ABSORPTION OF (-)-EPIGALLOTECTECHIN GALLATE INTO RAT PORTAL VEIN

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Following oral administration of (-)-epigallocatechin gallate to rats, the presence of (-)-epigallocatechin gallate was examined in the portal blood. A compound present in the blood was identified as (-)-epigallocatechin gallate by HPLC and mass spectrometry analysis. The results clearly demonstrate that (-)-epigallocatechin gallate is absorbed, at least in part, into rat portal blood.

**KEYWORDS** (-)-epigallocatechin gallate; absorption; portal blood; rat

![Fig.1. Structural Formula of (-)-EGCg](image)

(-)-Epigallocatechin gallate ((-)EGCg), which is a major polyphenol component in the tea leaf, has been proved to have a variety of physiological functions such as anti-tumor, hypotensive and anti-oxidative activity. However, information pertaining to (-)-EGCg metabolism is limited; hence it is important first of all to determine whether (-)-EGCg is actually absorbed into the body. In this paper, we describe the absorption of (-)-EGCg into rat portal blood after oral administration to rats.

**MATERIALS AND METHODS**

(-)-EGCg was extracted and isolated by our method. Two male Wistar strain rats (6 weeks of age) were maintained on a polyphenol-free-diet for a week. 100 mg of (-)-EGCg dissolved in 2 ml of water were administered orally to the rats after they were starved overnight. The rats were anesthetized with pentobarbital sodium 45 minutes after dosing, and blood was collected from the portal vein and heparinized. The plasma was separated by centrifuging the blood at 3000 rpm for 10 min, and then extracted three times with 10 ml of ethyl acetate. The extract was evaporated to dryness. One half of the residue was incubated with one unit of tannase (Sankyo Co., Ltd.) at 37 °C for 3 hours in one ml of 0.1M phosphate buffer (pH 6.8).

Analytical HPLC was carried out in a Waters liquid chromatograph apparatus equipped with a Millennium 2010 data system (Nihon Waters Ltd.). The conditions were as follows: column; Capcell-pak C-18 AG 120 4.6 mm I.D. x 250 mm L. (Shiseido Co., Ltd.), column temp.; 40 °C, solvent; acetonitrile-ethyl acetate-0.05%phosphoric acid aqueous solution 12:2:86 (by volume), flow rate; 1.0 ml/min, detection; UV 280 nm and wavelength in the range of 230 - 450 nm. Preparative HPLC was performed in a JASCO liquid chromatograph apparatus with a JASCO 870 UV detector (JAPAN SPECTROSCOPIC CO., LTD). The preparation conditions were as follows: column; Capcell-pak C-18 AG 120 20 mm I.D. x 250 mm L. (Shiseido Co., Ltd.), column temp.; 40 °C, solvent; acetonitrile-ethyl acetate-0.05% phosphoric acid aqueous solution 12:2:86 (by volume), flow rate; 10 ml/min, detection; UV 280 nm. The isolated compound was dissolved in 20 μl of acetone, and glycerol was added to the solution as matrix. Positive and negative ion FAB-MS analysis was carried out using a JEOL JMS DX-300 mass spectrometer (JEOL LTD.).

**RESULTS AND DISCUSSION**

The ethyl acetate fraction prepared from the portal plasma was first examined by analytical HPLC. A peak of whose
the retention time agreed with that of the standard (-)-EGCg was detected (Figure 2-A). Using the photodiode array detector, the UV spectrum of the compound was also found to be consistent with that of (-)-EGCg. Further, when the fraction was treated with tannase, which is capable of hydrolyzing galloyl ester, and analyzed by HPLC, the peak disappeared and two new peaks emerged (Figure 2-B). The two compounds were estimated to be gallic acid and (-)-epigallocatechin, respectively, judging from the comparison of the retention time and the UV spectra of the compounds with those of standard gallic acid and (-)-epigallocatechin. These results suggest that the compound in the ethyl acetate fraction was intact (-)-EGCg.

In order to verify that the compound was (-)-EGCg, it was isolated from the ethyl acetate fraction by preparative HPLC and analyzed by FAB-MS. The isolated compound showed quasimolecular ion peaks at m/z 481[M+Na]+ (positive) and at 457[M-H]- (negative). Consequently, the compound was identified as (-)-EGCg.

In these experiments, it was demonstrated that orally administrated (-)-EGCg is absorbed into portal blood via intestinal tract in the rat. Studies on the absorption of other tea catechins are now in progress.

REFERENCES

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