CAPSULES notes and applications from BASi

356

May 2000

Multi-Channel Electrochemical Detection for the Determination of (-)-Epigallocatechin Gallate in Rat Blood with the Culex™ Automated Blood Sampler

Purpose

Determination of the (-)-epigallocatechin gallate (EGCg) (***F1***) in rat blood by multi-channel EC detector and evaluation of the pharmacokinetics of EGCg with an automated blood sampler.

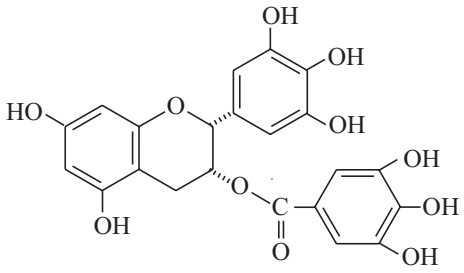

Figure 1. *The structure of (-)-epigallocatechin gallate.*

EGCg is an important tea catechin. The inhibitory activity of EGCg against tumorigenesis has been demonstrated in many animal models.

Method

LC with UV and chemiluminescence[1-4] have been used for the determination of EGCg in green tea and in biological samples, most of them with poor detection limit.

Conditions

LCEC System: BAS 480e chromatograph with a multi-channel amperometric detector (epsilon™, BAS) and ChromGraph version 2.00 software
Electrode: Four 2 mm glassy carbon working electrodes
Potential: +800, 700, 600, 500 mV vs. Ag/AgCl
Column: C18 5 m column (Discovery 150 x 2.1 mm, Supelco)
Mobile phase: 10 mM sodium acetate, 1% acetic acid, pH 3.5, 20% methanol (v/v)
Flow rate: 0.4 ml/min
Blood collecting system: An automated blood sampler (Culex™), including a rat containment system (Raturn™) and a fraction collector (HoneyComb™).

Sample Preparation

A total of 200 µl of rat blood solution, which contained 100 µl rat blood and 100 µl of physiological saline were transferred to a 1.7 ml centrifuge tube and centrifuged for 10 min at 4000 rpm. A total of 100 µl plasma solution was transferred to a 1.7 ml centrifuge tube and 900 µl of ethyl acetate was added, vortex-mixed for 3 min, and centrifuged for 3 min at 10,000 rpm. Following centrifugation, a 800 µl aliquot of the clear supernatant was transferred to a tube and dried by nitrogen. The residue was dissolved in 100 µl of 10 mM hydrochloric acid. A volume of 20 µl of the solution was injected into the LC system.

Preliminary Animal Study

Sprague-Dawley rats weighting 280-330 g were used. For the automated blood sampling experiments, the rats were implanted with a jugular vein cannula (0.3 I.D. x 0.6 O.D. x 81.3 mm L, polyurethane, BAS). After surgery, the rats were installed in the Raturn, then allowed to recover for one day with free access to food and water. EGCg (1 mg) was dissolved in 0.1 ml glycerol then diluted with 0.4 ml saline. The rats were dosed intraperitoneally (i.p.) at a single dose of 2 mg/kg. The blood was automatically withdrawn from the jugular vein and followed by a heparin/saline flush using the automated blood sampler, Culex. A total 200µl of blood and saline (1:1) was collected by the fraction collector.

Notes

Multi-channel electrochemical detection provides a selective and sensitive approach for the determination of natural products in blood [5]. By monitoring four potentials simultaneously one can easily determine the optimum potential during the method development and also verify peak purity by ratioing response at different energies for both standards and samples. In this study, a multi-channel detector with four glassy carbon electrodes was used. ***F2*** shows a chromatogram for EGCg extracted from rat plasma.

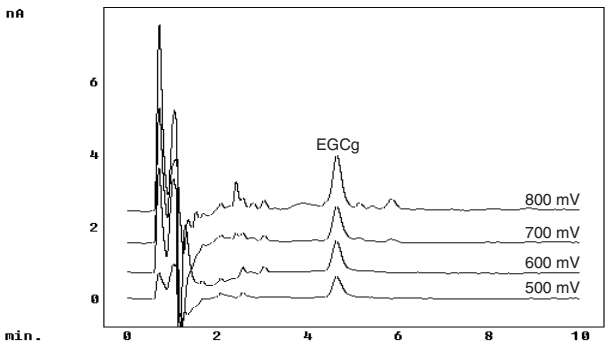

Figure 2. *Four channel detection of rat plasma spiked with 100 ng/ml of EGCg.*

Pharmacokinetic Studies

Using this method it was possible to quantify the blood concentration following a single dose of EGCg to rats with good accuracy and precision. Thus the pharmacokinetic properties of EGCg can be examined for intraperitoneal, oral and intravenous dosing. After administration of EGCg, blood samples were periodically collected by the automated blood sampler (Culex).

The proposed method was used for the determination of EGCg in rat blood. ***F3*** shows the blank plasma and a blood sample after 33 min of a single 2 mg/kg intraperitoneal dose administration of EGCg.

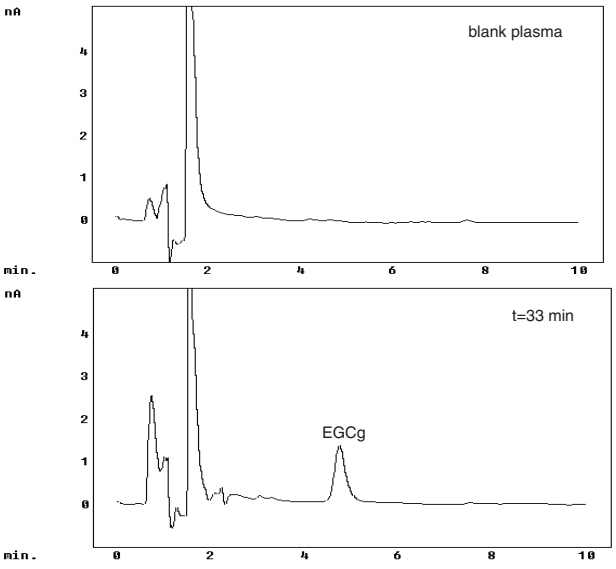

Figure 3. *Chromatograms of blank plasma and blood sample (t=33 min).*

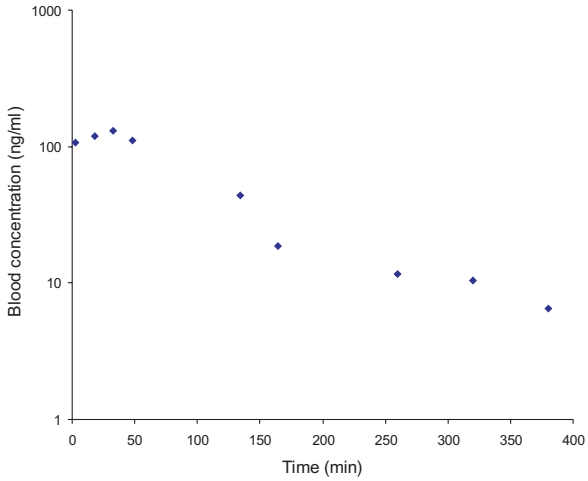

Figure 4. *Time course of EGCg concentration in blood after intraperitoneal administration of 2 mg/kg of EGCg to rat.*

F4 illustrates data for a single 2 mg/kg intraperitoneal dose of EGCg. The compound was rapidly absorbed to reach the maximum blood concentration. This method is being used to study EGCg kinetics using various routes of administration to better understand the potential role of this compound in human consumption of green tea.

References

1. *I. Sakata, M. Ikeuchi, I. Maruyama, T. Okuda, Yakugaku Zasshi, 111 (1991) 790-793.*
2. *L. Chen, M. J. Lee, H. Li, C. S. Yang, Drug Metab. Dispos., 25(1997) 1045-1050.*
3. *K. Nakagawa, T. Miyazawa, Anal. Biochem., 248 (1997) 41-49.*
4. *W. E. Bronner, G. R. Beecher, J. Chromatogr. A, 805 (1998) 137-142.*
5. *Y. Zhu, T. Huang, M. Cregor, H. Long, C. B. Kissinger, P. T. Kissinger, J. Chromatogr. B, 740 (2000) 129-133.*