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Protective effects of garlic powder against potassium dichromate-induced oxidative stress and nephrotoxicity

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Abstract

Potassium dichromate ($K_2Cr_2O_7$)-induced nephrotoxicity is associated with oxidative stress. In the present work the effect of garlic powder, a recognized antioxidant, on $K_2Cr_2O_7$-induced nephrotoxicity and oxidative stress was studied. Rats were fed a 2% garlic powder diet for 1 month. A single injection of $K_2Cr_2O_7$ (15 mg/kg) to rats induced tubule interstitial damage and an increase in the following markers of renal injury 2 days later: blood urea nitrogen (4.6-fold), serum creatinine (9.7-fold), proteinuria (35.9-fold), urinary excretion of *N*-acetyl-β-D-glucosaminidase (12.9-fold) and glutathione-*S*-transferase (2.3-fold) and a decrease of 65% in serum glutathione peroxidase activity. In addition, $K_2Cr_2O_7$ injection increased the following nitrosative and oxidative stress markers in kidney: 3-nitrotyrosine (1.9-fold), 4-hydroxy-2-nonenal (2.1-fold), malondialdehyde (1.8-fold) and protein carbonyl content (1.7-fold). It was found that garlic powder feeding was able to prevent by 44–71% the alterations in the markers of renal injury studied, by 55% the histological damage, and by 47–100% the increase in markers of oxidative and nitrosative stress. It is concluded that the ability of garlic powder to ameliorate $K_2Cr_2O_7$-induced renal injury is associated with its antioxidant properties. Our data support the use of garlic powder as a renoprotective agent.

Keywords: Garlic; Potassium dichromate; 3-Nitrotyrosine; 4-Hydroxy-2-nonenal; Malondialdehyde; Protein carbonyl content; Nephrotoxicity; Antioxidant; Oxidative stress

1. Introduction

Potassium dichromate ($K_2Cr_2O_7$) is a chemical compound widely used in metallurgy, chrome plating, chemical industry, textile manufacture, wood preservation, photography and photoengraving, refractory and stainless steel industries and cooling systems (Barceloux, 1999). The oxidation state and solubility of chromium (Cr) compounds determine their toxicity. In contrast to Cr(III), which is a naturally occurring form and an essential trace element for humans and others mammals, Cr(VI) compounds are highly toxic (Wang et al., 2006). $K_2Cr_2O_7$ is a hexavalent form of Cr and has been demonstrated to induce oxidative stress and carcinogenic in nature (Stohs and Bagchi, 1995; Norseth, 1981; Von Burg and Liu, 1993; Bagchi et al., 2002). The kidney is the principal route of Cr excretion and it has been reported that acute exposure induces an increase in Cr kidney content on $K_2Cr_2O_7$-treated rats (Pedraza-Chaverri et al., 2005). Exposition to Cr(VI)

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produced anatomical lesions at the level of the proximal tubular cells (Franchini et al., 1978) and lipid peroxidation in human kidney (Huang et al., 1999). Interestingly, evidences suggest that reactive oxygen species (ROS) are involved in Cr(VI)-induced cell injury (Sengupta et al., 1992; Liu and Shi, 2001; Stohs and Bagchi, 1995; Bagchi et al., 2002; Travacio et al., 2001). Cr reduction intermediates [Cr(V) and Cr(IV)], may be toxic as they involve ROS production (Stohs et al., 2000; Shi and Dalal, 1990,1994; ÓBrien and Kortenkamp, 1994) which may be generated during physiological conditions. *In vitro*, chromate reduction via hydrogen peroxide (H_2O_2) has been shown to produce hydroxyl radical ($OH^{\cdot}$) via a Fenton-like reaction (ÓBrien and Kortenkamp, 1994; Aiyar et al., 1991; Shi and Dalal, 1990; Liu et al., 1997; Tsou et al., 1996). In *in vivo* experiments have been shown that $K_2Cr_2O_7$ exposition induces oxidative and nitrosative stress measured as protein carbonyl content and 3-nitrotyrosine (3-NT) immunostaining (Barrera et al., 2003a,b; Pedraza-Chaverri et al., 2005). The role of oxidative stress in the renal damage induced by $K_2Cr_2O_7$ has been supported by the fact that some antioxidants such as α-tocopherol, ascorbic acid, and glutathione (GSH) (Appenroth and Winnefeld, 1998; Arreola-Mendoza et al., 2006; Na et al., 1992; Sugiyama, 1992; Hojo and Satomi, 1991; Standeven and Wetterhahn, 1991) and the previous induction of heme oxygenase-1 (Barrera et al., 2003a,b) are able to ameliorate $K_2Cr_2O_7$-induced nephrotoxicity and oxidative damage.

To our knowledge, the potential protective effect of garlic powder on $K_2Cr_2O_7$-induced nephrotoxicity has not been explored. Garlic is a particularly rich source of organosulfur compounds which are responsible for its flavor and aroma, as well as for its potential health benefits (Lawson, 1996, 1998; Reuter et al., 1996). γ-Glutamyl-*S*-alkyl-L-cysteines and *S*-alkyl-L-cysteine sulfoxides are found mainly in raw garlic cloves (Lawson, 1996). The most abundant organosulfur compound in raw garlic cloves is alliin (*S*-allylcysteine sulfoxide), which is present at 10 mg/g fresh garlic (Lawson, 1998). When garlic cloves are cut or when the powder of dried cloves becomes wet in a non-acid solution, the cysteine sulfoxides, which are odorless, are very rapidly converted to a new class of compounds, the thiosulfinates which are responsible for the odor of freshly chopped garlic. This is because cysteine sulfoxides, which are located only in the clove mesophyll storage cells, come in contact with the enzyme allinase or alliin lyase, which is located only in the vascular bundle sheath cells. Due to the abundance of alliin, the main thiosulfinate formed upon crushing garlic cloves is allicin (Lawson, 1996).

The antioxidant ability of garlic in several presentations is well known (Banerjee et al., 2003a; Rahman and Lowe, 2006) and has been associated with its protective effect in several experimental models (Thabrew et al., 2000; Pedraza-Chaverri et al., 2000a; Gedik et al., 2005; Ip et al., 1992; Liu et al., 1992; Pal et al., 2006; Reuter et al, 1996; Sener et al., 2005).

In fact, a protective effect of a diet with garlic powder has been observed in cardiac ischemia and reperfusion (Rietz et al., 1993), adriamycin-induced toxicity (Thabrew et al., 2000), gentamicin-induced nephrotoxicity (Pedraza-Chaverri et al., 2000a), azoxymethane-induced damage (Khanum et al., 1998), and hypercholesterolemic (Heinle and Betz, 1994; Kempaiah and Srinivasan, 2004b; Gorinstein et al., 2006; Durak et al., 2002) and high fat (Kempaiah and Srinivasan, 2004a) diet-induced oxidative damage. In addition, the antioxidant properties of garlic extracts have been shown *in vitro*. Extracts of garlic powder are able to inhibit Cu^{2+}-induced low-density lipoprotein oxidation (Lewin and Popov, 1994; Pedraza-Chaverri et al., 2004) and to scavenge $OH^{\cdot}$ (Lewin and Popov, 1994; Pedraza-Chaverri et al., 2006; Torok et al., 1994), superoxide anion ($O_2^{\cdot-}$) (Pedraza-Chaverri et al., 2006), H_2O_2 (Pedraza-Chaverri et al., 2006), and peroxynitrite ($ONOO^-$) (Pedraza-Chaverri et al., 2007). Based on the above information we made the hypothesis that garlic powder may reduce $K_2Cr_2O_7$-induced renal injury. The aim of this study was to examine the effect of a 2% garlic powder supplemented diet on $K_2Cr_2O_7$-induced nephrotoxicity and oxidative and nitrosative stress.

2. Materials and methods

2.1. Reagents

Guanidine hydrochloride, *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, 2,4,-dinitrophenylhydrazine (DNPH), streptomycin sulfate, 1-methyl-2-phenylindole, tetramethoxypropane, 1-chloro-2,4-dinitrobenzene (CDNB), GSH, glutathione reductase (GR), and nicotine-adenine-dinucleotide phosphate (NADPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid, HCl, H_2O_2, acetonitrile, and methanol were purchased from Mallinckrodt Baker (Xalostoc, México). Commercial kits for the measurement of blood urea nitrogen (BUN) and creatinine levels (Sera-pak plus urea and Sera-pak plus creatinine) were from Bayer (Tarrytown, NY, USA). Mouse monoclonal *anti*-4-hydroxy-2-nonenal (4-HNE) antibodies (Cat. #24325) were from Oxis International, Inc. (Portland, OR, USA). Mouse monoclonal antibodies against 3-NT (Cat. #189542) were purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). The secondary antibodies biotin SP conjugated AffiniPure donkey anti-mouse IgG (Cat. #715-065-151) were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA, USA). Declere was from Cell Marque (Hot Springs, AR, USA). ABC-kit Vectastain was from Vector Laboratories (Orton Southgate, Peterborough, UK). Diaminobenzidine substrate (Cat. #K3466) and Mayer's Hematoxylin (Lillie's Modification) (Cat. #S3309) were from DAKO Corporation (Carpinteria, CA, USA). A commercial natural garlic powder (Code Number 91374, Expiration date May 9, 2008) manufactured by Tone Brothers Inc. (Ankeny, IA, USA) was used. The nutritional information of this particular garlic powder is the following: calories: 0, calories from fat: 0, total fat: 0 g, trans fat: 0 g, saturated fat: 0 g, cholesterol: 0 mg, sodium: 0 mg, total carbohydrate: 0 g, dietary sugars: 0 g, fiber: 0 g, and protein: 0 g.

2.1.1. H_2O_2 scavenging activity of garlic powder

In previous papers, we have shown that a garlic powder from McCormick has *in vitro* reactive oxygen and nitrogen species scavenging properties (Pedraza-Chaverri et al., 2004, 2006, 2007). Therefore, with the purpose to evaluate the antioxidant ability of the garlic powder used in the present study, we measured its *in vitro* H_2O_2 scavenging ability (expressed as IC50) as previously described (Pedraza-Chaverri et al., 2006). This result was compared with that obtained from garlic powder from