Journal of Pharmaceutical and Biomedical Analysis 76 (2013) 134–138

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Phenolic acid metabolites derived from coffee consumption are unlikely to cross the blood–brain barrier

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ARTICLE INFO

Article history:
Received 21 August 2012
Received in revised form
11 December 2012
Accepted 12 December 2012
Available online 21 December 2012

Keywords:
Blood–brain barrier
Chlorogenic acids
Coffee
Metabolites
Permeation

ABSTRACT

Coffee drinking is well known for its stimulating effects on the brain and on cognition. In addition to the most active component, caffeine, coffee contains phenolic acids, which may also have some activity. Dihydrocaffeoyl-3-*O*-sulfate, caffeoyl-3-*O*-sulfate, dihydroferuloyl-4-*O*-sulfate, as well as dihydroferulic, dihydrocaffeic, 5-*O*-feruloylquinic and 5-*O*-caffeoylquinic acids, the major phenolic acid metabolites circulating in human plasma after coffee ingestion, were tested for their potential to enter the brain using a validated *in vitro* model of the blood brain barrier made of endothelial cells from bovine brain capillaries. As expected, caffeine showed a high rate of permeation across this barrier, but the phenolic acid metabolites exhibited a very low rate of permeation. The data suggest that none of these phenolic acid metabolites can be considered as potential candidate to enter the brain *in vivo* and so are unlikely to affect cognitive processes directly as proposed for caffeine.

1. Introduction

Coffee, one of the most consumed beverages in the world, has aroused more and more interest in the past few years because of its high content of hydroxycinnamic acids, being mainly chlorogenic acids, the quinic acid esters of caffeic, ferulic and *p*-coumaric acids [1]. In addition to their antioxidant activity, hydroxycinnamic acids and their esters are also demonstrated to have beneficial effects through other mechanisms of action, such as the modulation of gene expression [2] or the regulation of enzyme activity [3]. Even though controversy still persists, epidemiological studies rather suggest that coffee consumption is beneficial for human health and is associated with a decrease in brain disorders such as Parkinson and Alzheimer's diseases [4], effects in general attributed to caffeine [5]. *In vitro* and pre-clinical studies have also suggested the potential effects of 5-*O*-caffeoylquinic acid on brain protection against factors leading to neurodegenerative disorders such as neuronal cell death caused by oxidative damage [6], impairment of the blood–brain barrier following ischemia [7] or microglial activation caused by inflammation [8]. However, the capacity of hydroxycinnamic acids metabolites circulating in plasma after coffee ingestion to enter the brain has never been investigated. The blood–brain barrier (BBB), localized in the capillaries of the brain, is considered as the most important influx barrier for solutes to enter the brain [9]. It maintains homeostasis of the brain with its tight cell-cell junctions and lack of fenestration, limiting the entry of small hydrophilic molecules. Bioavailability of coffee hydroxycinnamic acids has been investigated in animals and human, allowing the clarification of their sites of absorption in the gastrointestinal tract as well as the identification of their plasma circulating metabolites. The current study investigated the capacity of the main circulating coffee hydroxycinnamic acids metabolites to enter the brain using a commercially available and validated *in vitro* model.

2. Materials and methods

2.1. Reagents

The *in vitro* blood–brain barrier model (BBB-RTU kit), including dishes, inserts, cells and culture media, was purchased from Cellial Technologies (Lens, France). Lucifer Yellow (LY), caffeine, ferulic, dihydroferulic and 3-(4′-hydroxyphenyl)propionic acids, Na_2HPO_4 and dimethyl sulfoxide (DMSO) were from Sigma (Buchs, Switzerland). 5-*O*-caffeoylquinic and dihydrocaffeic acids were from Extrasynthese (Genay, France). Dihydrocaffeoyl-3-*O*-sulfate, dihydroferuloyl-4-*O*-sulfate, caffeoyl-3-*O*-sulfate, feruloyl-4-*O*-sulfate and 5-*O*-feruloylquinic acid were from the Nestlé collection (92–98% purity). The IUPAC numbering was used for the chlorogenic acids.

Abbreviations: Avedev, average of the absolute deviations of data points from their mean; BBB, blood–brain barrier; LY, Lucifer Yellow; MCT, monocarboxylic acid transport; OAT, organic anion transporter.

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http://dx.doi.org/10.1016/j.jpba.2012.12.016

2.2. Cell culture

The *in vitro* BBB model used consisted of endothelial cells from bovine brain capillaries grown on collagen-coated semi-permeable polycarbonate filters (0.4 μm pore size diameter). The cells were received frozen from the supplier, already seeded on inserts, in 24-wells format dishes and kept at −80 °C until used. 4 days before the transport experiment, the cells were defrosted by the addition of culture medium and then processed as recommended by the supplier. Filters coated with collagen, but which did not contain cells, were also processed together with the plates containing cells from the beginning to the end of the experiment.

2.3. Permeability studies

The transport experiments were performed in Ringer Hepes Buffer (pH 7.4). Caffeine and ferulic, dihydrocaffeic, dihydroferulic, 3-(4′-hydroxyphenyl)propionic, 5-*O*-caffeoylquinic and 5-*O*-feruloylquinic acids were stored at −20 °C as stock solutions in DMSO (10 mM). The conjugates were stored at −20 °C as powders and re-suspended in DMSO just before use (10 mM). A stock solution of Lucifer Yellow (10 mM) was prepared in sterile water and stored at −20 °C. To perform transport experiments, culture medium was removed from the inserts and the cells rinsed with Ringer Hepes buffer at 37 °C. 400 μl of transport solution containing 20 μM Lucifer Yellow and 10 μM of compounds to be tested were put in the apical compartments. 800 μl of pre-warmed Ringer Hepes buffer were added to the lower compartments. A control was prepared using the buffer containing the vehicle only (0.2% DMSO). The transport experiments were performed at 37 °C, 5% CO_2/90% relative humidity during 1 h. At the end of the incubation time, samples were collected from apical (20 μl) and basal (200 μl) compartments and processed immediately for Lucifer Yellow quantification. After acidification with 2% 1 M acetic acid, the remaining volumes were stored at −20 °C until the analysis.

2.4. Lucifer Yellow quantification

The quantification of Lucifer Yellow was performed using a fluorimeter (Varioskan Flash, Thermoscientific, Zurich, Switzerland) with an excitation wavelength at 428–430 nm and an emission wavelength at 540 nm. The samples from the apical compartments were diluted 10 times using Ringer Hepes buffer before the analysis.

2.5. HPLC analysis

The analytical method used for the detection of coffee metabolites is based on the method reported by Lafay et al. [10] and modified to allow the detection of both aglycones and conjugates of phenolic acids. 50 μl of samples were analysed by HPLC coupled to an eight-electrode Coul-Array Model 5600 detector (ESA, Chelmsford, MA, USA) with potentials set at −100, 10, 70, 200, 270, 450, 720 and 770 mV. The separation was performed at 37 °C using a Nucleodur 5 μm/250 mm × 3.0 mm reverse phase column (Macherey-Nagel, Oensingen, Switzerland). Injections were carried out with an auto sampler maintained at 4 °C for the stability of the compounds. Solvent A was 50 mM Na_2HPO_4 in water (pH 1.5) and solvent B was acetonitrile/solvent A (1/1). The flow rate was 0.5 ml/min applying the following gradient: 15% B from 0 to 4 min; from 15 to 20% B between 4 and 12 min; from 20 to 22% B between 12 and 19 min; from 22 to 100% B between 19 and 40 min; 100% B from 40 to 45 min; back to initial conditions within 7 min. For caffeine analysis, 50 μl samples were injected on the same column but the elution was performed using MilliQ-water as solvent A and 100% methanol as solvent B. The flow rate was 0.5 ml/min, applying a gradient from 20 to 60% B between 0 and 10 min; from 60 to 70% B between 10 and 12 min; 70% B from 12 to 15 min; back to initial conditions within 5 min. Caffeine was detected by UV (274 nm) with a 1100 Agilent Diode Array Detector (Agilent Technologies, Morges, Switzerland). The internal standard 3-(4′-hydroxyphenyl)propionic acid was added at 0.5 μM to each sample before injection to normalize the measurements. Coefficient of linearity for the calibration curves were typically $R^2 > 0.99$. The limits of quantification (LOQ) were determined by triplicate analyses of standards spiked at low concentrations in the transport buffer and corresponded to the last point of the linear standard curves used. LOQ were 0.02 μM for ferulic and dihydroferulic acids and dihydrocaffeoyl-3-*O*-sulfate, 0.04 μM for dihydroferuloyl-4-*O*-sulfate, 0.08 μM for caffeoyl-3-*O*-sulfate, and dihydrocaffeic and 5-*O*-feruloylquinic acids, 0.09 μM for caffeine and 0.15 μM for feruloyl-4-*O*-sulfate and 5-*O*-caffeoylquinic acid. The method has been validated using caffeine, DHFA and DHCA3S, as representative molecules for each category of compound tested. The coefficient of variation values for the repeatability were 1.3, 2.2 and 1.7% respectively for caffeine, DHFA and DHCA3S. The coefficient of variation values for the intermediate reproducibility were 6.2, 9.0 and 8.4% respectively for caffeine, DHFA and DHCA3S. A chromatogram presenting the compounds spiked in the transport solution is shown on Fig. 1. The co-elution of DHFA and FQA was not an issue as their permeation through the BBB was studied in separate wells.

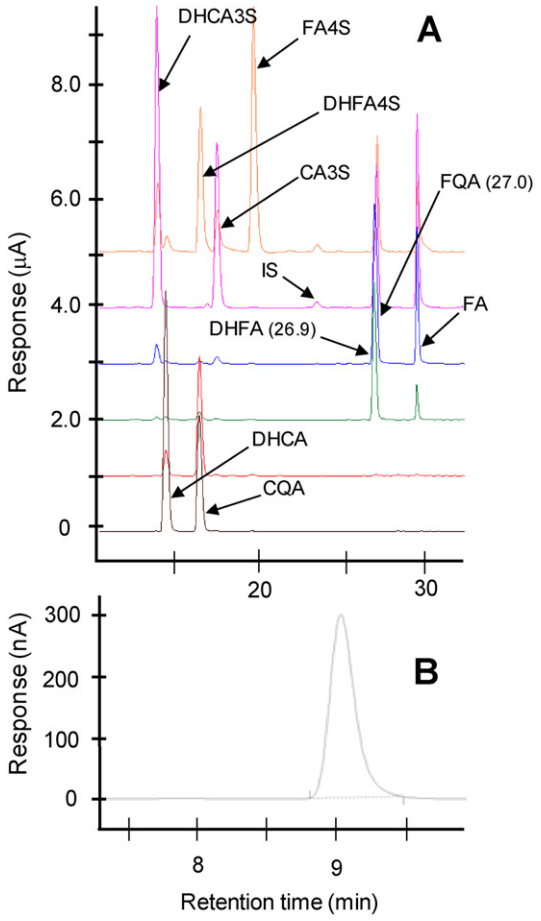

Fig. 1. Chromatograms of transport solution spiked with 10 μM DHCA, CA, DHFA, FA, FQA, CA3S, DHFA4S, FA4S, DHCA3S and 0.5 μM IS (A) or 5 μM caffeine (B). See text for details of the analytical method.