Pharmacokinetic Study of Catechin (Epigallocatechin Gallate) after Intraperitoneal and Oral Administration to Yellowtail Seriola quinqueradiata

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Abstract: This study was designed to determine the pharmacokinetics of (-)-Epigallocatechin gallate (EGCG) in yellowtail marine fish following oral gavage or intraperitoneal (IP) administration. After IP administration at a dose of 10 mg/kg body-weight, EGCG was detected in all plasma samples collected at 2 h post-dosing (2.22 ± 1.46 μg/ml). It was markedly lower by 4 h and 8 h post-dosing, and not detected at later time points (< 0.066 μg/ml). After oral gavage, EGCG was only detected in plasma at 2 h post-treatment, and it was not detected in any muscle or liver samples. A one-compartment analysis revealed a high elimination rate constant (0.24) and a short half-life (2.89 h). These results suggest that EGCG may be poorly absorbed and/or quickly eliminated in yellowtail.

Key words: Yellowtail Seriola quinqueradiata; (-)-Epigallocatechin gallate (EGCG); Pharmacokinetics

(-)-Epigallocatechin gallate (EGCG) is the most abundant catechin in green tea among epigallocatechin (EGC), epicatechin (EC), and epicatechin gallate (EGC) (Graham 1992). It has been shown to be one of the most powerful green tea polyphenol antioxidants in vitro and in vivo. Moreover, green tea polyphenols have suppressive effects on oxidation of lipid, deterioration of flesh color, and microbial growth, and they also slow resolution after rigor mortis in yellowtail fish during round ice storage (Ishihara et al. 2000, 2001, 2002). The potentials of EGCG as an antioxidant and as an immunostimulant were supported by findings in rainbow trout Oncorhynchus mykiss (Thawonsuwan et al. 2010). In addition, it has been found that the mechanism of their action as radical scavengers involves the donation of a hydrogen atom and/or electrons to stabilize radical species (Rice-Evans et al. 1996). As EGCG contains a lot of benefits for fish, pharmacokinetic studies of EGCG in fish are important. Our study is the first to determine these pharmacokinetics.

To prepare a test solution, 4.5 mg of EGCG (> 98.0% purity, C22H18O11·H2O, Tokyo Chemical Industry, Japan) dissolved in 4.5 mL of 0.9% NaCl solution. This EGCG solution was administrated intraperitoneally to 33 yellowtail fish, Seriola quinqueradiata (ca., 100 g) at a dose of 10 mg/kg body-weight. Blood (5 to 6 specimens in each group) was collected at 0 h (control group), 2 h, 4 h, 8 h, 24 h, 48 h, and 72 h post-treatment. At the same intervals, liver samples were also collected. To prepare the test diet, 45 mg of EGCG was added to 3.5 g of pulverized commercial diet (EP2, Marubeni-Nisshin Feed Co., LTD., Japan) and mixed with 7.5 mL distilled water. This EGCG containing diet was also administered by oral gavage to 33 yellowtail fish at a dose of 10 mg/kg body-weight. Blood and muscle samples were collected at same time-courses in IP administration. Plasma was obtained by centrifuging the blood at 12,000 rpm for 10 min at 4°C. Those samples were stored at -20°C until analysis. Our EGCG extraction in plasma protocol was slightly modified from the Fu et al. (2008) method. Briefly, a mixture of 400 μl of thawed plasma, 20 μl of 20% L(+)-ascorbic acid, and 20 μl of resorcinol (internal standard, IS) was extracted three times with 800 μl of ethyl acetate (EtOAc) (Tokyo Chemical Industry, Japan) each by well-mixing and centrifuging at 3,000 rpm for 10 min at 4°C. The upper organic phase was obtained and evaporated to dryness under a gentle nitrogen stream at 35°C. The residue was then reconstituted in 500 μl of 20% methanol (MeOH) aqueous solution and centrifuged at 3,000 rpm for 10 min at 4°C. The upper organic phase was obtained and evaporated to dryness under a gentle nitrogen stream at 35°C.
equipped with a LODS C18 column (length, 150 mm; inner diameter, 2.1 mm) (CERI, Tokyo, Japan) and a LODS protection column (2.0 mm L × 5 mm i.d.) (CERI) were used. An isocratic elution profile: a mobile phase mixture of MeOH and 20 mM H₃PO₄ (23:77, v/v), an injection volume of 1 μl and a flow rate of 0.2 ml/min, was monitored and quantified by UV detection at 270 nm. Mixtures of stock solution, EGCG (6 mg/ml) and resorcinol (IS) (75 μg/ml), were prepared in methanol and kept at −20°C. Peaks of EGCG and IS were detected at 5.9 ± 0.1 min and 4.3 min, respectively. The IS peak was used to verify extraction recovery. The EGCG concentration in samples was estimated from the EGCG concentration in standard solution. Finally, EGCG pharmacokinetic parameters in plasma after IP administration test were analyzed using GraphPad Prism 5 (GraphPad Software Inc., USA). This was done using fitting with a 1-compartment IP administration model: \( Y = D/V \times \exp(-C/V \times X) \), where \( Y \) is EGCG concentration (mg/l) at the time-course \( X \) (h) with an injection dose (D=10 mg/kg). The \( T_{1/2} \) and \( \ln(2) = \frac{\ln(2)}{C/V} \) for distribution volume, clearance, elimination rate constant, and half-life, respectively.

We found that there were a high elimination rate constant (0.24) and a short half-life (2.89 h) for EGCG in yellowtail fish. This may be due to low absorption and/or quick elimination of EGCG. No previous study has reported the pharmacokinetics of EGCG in fish. It is possible that plasma EGCG was not absorbed and/or quickly distributed into tissues, and excluded in fish. In rats, EGCG quickly distributes into the peripheral compartment and is excreted mainly through the bile after intravenous administration (Chen et al. 1997). In addition, the major elimination pathways of tea polyphenols are glucuronidation and sulfation (Lee et al. 1995). Thus, we suggest that EGCG metabolism may have driven the absence of EGCG in fish plasma following oral administration. Our results provide a basis for understanding the pharmacokinetics of EGCG in fish, and indicate that EGCG was poorly absorbed and/or quickly eliminated in yellowtail. To confirm our interpretation, it will be necessary to measure EGCG metabolites in the plasma and tissues of fish.

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References