ABSTRACT  A previous study in ileostomy patients indicated that dietary glucosides of the flavonoid quercetin are hydrolyzed efficiently in the intestinal lumen, followed by absorption of a large fraction of the quercetin aglycone. To determine the fate of quercetin, we administered 1.85 MBq (50 μCi) of 14C-quercetin both orally (100 mg, 330 μmol) and intravenously (iv; 0.3 mg, 1 μmol) to healthy volunteers. Serial plasma samples, urines and stools were collected for 72 h. Total radioactivity was determined by liquid scintillation spectrometry directly in plasma and urine and after repeated methanol extraction of stool homogenate samples. The oral absorption, based on total radioactivity, was surprisingly high, ranging from 36.4 to 53.0%. The biological half-life was very long, ranging from 20 to 72 h. The urinary recovery of total radioactivity ranged from 18.4 to 26.8% after the iv dose and from 3.3 to 5.7% after the oral dose. The corresponding fecal recoveries were only 1.5–5.0% and 1.6–4.6%, respectively. Thus, the total recovery of the 14C-quercetin doses, in particular after oral administration, was very low. In search for the unaccounted for fraction of the 14C-quercetin dose, we performed 14CO2 recovery studies in three volunteers (3 iv and 3 oral doses). At timed intervals, 14CO2 in expired air was trapped in hyamine hydroxide/thymolphthalein and analyzed for radioactivity. As much as 23.0–81.1% of the quercetin dose was recovered as 14CO2 in the expired air from these volunteers, after both oral and iv doses. The disposition of quercetin in humans is thus highly complex, requiring further studies.


KEY WORDS:  quercetin  intestinal absorption  enterohepatic recirculation  carbon dioxide formation  flavonoids  humans

Quercetin is one of the most prevalent as well as thoroughly studied dietary flavonoids. It is present in fruits, vegetables and beverages mainly as glucosides, with the highest content in onions, apples and red wine (1–3). Epidemiologic studies suggest that flavonoids are protective against coronary heart disease and stroke (4–6) as well as in certain cancers (7–9). Large numbers of in vitro studies suggest a variety of molecular targets for these effects (10). The numerous reports on quercetin’s potential beneficial effects on human health have more recently led to a proliferation of high dose quercetin nutritional preparations. However, a major concern has been the poor oral bioavailability of quercetin and most other flavonoids (11).

The original model of flavonoid absorption assumed that flavonoid glucosides were too polar to be absorbed from the small intestine and that absorption was dependent on the flavonoid glucosides were too polar to be absorbed from the small intestine, potentially independently of bacterial enzymes (17). This conclusion is supported by in vitro studies (16,18–20). After hydrolysis of the glucosides in the ileostomy fluid or plasma to support such a conclusion. In contrast, in vitro studies using human intestinal Caco-2 cell monolayers as a model of human intestinal absorption showed complete lack of absorption of the glucosides, mainly due to effective efflux by the multidrug resistance protein 2 transporter (MRP2) (14,15), whereas quercetin itself was easily absorbed (16). In a subsequent reinvestigation of the absorption of the quercetin glucosides in ileostomy patients, we found that the glucosides were efficiently hydrolyzed in the small intestine, potentially independently of bacterial enzymes (17). This conclusion is supported by in vitro studies (16,18–20). After hydrolysis of the glucosides in the ileostomy patients, it was calculated that the absorption of the quercetin aglycone may be as high as 65–81% (17).

On the basis of these observations, we focused our attention on the absorption and the biological fate of the quercetin aglycone. 14C-Labeled quercetin (Fig. 1) was administered both orally and intravenously (iv) to normal, healthy volunteers, greatly facilitating estimates of recoveries and fraction absorbed. A large fraction of the oral dose of quercetin was...
absorbed. Surprisingly, the main route of elimination of quercetin, by both routes of administration, was via exhalation of carbon dioxide.

SUBJECTS AND METHODS

Subjects and study design. Six healthy subjects (23–44 y; 70–110 kg) participated in the study. Two subjects were female; one was Asian and five were Caucasian. Written informed consents were obtained. The study was approved by the Institutional Review Board for Human Research. The oral and iv radiation doses were estimated to be ~1% of the annual whole-body background radiation in the United States. All subjects were studied in a Clinical Research Unit. The diet during and for 4 d before the study was low in flavonoids. Oral (6 subjects) and iv (4 subjects) quercetin doses, at least 10 d apart, were administered in the morning after an overnight fast. Breakfast was served 3 h later. Serial blood samples drawn at 0–72 h after the dose were centrifuged at 1000 g and homogenized with 1 mol/L acetic acid. Aliquots of all samples were stored at −20°C. Some subjects, samples of exhaled air were also collected (see below).

14C-Quercetin doses. The oral 14C-quercetin dose consisted of 112 mg quercetin dihydrate (100 mg, 330 μmol quercetin) and 10 mg ascorbic acid (Sigma, St. Louis, MO), dissolved in 9 mL ethanol, with 1.85 MBq (50 μCi) 14C-quercetin [1960 MBq/mmol; NCI Radiochemical Repository at Chemsyn Science Laboratories, Lenexa, KS] added in 50 μL dimethyl sulfoxide. Immediately before administration, 30 mL of Simple Syrup (Hunco, Texarkana, TX) and 1 mL vanilla extract were added with vigorous shaking to form a suspension. The oral dose was obtained by 500 mL of water.

For the iv 14C-quercetin doses, 2.5 mg 14C-quercetin [14.8 MBq] was dissolved in 4 mL 100% ethanol and sterilized by filtration. The solution was tested for sterility and pyrogens and stored at −80°C. Immediately before administration, 0.6 mL of this solution was added to 11.4 mL sterile saline and 10 mL [0.3 mg, 1 μmol 14C-quercetin, 1.85 MBq (50 μCi)] was infused with an equal volume of saline over 10 min. The infusion line was then rapidly flushed with 10 mL saline.

Sample analysis. Plasma and urine samples (in duplicates) were obtained directly after the addition of an equal volume of water and 10 mL Aquasol-2 scintillation cocktail (Packard, Meriden, CT). The radioactivity content in the fecal homogenates was estimated by freeze-drying duplicate 2-g aliquots and extracting them with 3 × 10 mL methanol. Aliquots of the methanol extracts were counted. The counts in the third extract were <1% of those in the first. Exhaled 14CO2 was measured by an adaptation of the erythromycin breath test (21). Hyamine hydroxide (1 mol/L in methanol, J. T. Baker, Phillipsburg, NJ) was mixed with an equal volume of 100% ethanol and 50 mL/L of 1% thymolphthalein. Aliquots (4.0 mL) of this blue solution were put into 20-mL glass scintillation vials and capped tightly. At timed intervals before and after the two 14C-quercetin doses, the subjects were instructed to blow bubbles through a pipet with a one-way valve into a collection vial until the solution turned colorless, at which point 2 mmol of CO2 was trapped (~1 min). The vials were then tightly capped; after addition of Aquasol-2 and dark-adaptation, they were counted for radioactivity. The predose counts were ~10 dpm/sample, whereas the peak counts after both oral and iv doses were 10,000–40,000 dpm/sample.

Calculations. The areas under the plasma concentration vs. time curves (AUC) were calculated by the trapezoidal rule to the last time point, 72 h. The plasma, urine and exhaled carbon dioxide half-lives were calculated by least-squares linear regression. The fraction of the oral dose absorbed (in %) was calculated as (AUCoral × Doseoral) / (AUCiv × Doseiv) × 100. For calculations of total amount of 14CO2 exhaled, the endogenous rate of production (ERP) of CO2 was calculated as 5 mmol CO2/m2 body surface area · min (21). The dpm exhaled during a collection interval was calculated as dpm measured (corrected for background) × ERP/2 (mmol). This value could then be expressed as a percentage of the administered dose (110 × 106 dpm) exhaled per hour. The AUC of the percentage of dose exhaled per hour vs. time was finally calculated by the trapezoidal rule to give the total fraction of the dose excreted as 14CO2.

RESULTS

After an oral dose of 100 mg 14C-quercetin (330 μmol) in six human subjects, an early peak plasma concentration of 270 ng/mL (890 nmol/L) was reached as early as 30 min after the dose (Fig. 2). At 8 h after the dose, there was a second peak of 350 ng/mL (1160 nmol/L). The plasma concentrations then declined exponentially over the entire study period of 72 h. After an iv dose of 0.3 mg 14C-quercetin (1 μmol) in four subjects, there was a rapid fall of the plasma concentrations of total radioactivity over the first 4 h after the bolus injection, indicating the distribution phase (22). The plasma concentrations then fell in parallel with those after the oral dose for the remainder of the study period.

The terminal elimination half-lives were quite long, ranging from 20 to 72 h (Table 1). There was no statistical difference between the two routes of administration in the limited number of individuals studied. The interindividual variability in AUC values for the oral doses was less than twofold, with even less variability for the iv doses (Table 1). For the four individuals who received the iv doses, the absorption, ranging from 36.4 to 53% of the dose, could for the first time be directly determined for quercetin. It should be noted that measured and calculated kinetic parameters in the single obese female subject (110 kg) did not differ from those obtained with the five subjects of normal weight (70–89 kg).

FIGURE 1 Chemical structure of quercetin. The 14C-label is in the 4-position.
The recoveries in urine and feces after the oral dose were surprisingly low, with 3.3–5.7% of the dose found in the urine and only 0.2–4.6% in the feces (Table 2). After the iv dose, the recoveries in urine were as high as 18.4–26.8% of the dose with only 1.5–5.0% found in the feces. Thus, the overall recoveries in urine and feces were; 3.5–8.3% after the oral and 21.3–30.2% after the intravenous dose. Further studies attempting to increase the recoveries of radioactivity, in particular from feces, compared with the procedure described in Methods, gave no improvement.

The time course of 14CO2 formation in three individuals receiving either an oral or an iv dose is shown in Figure 3. The curves for the two routes of administration were very similar except for higher peak levels after the oral dose. The large variability in the early part of the time courses is due mainly to the fact that in some individuals, 14CO2 started to appear in the expired air 4 h after the 14C-quercetin dose, whereas in others not until 8 h after the dose. The 14CO2 formation/exhalation accounted for 23.0–81.1% of the administered 14C-labeled quercetin doses, both oral and intravenous (Table 2).

DISCUSSION

A large number of studies have been devoted to determining the biological fate of quercetin, mainly as glucosides, the major form in which it appears in the diet. Unfortunately, very little information has been gained from these studies, except that some form of acid- or enzyme-hydrolyzable conjugates does reach the systemic circulation (11,13,23,24) and that some biological activity may be associated with such conjugates (23). Of greater significance may be observations consistent with efficient hydrolysis of the glucoside conjugates in the intestinal lumen to the quercetin aglycone (17,20), which then appears to be efficiently absorbed. The biological fate of the aglycone was the focus of the present study.

In four subjects, who received both an oral and an iv dose of 14C-quercetin, we could for the first time establish the oral absorption of this flavonoid, taking into account a variety of metabolic and chemical breakdown products. The absorption was surprisingly high, ranging from 36 to 53%. This had previously been suggested on the basis of studies in a preclinical absorption model, the Caco-2 cell monolayer (16). After administration of the quercetin glucosides in ileostomy patients, the absorption of quercetin after enzymatic hydrolysis of the glucosides was calculated to be as high as 65–85% (17). The dietary quercetin glucosides may thus, as suggested, act as more soluble quercetin prodrugs with favorable absorption (17).

The terminal elimination half-life for the total quercetin radioactivity was quite long, i.e., 20–72 h. This should be compared with that of quercetin alone, which was only 0.7–2.4 h after iv administration in two previous studies (22,25). When quercetin or quercetin glycosides were administered orally (24,26), half-lives for quercetin of 15–28 h have been reported. However, this was after acid or enzymatic hydrolysis of the samples and would thus be expected to reflect quercetin conjugates. The very long half-life observed in our study could be due to multiple factors. A high volume of distribution does...
not seem to be a significant contributor. The volume of distribution for quercetin itself is thus <0.5 L/kg (22,25) and for the total radioactivity in this study, ~1.4 L/kg. A more likely explanation is enterohepatic recirculation, which may replenish the plasma concentrations over an extended time period. There was very strong evidence for such recirculation after the oral dose, with all subjects showing a distinct second peak of plasma radioactivity 6–12 h after the dose, although there was only weak evidence after the iv dose (Fig. 2). An additional factor, which is the subject of a separate investigation (27), is the potential for covalent binding of quercetin to plasma proteins, presumably after initial enzymatic bioactivation.

The most challenging part of the disposition of quercetin in humans was the metabolic fate, including the route(s) of elimination, which required the use of radioactively labeled compound. The recoveries in both urine and feces after the oral dose were very low, amounting to <10% of the dose. The much higher recovery (~20%) in the urine after the iv dose was interesting. This may be an effect of the much lower dose given iv (0.3 mg) compared with the oral dose (100 mg). Such an effect may be explained by saturation of an efflux transporter in the kidney after the higher oral dose. However, the major fraction of both doses was still unaccounted for, suggesting an alternative route of elimination.

This was explored by measuring potential exhalation of radioactivity in the form of 14CO2, after both oral and iv doses. It was fortuitous that we were able to detect this major metabolic route because the 14C-quercetin used has only one of its 14 carbon atoms labeled, i.e., the one in the 4-position (Fig. 1). The total recoveries of 14C-quercetin in urine, feces and exhaled air in the individuals in Table 2 amounted to 46.7–20% in the urine after the iv dose (Fig. 2). An additional explanation is enterohepatic recirculation, which may replenish the total radioactivity in this study, ~1.4 L/kg. A more likely explanation is enterohepatic recirculation, which may replenish the plasma concentrations over an extended time period. There was very strong evidence for such recirculation after the oral dose, with all subjects showing a distinct second peak of plasma radioactivity 6–12 h after the dose, although there was only weak evidence after the iv dose (Fig. 2). An additional factor, which is the subject of a separate investigation (27), is the potential for covalent binding of quercetin to plasma proteins, presumably after initial enzymatic bioactivation.

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