Disposition of quercetin and kaempferol in human following an oral administration of Ginkgo biloba extract tablets

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SUMMARY

Ten adult volunteers with an average age 28 years were given a single oral dose of six tablets of Ginkgo biloba extract. Quercetin and kaempferol in different period of human urine were determined by using RP-HPLC. The results showed the elimination rate constant $k$ and the absorption rate constant $k_a$ of quercetin were slightly more than that of kaempferol; and the absorption half-life ($t_{1/2a}$), the elimination half-life ($t_{1/2}$) and $t_{max}$ of quercetin were less than that of kaempferol, the differences were, however, not statistically significant. The mean values of $k_a$ were 0.61 h$^{-1}$ and 0.55 h$^{-1}$, $t_{1/2a}$ 1.51h and 1.56h, $k$ 0.37 h$^{-1}$ and 0.30 h$^{-1}$, $t_{1/2}$ 2.17h and 2.76h, $T_{max}$ 2.30h and 2.68h for quercetin and kaempferol, respectively, which mean absorption and elimination of quercetin and kaempferol are 0.17% and 0.22%, respectively. Quercetin and kaempferol are excreted in the human urine mainly as glucuronides.

INTRODUCTION

(GBE) is a well-defined, complex product prepared from green leaves of Ginkgo. The extract mainly contains the Ginkgo flavone glycosides and terpenoids. Quercetin, kaempferal, and isorhamnnetin are the most important constituents in the group of flavonoids. Flavonoids exhibit a wide range of in vitro activities, including antioxidative, antiviral, vasodilatating actions, in particular their antioxidant properties, have been studied intensively, however, in vivo data on human absorption, bioavailability and metabolism after oral intake are scarce. In spite a high dietary intake of the flavonoid, only very low amounts (0.4-1%) of quercetin have been reported to be excreted in the urine of humans [1,2,3]. And it was aware that the metabolism of quercetin and kaempferol has been studied in animals, but very few data in human are available and our knowledge concerning the human systemic availability of quercetin and kaempferol after an oral administration of Ginkgo biloba extract is only sketchy. The objective of the present investigation was to clarify the metabolic forms and systemic availability of quercetin and kaempferol after oral administration of Ginkgo biloba extract tablets in healthy Chinese volunteers, using liquid-liquid extraction coupled with a reserved-phase high performance liquid chromatography assay with UV absorbance detection method.
MATERIALS AND METHODS

Materials
Quercetin (Q) and Kaempferol (K) were purchased from the National Institute for the Quality Control of Pharmaceutical and Biological Products (Beijing, China). Tablets of Ginkgo biloba extract (containing 1.134mg Q and 1.233mg K per tablet) were obtained from Zhejiang CONBA Pharmaceutical Manufacturing Company, Ltd (Hangzhou, China). Tetrahydrofuran, methanol and isopropanol were of HPLC grade, and sodium dihydrogen phosphate (NaH₂PO₄), concentrated phosphoric acid were all analytical grade.

All other chemicals and solvents were of an analytical or chromatographic grade and obtained from commercial sources.

Phosphate buffer used for the mobile phase was prepared with sodium dihydrogen phosphate and adjusted to pH 2.0 with concentrated phosphoric acid.

Instrumentation
Chromatographic determination of the quercetin and kaempferol were performed by using a Shimadzu HPLC system equipped with: LC-10AT VP pumps coupled to a manual injector with a 20 μl fixed loop, a platinum EPS C₁₈ 100A (250 mm x 4.6 mm i.d., 5μm) with a guard column (10mm x 4.6 mm, i.d., 5μm; packed with YWG-C₁₈ H₃Te), and a SPD10A VPUV-VIS detector. The chromatographic data were collected and processed on an Epper chromatopac station version (Zhejiang University, Hangzhou, China). The mobile phase consisted of phosphate buffer-tetrahydrofuran-methanol-isopropanol (70:15:10:20, v/v/v/v). The flow rate was set at 0.7ml/min. The chromatographic peaks of eluted components were monitored at 380nm, the quercetin and kaempferol concentrations were calculated by integration of their chromatographic peaks.

Drug administration and samples collection
This study was approved by the Ethics Committee of College of Pharmaceutical Sciences, Zhejiang University. Ten adult volunteers with an average age 28 years (22 to 32 years) and an average weight of 60 kg (56 to 65kg) participated in this study. The volunteers were judged to be in good health on the basis of their medical history, physical examination and laboratory profiles, which were performed within 2 weeks before the study. And they abstained from flavonoid-containing diets before and 12h after dosing. The volunteers were given 150ml of water at approximately 7:00a.m. after excreting urine. Blank urine was collected at approximately 7:40 a.m., and then they were given a single oral dose of GBE (six tablets) with 150ml of water on an empty stomach. During the investigation period, the intake of other drugs and of alcohol was not allowed. Urine samples were collected just before dosing and at 1, 2, 4, 6, 8, 10 and 12 hours after dosing. The volumes of the urine samples were measured immediately after collection and the urine samples were stored at -20°C until analysis.

Assay procedure
The urine samples were allowed to stand and warmed to room temperature, then filtered. 4.0ml of the filtrates was taken for analysis. 1ml 6mol/L hydrochloric acid was added to urine samples for hydrolyzing the glucuronides of quercetin and kaempferol. The mixture was heated at 80°C for 30 min. The urine sample hydrolyzed was extracted with 5.0ml ether by vortex-mixed for 5min then centrifuged for 10min at 3500rpm after cooled down to room temperature. 4.0ml of upper organic layer was quantitatively transferred into another test tube and evaporated to dryness with N₂. The residue was reconstituted in 100μl mobile phase before analysis. 20μl of the sample was injected into the HPLC system.

Calculation
The apparent terminal elimination rate constant, k, was calculated through least-squares regression analysis of urine excretion rate-mid-point time date over the terminal log-linear disposition phase. The absorption rate constant, kₐ, was calculated based on Wagner-Nelson method. The elimination half-life (t₁/₂) was calculated as 0.693/k, and the absorption half-life (t₁/₂a) was calculated as 0.693/ kₐ. The time to peak concentration (Tₘₐₓ) was calculated by