Antioxidant Activity of a Novel Extract from Bamboo Grass (AHSS) against Ischemia-Reperfusion Injury in Rat Small Intestine

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Production of free radical species in cells and body tissues is known to cause many pathological disorders. Therefore, free radical scavengers play an important role in the prevention of various human diseases. Bamboo grass, *Sasa senanensis*, is a native Japanese plant. *Sasa* has been used for medicine in Japan for many centuries. In this study, we investigated the antioxidative activity of Absolutely Hemicellulose Senanensis (AHSS), a novel extract from *Sasa*. In the first part of this study, we found that AHSS has antioxidative activities by the assay using superoxide anion-2-methyl-6-methoxyphenylethynylimidazopyrazynone (MPEC) reaction kit. We then confirmed its antioxidative activity using a rat ischemia and subsequent reperfusion (I/R) injury model. Breakdown of the intestinal wall caused by intestinal I/R was attenuated by pretreatment with AHSS. Moreover, AHSS inhibited the production of lipid peroxide by intestinal I/R. AHSS could be an important source of ingredients for use in functional foods and other applications.

Key words *Sasa*; anion-2-methyl-6-methoxyphenylethynylimidazopyrazynone; lipid peroxide; radical scavenger; functional food

Production of free radical species in cells and body tissues is known to cause many pathological disorders. Reactive oxygen species can damage biological molecules such as proteins, lipids and DNA. Reactive oxygen species are generated as byproducts of normal cell aerobic respiration, which is essential to life. Exposure to free radicals from external sources such as cigarette smoke, pollutants, chemicals and environmental toxins may also occur. Therefore, free radical scavengers play an important role in the prevention of various human diseases. Diets rich in fruits and vegetables have been considered to be excellent sources of antioxidants. Bamboo grass, *Sasa senanensis*, is a native Japanese plant that grows only in the Japanese Archipelago and in the northern parts of Saghalien and the Korean Peninsula. *Sasa* leaves have been known for many centuries in Japan to have strong antibiotic activity. *Sasa* leaves have been used for medicine and for wrapping sushi and chimaki (rice cake) to prevent putrefaction. The biochemical substance “Absolutely Hemicellulose Sound Senanesis sasa” (AHSS) has been extracted as described in a previous report with some modification.

The intestinal mucosa is extremely sensitive to reactive oxygen species. In this study, we used a rat mesenteric ischemia and subsequent reperfusion (I/R) injury model as a model of oxidative injury. The aim of this study was to determine the antioxidative activity of a novel extract, AHSS, from *Sasa* grass. We tested whether AHSS could attenuate I/R-associated intestinal injury.

MATERIALS AND METHODS

**Chemicals** AHSS was kindly supplied by Biomedical Laboratory Co., Ltd. (Horokanai, Japan). Allopurinol was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents were of the highest grade available and used without further purification.

**Measurement of Antioxidant Activity** The assay was carried out by using superoxide anion-2-methyl-6-methoxyphenylethynylimidazopyrazynone (MPEC) reaction kit (ATTO Corp. Osaka, Japan) according to the manufacturer’s instructions. Light emission induced by xanthine oxidase (XO) is inhibited by XO inhibitor or radical scavenger. Light emission was measured with multilabel counter Wallac 1420 ARVOse (Perkin Elmer, Wellesley, MA, U.S.A.).

**Animals** Male Wistar rats, aged 7 to 9 weeks (300—350 g in weight), were obtained from NRC Haruna (Gunma, Japan). The housing conditions were described previously. The rats were housed at least 1 week at 23±3°C and 50±10% relative humidity and were maintained on a 12 h light/dark cycle. During the acclimatization the rats were allowed free access to food and water. The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the “Guide for the Care and Use of Laboratory Animals”.

**Intestinal I/R Model** Surgical procedures were carried out as described in a previous report with some modifications. Wistar rats were anesthetized with sodium pentobarbital (40 mg/kg weight, i.p.). Through a midline laparotomy, the superior mesenteric artery was isolated and a bulldog arterial clamp was applied at the aortic origin. The abdomen was then covered with a sterile plastic wrap. After 60 min of intestinal ischemia, the arterial clamp was removed. Allopurinol was administrated intraperitoneally (50 mg/kg weight) for 1 h before the induction of ischemia. AHSS was administered orally (125 mg/kg weight) for 4 h before the induction of ischemia.

**Tissue Sampling** The 5-cm-long intestine was excised, the contents were removed, and the intestine was cleansed in ice-cold saline. The intestine was then homogenized in 2.5 ml saline using a glass Teflon homogenizer with 20 strokes. Protein was measured by the method of Lowry et al. with bovine serum albumin as a standard.

**TBA Analysis** The amount of lipid peroxide in the intestine was determined as that of malondialdehyde (MDA) by
the method of Ohkawa et al. with some modification.\textsuperscript{14}) Thiobarbituric acid (TBA) solution was composed of 2.6 mM TBA, 918 mM trichloroacetic acid, 0.3 mM HCl, and 1.8 mM 2,6-di-tert-butyl-4-methylphenol (BHT) in 22% ethanol. The reaction mixture contained 0.2 ml of tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid solution (pH 3.5), and 1.5 ml of 0.8% aqueous solution of TBA. The mixture was heated at 95 °C for 60 min. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of \textit{n}-butanol were added, and the mixture was shaken vigorously. After centrifugation at 3000 × g for 10 min, the absorbance of the organic layer (upper layer) was measured at 535 nm with 1,1,3,3-tetraethoxypropane as a standard.

**Histological Study** Samples of the intestine were taken after reperfusion and immediately fixed in 10% buffered formalin. The fixed tissue was embedded in paraffin and thin-sectioned (5.0 μm). Slides were stained using hematoxylin and eosin to evaluate the intestinal morphology under a light microscope for classification.

**Data Analysis** Statistical significance was evaluated using unpaired Student’s \textit{t}-test. A value of \(p<0.05\) was considered significant.

**RESULTS AND DISCUSSION**

The hypothesized role of oxidation in development of disease has promoted interest in the role of antioxidants in treatment and prevention. Intake of antioxidants from nutrients as well as nonnutrients is related to health outcomes.\textsuperscript{15} It has been reported that there is an inverse correlation between antioxidant intake and risk of gastric cancer.\textsuperscript{16} Vegetables, fruits and oil seeds have been considered to be the major sources of antioxidants in our daily diet.\textsuperscript{17—19}

In Japan, Bamboo grass has been known for many centuries to have strong antibiotic activity and has been used for medicine. In this study, we compared the antioxidative activity of AHSS, a novel extract from 	extit{Sasa} grass obtained by a multistep-pressure extraction method, with that of allopurinol, an inhibitor of XO.

In the first part of this study, we investigated the antioxidant activities of allopurinol and AHSS using MPEC. Allopurinol inhibited the light emission induced by XO with concentration-dependent manner (Fig. 1A). AHSS, as well as allopurinol, inhibited the light emission induced by XO with concentration-dependent manner (Fig. 1B). These results suggest that AHSS is a radical scavenger or an inhibitor of XO.

We then confirmed the antioxidant activities of allopurinol and AHSS using rat I/R injury model. It has been reported that overproduction of lipid peroxide, one of the reactive oxygen radicals, contributes to small intestinal injury.\textsuperscript{9,10} XO is one of the sources of free radical species in the ischemic small intestine.\textsuperscript{20} Allopurinol prevented the decrease in protein caused by intestinal I/R (Fig. 2). The scavenging capacity of allopurinol was evaluated by measuring the amount of lipid peroxide. The increase in the amount of lipid peroxide after I/R was significantly inhibited by treatment with allopurinol (Fig. 3). We then investigated the effect of AHSS on the protein content. Decrease of protein caused by intestinal I/R was attenuated by treatment with AHSS, as well as by treatment with allopurinol (Fig. 2). Moreover, AHSS inhibited the production of lipid peroxide induced by intestinal I/R (Fig. 3).

Shortening of the villi is a typical histological finding after I/R injury. The loss of villi was attenuated by pretreatment

![Fig. 1. Dose-Response Relationship for the Inhibition of XO-Induced Light Emission by Allopurinol (A) and AHSS (B)](image)

Each value represents the mean with S.D. of 3 preparations. \(\ast p<0.05\), significantly different from that in the absence of allopurinol or AHSS (control).

![Fig. 2. Effects of Allopurinol and AHSS on Protein Content in the Small Intestine after I/R](image)

Each column represents the mean with S.E. of 4 to 8 preparations. \(\ast p<0.05\), significantly different from nonischemia control animals. \(\dagger p<0.05\), significantly different from animals not treated with allopurinol or AHSS (I/R animals).

![Fig. 3. Effects of Allopurinol and AHSS on the Amount of Lipid Peroxide in the Jejunum after I/R](image)

Each column represents the mean with S.E. of 4 to 8 preparations. \(\ast p<0.05\), significantly different from nonischemia control animals. \(\dagger p<0.05\), significantly different from animals not treated with allopurinol or AHSS (I/R animals).
with AHSS (Fig. 4). Taking all of the results presented in this paper into consideration, AHSS could be an important source of ingredients for use in functional foods and other applications. However, the components of AHSS that are important antioxidants for protection of nutritional compounds against oxidation have not been elucidated yet. Further studies are needed to determine the bioactive compounds.

In summary, we have demonstrated that AHSS can prevent I/R-associated intestinal injury. In addition to I/R-associated intestinal injury, oxidative stress is an important factor in cancer, heart disease, and neuronal degeneration such as Alzheimer’s and Parkinson’s diseases. It is possible that chronic supplementation with AHSS will prevent these diseases. Further study is needed to determine whether long-term administration of AHSS can prevent these diseases.

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**REFERENCES**