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The garlic ingredient diallyl sulfide inhibits cytochrome P450 2E1 dependent bioactivation of acrylamide to glycidamide

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Abstract

Genotoxic effects of acrylamide are supposed to result from oxidative biotransformation to glycidamide. After incubation of rat liver slices with acrylamide we detected free glycidamide using a liquid chromatography tandem mass spectrometric method. Glycidamide formation was diminished in the presence of the cytochrome P450 2E1 inhibitor diallyl sulfide (DAS), which is a specific ingredient of garlic. This may be relevant to human health since the suggested carcinogenic risk of dietary acrylamide may be reduced by concomitant intake of garlic.

Keywords: Acrylamide; Glycidamide; Diallyl sulfide; Garlic; Rat liver slices; Cytochrome P450 2E1

1. Introduction

Based upon findings of carcinogenicity studies in rats (Johnson et al., 1986; Friedman et al., 1995), genotoxic effects in cultured mammalian cells and in somatic cells of treated animals (Besaratinia and Pfeifer, 2003), acrylamide is considered to be a probable human carcinogen (IARC Monographs, 1993). The potential carcinogenicity attained considerable interest with the discovery of high concentrations of acrylamide in common heated starch-rich foodstuffs (e.g. French fries, potato chips, cakes, bread) (Tareke et al., 2002) formed by Maillard reaction from reducing sugars and asparagine at processing temperatures above 120 °C (Mottram et al., 2002; Taubert et al., 2004).

Experimental studies (Besaratinia and Pfeifer, 2003) and investigations of in vivo genotoxicity (Manjanatha et al., 2005) have demonstrated a dose dependent increase of mutation frequency after exposure to acrylamide. There is evidence that the genotoxicity of acrylamide predominantly results from metabolic conversion to its epoxide derivative glycidamide (Twaddle et al., 2004; Doerge et al., 2005a) and subsequent formation of glycidamide–DNA adducts (Gamboa da Costa et al., 2003; Doerge et al., 2005b; Ghanayem et al., 2005a). Paulsson et al. (2003) reported that in mice the induction of micronuclei per unit of glycidamide in blood, a measure of genotoxicity, was identical when glycidamide was directly administered or when it arose as a metabolite from acrylamide administration. Recently, glycidamide was shown to be an inducer of genotoxicity or mutagenicity in V79 cells and human lymphocytes, while acrylamide was inactive in these models (Baum et al., 2005). Sumner et al. (1999) found cytochrome P450 2E1 (CYP2E1) to be the specific liver enzyme

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involved in this reaction in mice. Furthermore, in mice pretreated with 1-aminobenzotriazole, an inhibitor of CYP2E1, acrylamide induced mutations in spermatids were substantially reduced (Adler et al., 2000).

Two major metabolic pathways for acrylamide have been reported (Calleman, 1996; Dybing et al., 2005). One pathway is conjugation with glutathione to form the urinary metabolites *N*-acetyl-*S*-(3-amino-3-oxypropyl) cysteine and *N*-acetyl-*S*-(2-carbamoylethyl) cysteine. The second pathway is epoxidation to glycidamide. Most of glycidamide is metabolized by conjugation with glutathione to form mercapturic acids or metabolized by epoxide hydrolase (Friedman and Chemistry, 2003; Boettcher et al., 2005). However, only free unchanged glycidamide is supposed to account for the genotoxicity of acrylamide by formation of promutagenic DNA adducts (Gamboa da Costa et al., 2003; Doerge et al., 2005b; Segerback et al., 1995).

Administration of garlic (Allium sativum) has been shown to reduce the incidence of various chemically induced tumors in animal models (Milner, 1996). Epidemiologic studies indicate that frequent consumption of garlic or garlic extracts is associated with reduced cancer risk (Fleischauer and Arab, 2001). One of the primary constituents of garlic suggested to be responsible for this anticarcinogenic action is allyl sulfides that are arising from decomposition of the native cysteine sulfoxide alliin (Amagase et al., 2001). Allyl sulfides are thought to exert their protective effects in part by inhibition of CYP2E1, thereby preventing the formation of genotoxic oxidative metabolites from xenobiotics (Milner, 2001; Yang et al., 2001). A potent inhibition of CYP2E1-mediated bioactivation of procarcinogenes was reported for diallyl sulfide (DAS) as well as its metabolites diallyl sulfoxide (DASO) and diallyl sulfone ($DASO_2$) (Brady et al., 1991).

Using an in vitro model (rat liver slices), we wanted to test the hypothesis that biotransformation of acrylamide leads to free glycidamide and that the glycidamide formation is inhibited by DAS.

2. Experimental

2.1. Reagents

Glycidamide and D3-glycidamide (purity > 98% w/w) were synthesized from acrylamide and D3-acrylamide, respectively by H_2O_2 oxidation of acrylonitrile, as described (Payne and Williams, 1961). Deuterated acrylamide ([2,3,3-$^2H_3$]-acrylamide, 98% purity w/w) was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Acrylamide (purity > 99% w/w) and all other chemicals were from Sigma.

2.2. Incubation of rat liver slices

Metabolism experiments were performed using precision-cut rat liver slices. The liver was dissected out of ether sacrificed male Wistar rats weighing 200–300 g and placed into ice-cold Krebs–Henseleit–HEPES buffer. Preparation of liver slices (thickness about 250 μm) was performed directly out of the liver lobes with a Krumdieck tissue slicer, as described previously (Lupp et al., 2001).

Four slices each were placed in bidirectionally shaking Erlenmeyer flasks, filled with 5 ml carbogen aerated William's Medium E (pH 7.4, 37 °C) supplemented with insulin (1 μmol/l), L-glutamine (2 mmol/l) and ampicillin (10 mg/l). Slices were incubated for 0.5, 1, 2, 4, and 24 h, respectively with 1.4 μmol/l acrylamide in the presence of either 100 μmol/l or 1000 μmol/l diallyl sulfide or DAS free solvent (dimethylsulfoxide, 0.2% final concentration). All experiments were performed in duplicate. Glycidamide was determined in the medium. Incubation of glycidamide with DAS for 24 h in William's Medium E had no effect on glycidamide concentration, which excludes a direct interaction between glycidamide and DAS.

2.3. LC–MS/MS detection of glycidamide

Analysis was performed on a triple-quadrupole tandem mass spectrometer (TSQ Quantum Ultra, Thermo Electron, Dreieich, Germany) equipped with a thermostated (5 °C) Surveyor autosampler and a thermostated (30 °C) Surveyor HPLC system (Thermo Electron) operating in positive electrospray ionization (ESI^+) mode. Spray voltage was set at 4000 V and capillary temperature was kept at 350 °C. Nitrogen sheath gas and auxiliary gas pressure were 40 and 4 psi, respectively. Argon collision gas pressure was 1.0 Torr. The multiplier gain was tuned to 6 Mio to achieve maximal sensitivity. Fifteen microlitre aliquots of William's Medium E samples were injected onto a 5 μm Hypersil BDS C_{18} column (50 mm × 2.1 mm; Thermo Electron), and eluted isocratically at a flow rate of 0.25 ml/min (run time 5.0 min). The mobile phase consisted of 5% (v/v) acetonitrile/0.1% formic acid and 95% (v/v) deionized water/0.1% formic acid.

Precursor ion $[M+H]^+ \rightarrow$ product ion transition (single reaction monitoring (SRM)) used for quantification of glycidamide was m/z 88 → 44 (collision energy 20 eV). Detection of the internal standard (IS) D3-glycidamide (500 ng/ml) was performed by monitoring the m/z 91 → 44 transition (collision energy 20 eV). Response ratios of glycidamide versus IS were linear over a concentration range of 0.1–75 ng/ml (0.00115–0.86128 μmol/l) glycidamide ($r^2 > 0.990$). The limit of detection (LOD) was 0.1 ng/ml (0.00115 μmol/l) glycidamide.

3. Results and discussion

Incubation of rat liver slices with acrylamide resulted in a time dependent formation of free gly-