm.

Statistical analysis

The software package INSTAT (Graphpad, San Diego, CA) was used to analyze the data for Student's *t*-test. Significance was considered at $p < 0.05$.

Results and discussion

The inhibitory effects of DAS, DADS, DPS and DPDS on NADPH and ascorbate-induced liver microsomal lipid peroxidation are presented in Table 1. DADS completely inhibited both NADPH and ascorbate-induced lipid peroxidation at 0.5 μM concentration. DPDS caused 70% inhibition of lipid peroxidation induced by NADPH but was ineffective in ascorbate-induced lipid peroxidation. DAS and DPS were ineffective inhibitors of microsomal lipid peroxidation at the concentrations tested. Doxorubicin (20 μM) stimulated NADPH-dependent hepatic microsomal lipid peroxidation *in vitro*. The value for malonaldehyde in the absence of doxorubicin was 22.7 ± 3.8 nmol/mg protein, whereas a significantly higher ($p < 0.05$) malonaldehyde value of 34.2 ± 2.9 nmol/mg protein

Table 1. Effects of oil-soluble organosulfur compounds present in garlic on lipid preoxidation in rat liver microsomes[a]

Compound	Percent inhibition	
	NADPH[b]	Ascorbate[c]
DAS	33.7	0
DADS	100	100
DPS	46	0
DPDS	70	0

[a]Values represent mean calculated from five experiments run in triplicate. The final concentration of various compounds was 0.5 mM.
[b]Control value for lipid peroxidation in the absence of inhibitors was 24 ± 3 nmol malonaldehyde/mg protein.
[c]Control value for lipid peroxidation in the absence of inhibitors was 19 ± 1.8 nmol malonaldehyde/mg protein.

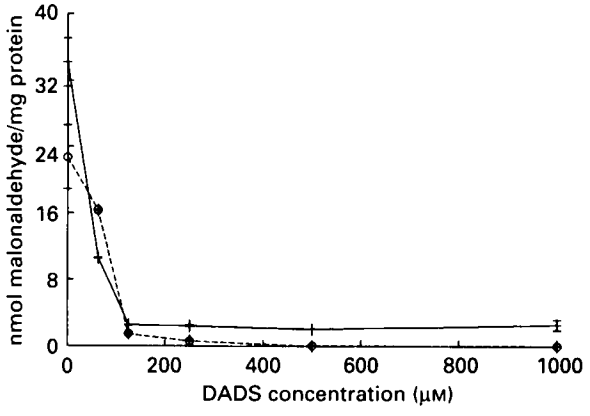

Figure 1. Effects of DADS on doxorubicin-induced lipid peroxidation. Each point represents mean ± SD derived from five experiments performed in triplicate: +, with doxorubicin; ○ without doxorubicin.

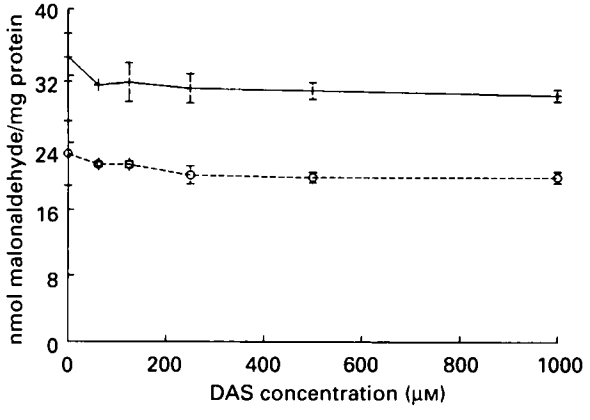

Figure 2. Effects of DAS on doxorubicin-induced lipid peroxidation. Each point represents mean ± SD derived from five experiments performed in triplicate: +, with doxorubicin; ○, without doxorubicin.

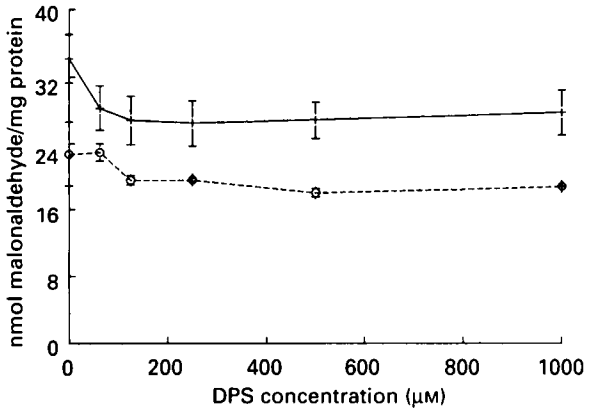

Figure 3. Effects of DPS on doxorubicin-induced lipid peroxidation. Each point represents mean ± SD derived from five experiments performed in triplicate: +, with doxorubicin; ○, without doxorubicin.

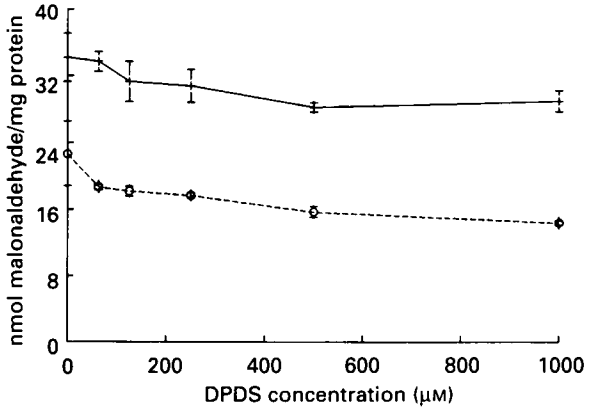

Figure 4. Effects of DPDS on doxorubicin-induced lipid peroxidation. Each point represents mean ± SD derived from five experiments performed in triplicate: +, with doxorubicin; ○, without doxorubicin.

was measured in incubation containing 20 μM doxorubicin. DADS is an effective inhibitor of doxorubicin-induced microsomal lipid peroxidation. The IC_{50} value for DADS was found to be 50 μM (Figure 1). DAS, DPS and DPDS were ineffective in inhibiting doxorubicin-induced lipid peroxidation (Figures 2-4).

These results indicate that DADS is the most effective inhibitor among oil-soluble organosulfur compounds present in garlic of microsomal lipid peroxidation caused by either NADPH, doxorubicin or ascorbate. DAS, DPS and DADPS are ineffective antiperoxidants present in garlic. Previous studies have also demonstrated DADS as an effective component in garlic for skin cancer prevention[14] and enhancing glutathione levels glutathione-S-transferase activity.[17] DADS appears to be the active component of garlic acting as the most effective antiperoxidant. The administration of DADS together with doxorubicin may decrease the cardiac toxicity because of its antiperoxidant effects, and thus may enhance the clinical effectiveness of doxorubicin and other similar drugs for the treatment of a variety of cancer. However, further studies including the effects of DADS and doxorubicin combination on cardiac peroxides and various cardiovascular functions are needed to fully understand the effectiveness of this combination.

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