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S-allylcysteine scavenges singlet oxygen and hypochlorous acid and protects LLC-PK_1 cells of potassium dichromate-induced toxicity

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Abstract

It has been found that *S*-allylcysteine (SAC), a garlic-derived compound, has *in vivo* and *in vitro* antioxidant properties. In addition, it is known that SAC is able to scavenge different reactive oxygen or nitrogen species including superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), and peroxynitrite anion ($ONOO^-$) although the IC_{5O} values for each reactive species has not been calculated and the potential ability of SAC to scavenge singlet oxygen ($^1O_2$) and hypochlorous acid (HOCl) has not been explored. The purposes of this work was (a) to explore the potential ability of SAC to scavenge $^1O_2$ and HOCl, (b) to further characterize the $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$, and $ONOO^-$ scavenging ability of SAC by measuring the IC_{50} values using *in vitro* assays, and (c) to explore the potential ability of SAC to ameliorate the potassium dichromate ($K_2Cr_2O_7$)-induced cytotoxicity in LLC-PK1 cells in which oxidative stress is involved. The scavenging activity was compared against the following reference compounds: *N*-acetylcysteine for $O_2^{\cdot-}$, sodium pyruvate for H_2O_2, dimethylthiourea for $OH^{\cdot}$, lipoic acid and glutathione for $^1O_2$, lipoic acid for HOCl, and penicillamine for $ONOO^-$. It was found that SAC was able to scavenge concentration-dependently all the species assayed with the following IC_{5O} (mean ± SEM, mM): $O_2^{\cdot-}$ (14.49 ± 1.67), H_2O_2 (68 ± 1.92), $OH^{\cdot}$ (0.68 ± 0.06), $^1O_2$ (1.93 ± 0.27), HOCl (2.86 ± 0.15), and $ONOO^-$ (0.80 ± 0.05). When the ability of SAC to scavenge these species was compared to those of the reference compounds it was found that the efficacy of SAC (a) to scavenge $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$, and $ONOO^-$ was lower, (b) to scavenge HOCl was similar, and (c) to scavenge $^1O_2$ was higher. In addition, it was found that SAC was able to prevent $K_2Cr_2O_7$-induced toxicity in LLC-PK1 cells in culture. It was showed for the first time that SAC is able to scavenge $^1O_2$ and HOCl and to ameliorate the $K_2Cr_2O_7$-induced toxicity.
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Keywords: *S*-allylcysteine; Scavenging activity; Peroxynitrite; Singlet oxygen; Hypochlorous acid; Oxidative stress; $K_2Cr_2O_7$

1. Introduction

S-allylcysteine (SAC) (Nagae et al., 1994) is a water-soluble and non-toxic garlic compound and the most abundant organosulfur compound in aged garlic extracts (AGE) (Lawson, 1996). The concentration of SAC was the highest among the organosulfur compounds measured in a commercial preparation of aged garlic extract (0.62 mg/g) and after 720 days of aging of chopped garlic in 20% ethanol (7.2 mg/g) (Lawson, 1996). It has been clearly shown that SAC has antioxidant properties both *in vivo* (Numagami et al., 1996; Numagami and Ohnishi, 2001; Mostafa et al., 2000; Maldonado et al., 2003; Perez-Severiano et al., 2004a,b; Herrera-Mundo et al., 2006; Kim et al., 2006a,b) and *in vitro* (Kim et al., 2001; Yamasaki et al., 1994; Ho et al., 2001; Ide and Lau, 1997, 1999, 2001; Geng

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et al., 1997; Ide et al., 1997; Imai et al., 1994; Kim et al., 2006a,b). *In vivo*, SAC ameliorates (a) edema formation in the ischemic rat brain, by a mechanism that seems to involve the inhibition of lipid peroxidation (Numagami et al., 1996; Numagami and Ohnishi, 2001), (b) hippocampal damage induced by ischemia and reperfusion in gerbils (Kim et al., 2006a), (c) the histological damage in heart and liver of mice treated with doxorubicin, an anticancer drug (Mostafa et al., 2000), (d) gentamicin-induced nephrotoxicity and oxidative stress (Maldonado et al., 2003), (e) oxidative damage and learning deficits induced by amyloid-beta peptide (Perez-Severiano et al., 2004b), (f) quinolinic acid-induced neurotoxicity (Perez-Severiano et al., 2004a), (g) 3-nitropropionic acid-induced neurotoxicity and oxidative stress (Herrera-Mundo et al., 2006), (h) acetaminophen-induced hepatotoxicity in mice (Mizuguchi et al., 2006), (i) carbon tetrachloride-induced oxidative stress and pulmonary fibrosis in rats (Hsu et al., 2006), and (j) learning deficits in senescence-accelerated mice (Nishiyama et al., 2001). Furthermore, Hsu et al. (2004) have found that SAC has antioxidant activity in Balb/cA mice which was measured by several parameters.

In vitro, SAC is able to scavenge superoxide anion ($O_2^{\cdot-}$) (Kim et al., 2001; Maldonado et al., 2003), H_2O_2 (Ide and Lau, 2001; Maldonado et al., 2003), $OH^{\cdot}$ (Kim et al., 2001; Chung, 2006), and $ONOO^-$ (Kim et al., 2006b). SAC is also able to ameliorate (a) $ONOO^-$ induced hemolysis (Morihara et al., 2005), (b) nitropropionic acid-induced lipid peroxidation and mitochondrial dysfunction in rat brain synaptosomes (Perez-de la Cruz et al., 2006), (c) H_2O_2-induced endothelial cell injury and lipid peroxidation (Yamasaki et al., 1994; Ide and Lau, 2001), (d) low-density lipoprotein oxidation (Higuchi et al., 2003; Ide and Lau, 1997, 2001; Ho et al., 2001; Ide et al., 1997; Huang et al., 2004; Nishimura et al., 2004, 2006), and (e) the damage induced by oxygen and glucose deprivation in human neuroblastoma cell line (SK-N-SH) (Kim et al., 2006a). In addition, it has been shown that SAC have protective effect on (a) amyloid-beta peptide-induced apoptosis *in vitro* (Peng et al., 2002), (b) neurotoxicity induced by amyloid beta-peptide and ibotenic acid in organotypic hippocampal cultures (Ito et al., 2003a), (c) amyloid beta-protein-induced cell death in nerve growth factor-differentiated PC12 cells (Ito et al., 2003b), and (d) cultured rat hippocampal neurons from amyloid beta-protein- and tunicamycin-induced neuronal death (Kosuge et al., 2003). Furthermore, Geng et al. (1997) have shown that SAC may block H_2O_2-induced nuclear factor kappa B activation.

On the other hand, it is known that potassium dichromate ($K_2Cr_2O_7$)-induced nephrotoxicity (Pedraza-Chaverri et al., 1995) is associated with oxidative stress (Barrera et al., 2003a,b; Pedraza-Chaverrí et al., 2005) which has been supported by the fact that some antioxidants (Hojo and Satomi, 1991; Standeven and Wetterhahn, 1991; Na et al., 1992; Sugiyama, 1992; Appenroth and Winnefeld, 1998) and the previous induction of heme oxygenase-1 are able to ameliorate $K_2Cr_2O_7$-induced nephrotoxicity. To our knowledge, it has not been explored the potential protective effect of SAC on $K_2Cr_2O_7$-induced nephrotoxicity.

Interestingly, the potential ability of SAC to scavenge singlet oxygen ($^1O_2$) and hypochlorous acid (HOCl) has not been studied. In addition the IC_{50} values of SAC for each one of the reactive oxygen species (ROS) have not been calculated. Based on the above mentioned information, the purposes of this work was (a) to study if SAC has the ability to scavenge $^1O_2$ and HOCl, (b) to further characterize the scavenging properties of SAC against $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$, and $ONOO^-$, and (c) to know if SAC is able to prevent $K_2Cr_2O_7$-induced toxicity in LLC-PK1 cells. The ROS scavenging properties were compared against those of reference compounds.

2. Materials and methods

2.1. Reagents

N-acetylcysteine (NAC), L-cysteine, glutathione (GSH), sodium pyruvate, 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), sodium borohydride, dimethyl thiourea (DMTU), DL-penicillamine, dimethyl sulfoxide (DMSO), lipoic acid, histidine, ascorbic acid, xylenol orange, butylated hydroxytoluene, *N*,*N*-dimethyl-4-nitrosoaniline, xanthine, xanthine oxidase, nitroblue tetrazolium (NBT), potassium nitrite (KNO_2), manganese dioxide (MnO_2), diethylene-triamine-pentaacetic acid (DTPA), sodium carbonate (Na_2CO_3), allyl bromide, ammonium iron (II) sulfate hexahydrate ($(NH_4)_2Fe(SO_4)_2 6H_2O$), bovine serum albumin (BSA), Tris–HCl, mercaptoethanol, bromophenol blue, Coomassie brilliant blue, were from Sigma-Aldrich (St. Louis, MO, USA). Dihydrorhodamine 123 (DHR 123) was purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). H_2O_2, trichloroacetic acid, ethylenediaminetetraacetic acid (EDTA), and sodium hypochlorite (NaOCl) were from JT Baker (Xalostoc, Edo. México, México). HPLC-grade methanol, and all other chemicals were reagent grade and commercially available.

2.2. Synthesis of SAC

SAC was synthesized by the reaction of L-cysteine with allyl bromide and purified by recrystallization from ethanol-water as previously described (Lawson et al., 1991; Maldonado et al., 2003).

2.3. Superoxide anion scavenging assay

Xanthine–xanthine oxidase system was used to determine the $O_2^{\cdot-}$ scavenging capacity of SAC. $O_2^{\cdot-}$ in the assay system and xanthine oxidase activity were measured as NBT reduction and uric acid production, respectively (Pedraza-Chaverrí et al., 2006; Floriano-Sánchez et al., 2006), using a DU-64O series Beckman spectrophotometer. This system is useful to test for $O_2^{\cdot-}$ scavenging capacity only when the extracts or compounds used do not interfere with the xanthine oxidase activity. A compound with $O_2^{\cdot-}$ scavenging capacity should decrease NBT reduction without interfering with xanthine oxidase activity measured as uric acid production. Eight hundred μL of the following reaction mixture: 0.116 mM xanthine, 20 mM Na_2CO_3, 29 μM NBT, and 18 mM phosphate buffer (pH 7.0) were mixed with 100 μL of 50 mM phosphate buffer (pH 7.0) (0% scavenging tube) or with 100 μL of different concentrations of SAC (from 0 to 50 mM) or NAC (0–15 mM). The reaction was started by the addition of 100 μL of xanthine oxidase (168 U/L). Optical density was registered both at 295 nm (for uric acid production) and 560 nm (for $O_2^{\cdot-}$ in the assay system). Scavenging percent was obtained from the optical densities at 560 nm.

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