Arch Pharm Res Vol 34, No 5, 801-810, 2011
DOI 10.1007/s12272-011-0514-4

Archives of Pharmacal Research
www.springer.com/12272

Comparative Evaluation of the Antioxidant Effects of the Natural Vitamin C Analog 2-O-β-*D*-glucopyranosyl-L-ascorbic Acid Isolated from *Goji* Berry Fruit

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(Received June 12, 2010/Revised September 6, 2010/Accepted September 30, 2010)

2-O-β-*D*-Glucopyranosyl-*L*-ascorbic acid (AA-2βG) is a natural derivative of vitamin C (L-ascorbic acid, AA) isolated from *Goji* berry (*Lycium barbarum* L.) fruit. We evaluated the antioxidant activities of AA-2βG and AA using *in vitro* and *in vivo* model systems. *In vitro* radical scavenging assays demonstrated that AA-2βG was capable of scavenging 1,1-diphenyl-2-picryl-hydrazyl and hydroxyl peroxide and inhibiting H_2O_2-induced hemolysis better than AA. AA-2βG and AA had similar hydroxyl radical scavenging capabilities, but AA-2βG was incapable of scavenging superoxide anion radicals, and its capacity to scavenge nitrite (NO_2^-) was lower than that of AA. The overall *in vitro* reduction capability of AA-2βG was also significantly lower than that of AA. Moreover, *in vivo* studies demonstrated that AA-2βG was capable of protecting the liver against carbon tetrachloride-induced acute liver injury in mice. These results suggest that AA-2βG is an important antioxidant component of *Goji* berry fruit, which may share similar but distinct antioxidant mechanistic properties with AA. This study furthers our understanding of the mechanisms of *Goji* berry fruit pharmacological activities on antiaging and antitumor properties as a traditional medicine and dietary supplement.

Key words: AA-2βG, Vitamin C, Analog, Antioxidant, *Lycium barbarum* L.

INTRODUCTION

Our bodies require the uptake of antioxidants to counterbalance the damaging effects of free radicals from the environment and cellular metabolic processes. A main source of antioxidants in the body comes from the diet and/or dietary supplements. Dietary antioxidants act as either antioxidants or pro-oxidants in cellular redox reactions (Oyagbemi et al., 2009). Phytochemicals and vitamins C and E are the main natural antioxidants found in fruits and vegetables, which affect cellular signal transduction and regulate the balance between cellular proliferation and apoptosis (Balsano and Alisi, 2009). Vitamin C (L-ascorbic acid, AA), a well-known essential nutrient in the body and is one of the most important and powerful natural antioxidants. Numerous studies have demonstrated the ability of AA to reduce oxidative stress and regulate cell cycle progression during oxidative stress. A high intake of AA and/or AA-rich foods has a statistically significant protective effect on preventing many different types of cancers (Sasazuki et al., 2008).

Recent *in vitro* and *in vivo* studies using high dose AA, administered by oral and intravenous routes, demonstrated a selective cytotoxicity of AA against tumor cells, suggesting that high concentrations of AA in the body are important for antitumor activity (Chen et al., 2008; Ohno et al., 2009). However, AA is highly water-soluble, and although it is easily absorbed, it is poorly stored in the body. This characteristic of AA makes it difficult to sustain high concentrations of AA within the body for therapeutic interventions. Therefore, a more stable AA analog or derivative would be an ideal alternative to replace traditional AA. 2-O-β-D-Glucopyranosyl-L-ascorbic acid (AA-2βG) is a novel

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stable AA analogue with pro-AA activity that has recently been discovered from the fruit of *L. barbarum* L. (Toyoda-Ono et al., 2004; Toyada-Ono et al., 2005). The dry fruit of *L. barbarum* (also called the *Goji* berry fruit in traditional medicine) is an important component of traditional medicines and medical diets and has exhibited multiple pharmacological functions including antioxidant, antiaging, immune promoting, and antitumorigenic activity (Yu et al., 2007; Amagase et al., 2009; Potterat, 2010; Reeve et al., 2010). The content of AA-2βG in the *Goji* berry fruit is up to 0.5% of the dry fruit, making *Goji* berry fruit a natural plant source for AA-2βG, which could serve as a stable AA substitute.

Several components from *Goji* berry fruit including AA-2βG, polysaccharose, and glycine betaine have been identified as an important class of bioactive natural products, possessing many important pharmacological properties. As a natural AA analogue, AA-2βG has high antioxidant activity and may play pharmacological roles in antioxidant, antiaging, and antitumorigenic activities (Takebayashi et al., 2006, 2007, 2008, 2010; Zhang et al., 2011). However, the AA-2βG antioxidative mechanism remains poorly understood due to a lack of *in vivo* investigations. In this report, we elucidated the AA-2βG antioxidant mechanism by comparing the capacity of AA-2βG to scavenge varied oxidants *in vitro* and evaluate its protective effects against carbon tetrachloride (CCl_4)-induced liver injury in an *in vivo* mouse model. The results will increase our understanding of the AA-2βG antioxidative mechanism and underline its importance as a dietary supplement in *Goji* berry fruit.

MATERIALS AND METHODS

Chemicals, detection kits, and animals

All chemicals were purchased from Sigma, unless otherwise indicated. The hydroxyl radical (OH), superoxide anion radical (O_2^-), alanine aminotransferase (ALT), aspartic acid aminotransferase (AST), superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) detection kits were obtained from Nanjing Jiancheng Bio-engineering Institute. Twelve-week-old male Kunming mice were purchased from the Ningxia Medical University animal facility. All animal experiments were performed using the established NIH and Ningxia University guidelines for the care and use of laboratory animals.

Isolation of AA-2βG from *Goji* berry fruit

The isolation and purification of AA-2βG was conducted as previously described (Toyoda-Ono et al., 2004; Zhang et al., 2011).

Evaluation of total reduction capability

Total antioxidant activity was determined by measuring the reduction capacity of antioxidants reducing Fe^{3+} to Fe^{2+}; Fe^{2+} forms a stable complex with phenanthroline, which can be determined by colorimetry (Gülcin et al., 2005). One unit of reduction capacity was defined as an increase in absorbance by 0.01 at $A580_{nm}$ in a 1.0 mL reaction mixture at 37°C for 1 min. Reactions without antioxidants (AA or AA-2βG) or standards served as controls. All reduction capabilities are reported as the mean of triplicate analyses.

Evaluation of radical scavenging activity (RSA)

The RSA of AA and AA-2βG against 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined by evaluating the RSA stoichiometric factor (RSA(n)). This factor was defined as the number of free radicals scavenged by each molecule of antioxidant in 120 min. The number of DPPH radicals scavenged by the antioxidants was calculated using a previously reported equation (Takebayashi et al., 2008): $RSA(n) = (\Delta A_{120}/A_0) \times (DPPH)/(AA \text{ or } AA\text{-}2\beta G)$, where ΔA_{120} represents the difference in absorbance between the reaction and control solutions at 120 min; A_0 represents the initial absorbance of the control; DPPH, the DPPH radical at a 100 μM concentration; AA or AA-2βG, the AA or AA-2βG antioxidant concentration (20 μM).

DPPH scavenging assay

The DPPH radical-scavenging assay was conducted as previously described with modifications (Fujinami et al., 2001). Briefly, AA-2βG and AA were dissolved in 60% ethanol/40% citrate buffers at various pH values (10 mM, pH 3.0, pH 4.0, pH 5.0, or pH 6.0). DPPH in ethanol was mixed with the above antioxidant solutions containing either AA-2βG (10-80 μM) or AA (10-80 μM) at a final concentration of 100 μM. The mixture was incubated at 25°C for 20 min. DPPH scavenging activity was measured by the changes in absorbance at 520 nm on a spectrophotometer (Mettler Toledo Inc.). Scavenging activity is presented as a percentage of scavenging efficiency. The percent DPPH scavenging efficiency was calculated using the following equation: $\text{DPPH scavenging efficiency } (\%) = (A_0 - A_S)/A_0 \times 100$, where A_0 and A_S represent the control or sample (AA or AA-2βG) absorbance, respectively. The 50% effective concentration (EC_{50}) value was determined as the concentration of each sample required to give 50% of the blank control absorbance.