Note

Stability and Bioavailability of Antioxidants in Garland (Chrysanthemum coronarium L.)

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The stability and bioavailability of the major antioxidants in garland (Chrysanthemum coronarium L.), chlorogenic acid, 3,5-dicaffeoylquinic acid and 4-succinyl-3,5-dicaffeoylquinic acid, were investigated together with caffeic acid. These compounds were stable in artificial digestive juice, but more than 90% of them disappeared from plasma within 30 min after intravenous injection into rats. When they were orally administered, only caffeic acid could be detected.

Key words: garland (Chrysanthemum coronarium L.); antioxidant; chlorogenic acid; artificial digestive juice; administration test

The metabolism and bioavailability of such functional ingredients in foods as antioxidants and antimitagens have recently attracted attention in addition to their activity in vitro. Food ingredients usually have to undergo the processes of cooking, ingestion, absorption and metabolism before being transported to each organ and tissue in the body. They are exposed to seasonings and/or heat during cooking, and then to acidic gastric juice, alkaline intestinal juice, etc. in the digestive organs. They consequently have to be stable under these conditions for their effective uptake into the body. Most of the minor functional ingredients of plant origin can be "strangers" to the human body and may not be absorbed in an intact form due to conjugation and enzymatic degradation when they pass through the highly selective digestive and metabolic system. It is, therefore, essential for an evaluation of their biological functions to know whether the compounds actually reach the tissues where they could desirably exert their activities after clearing the obstacles.

Chlorogenic acid, a well-known natural antioxidant with other nutritional effects,† and its derivatives are widely distributed among vegetables and fruits. Garland (Chrysanthemum coronarium L.) contains chlorogenic acid and its related compounds, 3,5-dicaffeoylquinic acid (SP-1) and 4-succinyl-3,5-dicaffeoylquinic acid (SP-2), as its major antioxidants† (Fig. 1). About 50% of the total amount of these antioxidants remains after the usual cooking procedure for garland.† However, there are few data on bioavailability of phenylpropanoids, including chlorogenic acid and its derivatives, although many studies have been reported on the bioavailability of flavonoids.† We investigate here the fate of the three antioxidants and caffeic acid (Fig. 1), which is one of their moietyes, after ingestion.

A stability test against artificial digestive juice was carried out according to the method of Hazell and Johnson.† The artificial gastric juice used was 0.2% NaCl and 9952 U/ml of pepsin in 0.063 M HCl, and the artificial intestinal juice was 0.4% pancreatin and 2.5% bile extract in 0.1 M NaHCO3. Ten milligrams of each caffeic acid, chlorogenic acid, SP-1 and SP-2 were respectively dissolved in 0.1 ml of methanol, and the solution was added to 3 ml of the artificial gastric juice. Each sample was incubated for 2 hours at 37°C on a shaker. After the pH had been adjusted to 7 with 0.5 M NaOH, the sample was mixed with 5 ml of artificial intestinal juice and incubated for a further 2 hours at 37°C on a shaker. After filtration through a Cosmonice cartridge of filter 0.45 μm pore size (Nacalai Tesque, Kyoto, Japan), the antioxidants in the solution were analyzed by HPLC in an ODS column (YM-C-Pack Pro C18, 75 × 2.0 mm I.D.; YMC, Kyoto, Japan) with a mobile phase consisting of acetonitrile-water-acetic acid (5:93:2 v/v/v) for caffeic acid and chlorogenic acid, and 15:83:2 v/v/v for SP-1 and SP-2) at 40°C and 0.3 ml/min. Each antioxidant was detected at 326 nm, and the concentration was determined from a calibration curve obtained from a standard solution of the compound. The results revealed that all of these compounds were quite stable in the artificial digestive juice, with nearly 100% of them being detected after the treatment (Fig. 2).

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Administration tests on rats were entrusted to Japan SLC Inc. (Shizuoka, Japan). The experimental design was approved by the Animal Experiment Committee of this company, and the laboratory animals were cared for according to the ethical guidelines for animal feeding. These tests were conducted on 3 males SD rats of 7 weeks old which weighed about 200 g and had been fasted overnight. The concentration of each antioxidant in its test solution was 10 mg/ml, and 0.2 ml was administered by intravenous injection and 1.0 ml orally. Blood samples (0.5 ml) were periodically collected up to 30 min and 480 min after the intravenous and oral administration, respectively. The plasma was separated by centrifugation and stored at \(-30^\circ\text{C}\) until needed for analysis.

Each plasma sample (0.2 ml) was diluted with 1.8 ml of 5% acetic acid and poured into a solid-phase cartridge column (Sep-pak Plas C18; Waters, Massachusetts, U.S.A.) which had been treated with methanol, water and 5% acetic acid. After the cartridge had been washed with 1.8 ml of 5% acetic acid, the antioxidants were eluted with 1.8 ml of methanol. The eluate was evaporated to dryness, and the resulting residue was dissolved in 0.1 ml of 5% acetic acid and then passed through a cartridge filter for the HPLC analysis as already described.

The intravenous administration test indicated that...
more than 90% of the administered compounds had disappeared from the plasma within 30 min (Fig. 3). The concentration of the larger molecules tended to be lower than that of the smaller ones at each collection time (caffeic acid > chlorogenic acid > SP-1 > SP-2). This may be attributable to a difference in solubility or rate of metabolism of these compounds in the blood or digestive organs.

The oral administration test only detected caffeic acid in the blood, which has been reported to be absorbed by oral administration in rats and rabbits (Fig. 4). Chlorogenic acid, SP-1, and SP-2 were, however, below the level of detectability after the administration. Czok et al. have reported that chlorogenic acid could not be detected in the blood after oral administration to rats. It can be assumed that these compounds are hard to absorb and/or rapidly conjugated or degraded, even if they have passed through the digestive organs.

Although the major antioxidants of garland were quite stable in the simulated digestive juice, they did not appear in the rat plasma after they had been orally administered. It is, therefore, necessary to examine their metabolism for a practical evaluation of their activity after ingestion.