Effect of water temperature and microcystins on the pharmacokinetics of epigallocatechin gallate (EGCG) in snakehead fish (*Ophiocephalus argus Cantor*)

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Abstract: The study was conducted to evaluate the effect of water temperatures and intraperitoneal administration of microcystins (MCs) on the pharmacokinetics characteristics of epigallocatechin gallate (EGCG) in snakehead fish (*Ophiocephalus argus Cantor*) by RP-HPLC method. The results showed that EGCG was absorbed and eliminated more faster at 20ºC than at 10ºC. Then the detected results also showed that EGCG was significantly absorbed fast in MCs-treated group than in control group. However, EGCG elimination became very lowly in MCs-treated group. Therefore, the pharmacokinetic characteristics of EGCG were significantly affected by water temperature and MCs toxin.

1 Introduction

Snakehead fish *(Ophiocephalus argus Cantor)* belong to omnivorous and carnivorous fish. Because of intensive farming and aquatic water polluting, the fish diseases become serious. MCs is a cyanobacterial toxin that often appears in eutrophic water and does great damage to the aquatic animals [1].

Epigallocatechin gallate (EGCG) is a kind of tea polyphenols, which widely exists in tea [2-3]. And the chemical structure of EGCG was also showed in Figure1. In recent years, many studies showed that EGCG exhibited some biological activities such as anticancer, antinutagenicity, prevention of cardiovascular disease and regulation of endocrine in some rodents [4-10]. Now catechin EGCG have been used as food additives and healthy products. Therefore, snakehead fish was used to study the effect of different water temperatures and intraperitoneal administration of microcystins (MCs) on the EGCG pharmacokinetics, which lastly served as reference for the dosage regimen of catechin EGCG applied to fish farming.

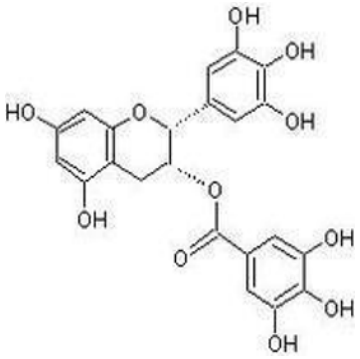

Figure 1. Chemical structure of EGCG

2 Materials and Methods

2.1. Chemicals and Preparation

The standard EGCG (purity≥98%) was purchased from Hangzhou Hetian Bio-technology Co., Ltd, China. Microcystins (MC-LR) (purity≥95%) was purchased from Express Technology Co., Ltd. Other chemical agents, including methanol and acetonitrile for the analysis of EGCG were of the HPLC grade, which were all obtained from Hangzhou Milk Chemical Instrument Co., Ltd, China. EGCG was dissolved in methanol to a concentration of 50 mg/mL as stock solution, which was stored at 4 ºC prior to the experiment.

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2.2. Animals

Two hundred-ten healthy snakehead fish (mean body 450±30 g) were obtained from YUETENG pisciculture (Hangzhou, China) and kept in flow through glass tank and acclimatized for one week before the experiment. These snakehead fish were randomly divided into three groups. The first group and the second group were used to study EGCG pharmacokinetics disposing progress at different water temperatures (20 ºC and 10 ºC). And the third group was controlled at 20±1ºC, which all fish was also intraperitoneally administered with EGCG after microcystins (MCs) treatment. The water quality in tanks was tested daily. All the fish were supplied with a catechin EGCG-free commercial diet before administration. On the day before study, the fish were not fed.

2.3. RP-HPLC Method

The HPLC system was SHIMADZU-20AT series equipment, with a UV detector and a Zhida N2000 instrument workstation written by Zhejiang University. EGCG separation was achieved on a Hypersil BDS C_{18} reversed-phase column (5μm, 250 mm×4.6 mm). The mobile phase was composed of acetonitrile and 0.1% citric acid solution (10:90, V/V) at a flow-rate of 1.0 mL/min. The column temperature was kept at 35 ºC and the detector wavelength was set at 278 nm. The 20μL were injected directly into the RP-HPLC system.

2.4. Assay of EGCG in Plasma

A plasma sample (0.6mL) was transferred to a 10 mL tapered glass tube and vortex-mixed for 1 min, then 3mL ethyl acetate was added. The tube was vortex-mixed for 3 mins. After centrifugation (6000 rpm) for 5 mins, the organic phase was transferred to a glass test-tube. And this extraction was repeated twice. All the organic phase were combined and evaporated to dryness at 45 ºC under a stream of nitrogen gas. The residue was reconstituted with 0.2mL acetonitrile and water (1:4, V/V), vortex-mixed for 1min and centrifuged (18000 rpm) for 5mins. Then 20 μL were injected directly into the RP-HPLC system.

2.5. Pharmacokinetics of EGCG

Fish in the first and second groups were given a single dose of 150 mg/kg body weight (50mg/mL). The fish in the third group were intraperitoneally administered EGCG at a single dose of 150 mg/kg body weight after they were treated with intraperitoneal administration of MC-LR at 50 μg/kg for a consecutive week. After administration of EGCG, five fish were sampled per each time point. 2 mL of blood was randomly taken from the caudal vein at 0.083h, 0.17h, 0.5h, 1h, 2h, 4h, 8h, 12h, 24h, 48h, 96h, 120h and 144h. The plasma was stored at -20 ºC after sampling until assayed. The parameters were calculated using the DAS3.0 computer program written by the Chinese Society of Mathematical Pharmacology.

2.6. Statistics

All data were expressed as means ± standard deviation. A probability of $P < 0.05$ was considered statistically significant using paired samples T test of SPSS 18.0.

3 Results

3.1. Chromatogram

By comparing the following chromatograms, EGCG and impurities are well separated. And there are no impurities in blank plasma.

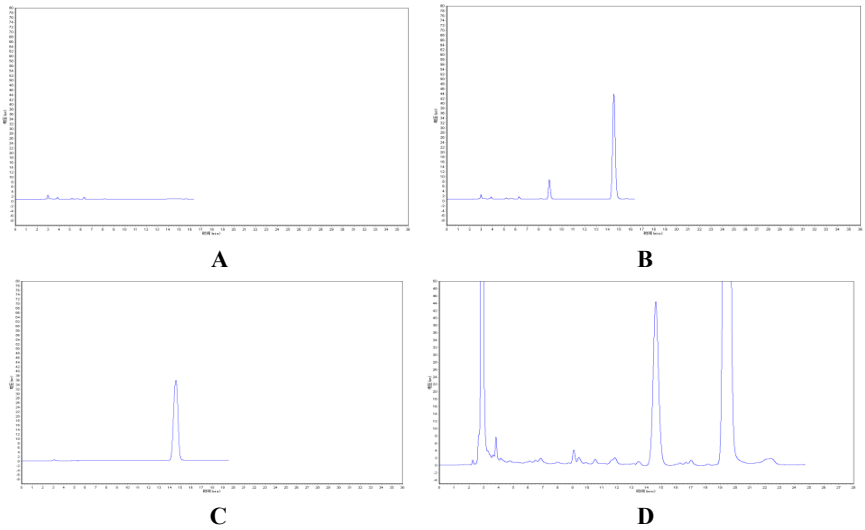

Figure 2. Chromatograms of EGCG (A) Blank plasma chromatogram; (B) Plasma spiked with nuciferine standard chromatogram; (C) Nuciferine standard chromatogram; (D) EGCG plasma chromatogram after administration.

3.2. Calibration and validation of the method

The standard curve equation of serum is Y = 12010X + 1566.7 (r =0.9999).

The intra-day and inter-day precisions were less than 10.0% for all three concentrations. All the datum of EGCG proved good precision and extraction recovery of the RP-HPLC method developed (Table 1).

Table 1. Extraction recovery and precision of EGCG (n=5)

Sample	Spiked Concentration $mg \cdot L^{-1}$（kg^{-1}）	Recovery (%)	Precision（%）	
			Intra day	Inter day
Plasma	0.5	85.31±7.19	6.72	8.43
	5.0	92.38±7.45	5.74	8.06
	50.0	95.36±6.72	6.48	7.05

3.3. Pharmacokinetics of EGCG

The plasma-concentration vs. time curves were given in Figure 3. And all the parameters for EGCG were presented in Table 2, which were calculated with DAS3.0 software.

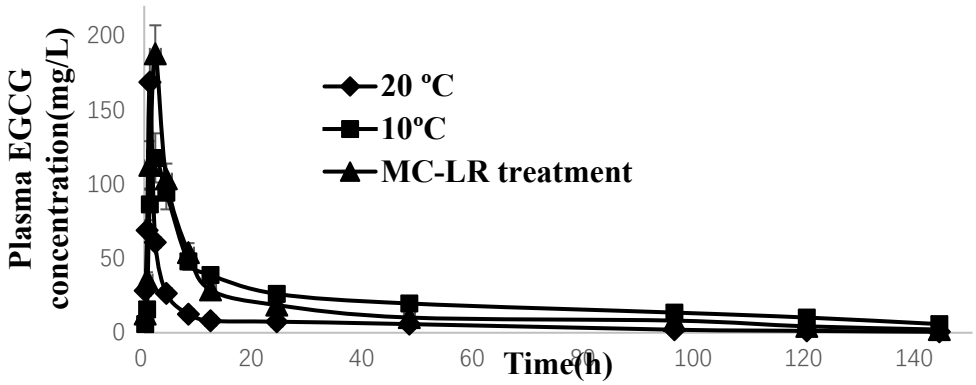

Figure 3. EGCG concentrations in plasma at different group

Table2. Pharmacokinetics parameters of EGCG at different groups

Parameters	Unit	20ºC	10ºC	MC-LR treatment
T1/2Ka	h	0.50	0.42	0.16
T1/2α	h	0.63	8.49	4.72
T1/2β	h	35.08	96.65	53.77
$AUC_{0-\infty}$	mg·h/L	963.19	4068.27	2518.98
Tmax	h	0.94	2.20	0.99
Cmax	h	130.76	90.48	141.75

4 Discussion

Recent years, some reports on the metabolism and pharmacokinetics of EGCG are also published in mammals [11-12]. And fish are cold-blooded animals, their physiological characteristics are deeply influenced by the water temperature [13-15]. From the results we found that the EGCG absorption and elimination were slower at a lower temperature (10 ºC). Though the absorption was faster at higher temperature, the elimination was faster, too. MCs is a kind of cyanobacteria toxin, which can enter animals through many ways and seriously threaten the health of animals. The results showed that the pharmacokinetic characteristics of EGCG were significantly affected by MCs pretreatment. The $T_{1/2Ka}$ became faster in MCs-treated snakehead fish, while EGCG elimination became very lowly. It was suggested that blood vessel of snakehead fish have been damaged by MCs, which was in accordance with aquatic toxicity induced by MCs.

Therefore, the EGCG pharmacokinetics progress was markedly affected by the water temperature and MCs toxin. In the future, the application of catechins to farming aquaculture should be with a consideration for the season and MCs effect on the physiological characteristics of fish.

Acknowledgement

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