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Disposition of Quercetin in Man after Single Oral and Intravenous Doses*

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Summary. The pharmacokinetics of quercetin, a flavonoid, have been studied in 6 volunteers after single intravenous (100 mg) and oral (4 g) doses. The data after iv administration were analyzed according to a two compartment open model with half lives of 8.8 ± 1.2 min for the αphase and 2.4 ± 0.2 h for the βphase (predominant half life), respectively. Protein binding was >98%. The apparent volume of distribution was small at 0.34 ± 0.03 l/kg. Of the intravenous dose 7.4 ± 1.2% was excreted in urine as a conjugated metabolite, and 0.65 ± 0.1% was excreted unchanged. After oral administration no measurable plasma concentrations could be detected, nor was any quercetin found in urine, either unchanged or in a metabolized form. These results exclude absorption of more than 1% of unchanged drug. Recovery in faeces after the oral dose was 53 ± 5%, which suggests extensive degradation by microorganisms in the gut. The data obtained show that oral administration of flavonoids may be of questionable value.

Key words: Quercetin, flavonoids, pharmacokinetics, absorption, disposition, metabolism, man.

Between 600 and 800 flavonoids have been isolated (1) and numerous investigations have been carried out to show their clinical effectiveness, especially in the treatment of peripheral vascular diseases. A recent report of a symposium on flavonoids (2) stressed their various effects on blood vessels and connective tissue. Excellent studies by Griffiths, Das and their co-workers (3-8) have described the metabolism of radio-labelled catechin, apigenin, myricetin and flavanone in the rat, guinea pig and man. Although Das (8) demonstrated that after oral administration of catechin, as much as 7.5% appeared unchanged in urine, the bioavailability of flavonoids has been questioned by some investigators (9-11), in particular of quercetin, rutin and hydroxy-ethylrutosides. All the results available now have been based on urine data, and detectable concentrations of any flavonoid in human plasma have never been reported.

The present study was designed to investigate the pharmacokinetics of quercetin, a key flavonoid, in man after intravenous and oral administration, using a specific and highly sensitive assay method recently developed in this laboratory (12).

MATERIALS AND METHODS

1. Experimental Design

6 volunteers (4 male, 2 female) participated in the study after giving informed consent. They were between 21 and 32 years of age and were considered healthy from the results of a history, physical examination and routine laboratory tests.

A. Intravenous Administration. Quercetin 100 mg was administered intravenously by an infusion pump to 6 subjects over a period of 5 minutes. Blood pressure

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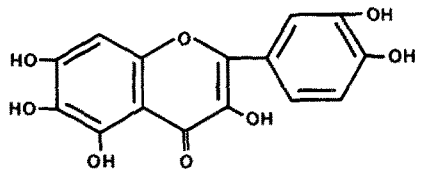

Fig. 1. Structural formula of quercetin

and heart rate were monitored during the infusion period and for 1 hour thereafter. Any drug remaining in the ampoule and the infusion system was washed out and the amount determined for subtraction from the total dose. Blood samples 12 ml were obtained before and 5, 10, 20, 40, 80, 160, 320 and 540 min after the infusion. Urine was collected in fractions of 0-80, 80-160, 160-320, 320-540 min and 540 min to 24 h.

B. Oral Administration. After fasting overnight, on separate occasions 4 of the 6 volunteers received single doses of quercetin 4 g (gelatin capsules each of 500 mg quercetin), together with 250 ml of water. Blood was taken 20, 40, 80, 160, 320, 540 min and 24 h after ingestion and urine collected as described for intravenous administration. In addition, faeces were collected over 72 hours and, following 500 or 1000 fold dilution of an aliquot, assayed for quercetin by the same procedure as used for plasma or urine.

2. Chemical Methods

Tetraphenyldiboroxide was a gift from Gebr. Schwabe, Karlsruhe, Germany. Quercetin was also kindly supplied by Gebr. Schwabe as ampoules containing 100 mg of the compound in pure ethanol, to be mixed with 20 ml of an alkaline (pH 8.5) aqueous solution no more than 1 hour before use. Quercetin crystals were purchased from Serva, Heidelberg, Germany, and packed into gelatin capsules. Both the iv and the oral preparation were tested for purity by their fluorescence spectra (12) and thin layer chromatography (toluene/formic acid/formic acid ethyl ester, 50:10:40); and the content of the ampoules was checked by the method employed in the study (12).

A. Quercetin. Quercetin concentrations were measured according to a recently published fluorimetric method which allows determination of 0.1 µg/ml of the compound in biological fluids (12). In brief, to 2 ml of plasma or urine 1.5 ml of a 1 M sodium citrate buffer pH 2.0 were added, and the compound extracted into 8 ml of water-saturated methylethylketone. 6 ml of the organic solvent were evaporated to near dryness, the residue dissolved in 0.5 ml of acetone and reacted with 0.6 mg of tetraphenyldiboroxide. The complex formed was adjusted to pH 3.0 with 2 ml of a 0.2 M sodium citrate buffer and extracted into 6 ml of diisopropylether. An aliquot of the organic layer was used for fluorimetric measurement at excitation and emission wavelengths of 445 and 500 nm, respectively.

B. Quercetin Conjugate. Identification of conjugated quercetin was achieved in the following ways:
1. A 5 ml aliquot of urine (Subject 2, 0-80 min) was adjusted to pH 5.3 with an equal volume of a 0.2 M acetate buffer and incubated for 12 hours with 0.2 ml of a β-glucuronidase-arylsulphatase solution (Boehringer, Mannheim, Germany) at 37°C in a metabolic shaker. Assaying the same urine before and after incubation gave the amount of free and total quercetin, respectively. The amount of the conjugate was obtained by subtracting the free quercetin from the total quercetin formed after hydrolysis. A blank urine incubated in the same way did not reveal any fluorescence interfering with quercetin.

The time course of the hydrolysis showed that the reaction was completed after 10 hours. Quercetin added to a blank urine was not degraded after 10 hours. Acid hydrolysis in boiling water for 30 minutes gave the same results, but this method was abandoned because of the high blank fluorescence, which inhibited estimation of low quercetin concentrations.

Urine 2 ml from Subject 2's 0-80 min collection period, and 2 ml of hydrolyzed urine from the same sample were each diluted with 18 ml of distilled water and placed on 10 x 60 mm glass columns packed with polyamide 80-100 mesh (Serva, Heidelberg, Germany). Elution of quercetin and its conjugate was performed with methanol/1 N acetic acid (20:1), the eluate evaporated to near dryness, the residue applied to a silica gel thin layer plate and developed in the system toluene/formic acid/formic acid ethyl ester (50:10:40). In addition to quercetin, which showed an R_f of 0.45, in the unhydrolyzed urine sample large amounts of a compound with the colour characteristics of quercetin remained at the origin. When the urine had been hydrolyzed, the origin was free of this compound. Quercetin from either sample

was subsequently eluted from the plate with acidified methanol and analyzed fluorimetrically as described under 'Methods'. The results were identical with those obtained by the original extraction method and exhibited a 10 times higher concentration of quercetin in the hydrolyzed than the unhydrolyzed urine.

3. Protein Binding

Protein binding was determined by gel chromatography on Sephadex G 25 according to the procedure of Krieglstein and Kuschinsky (13). A fresh pooled plasma solution from healthy volunteers with a quercetin concentration of 8 µg/ml was used.

RESULTS

Administration of quercetin by either route was well tolerated by all subjects.

1. Plasma Levels

Individual plasma levels after intravenous injection of 100 mg of quercetin are shown in Table 1; and the typical time course of the plasma level in two subjects plotted on a semilogarithmic scale is shown in Fig. 2. In all subjects there was a rapid decline of the first slope over 20-40 min, followed by a second and less rapidly declining slope, which proved to be linear at least over 120 min or 3 time points of measurement, until the lower limit of sensitivity of the method was reached. Every plasma level curve could be followed at least for 160 min, in three volunteers for 320 and in one for 540 min. The plasma concentration 10 min after injection was 3.69 ± 0.47 µg/ml (mean ± S.E.), and after 40 min 0.76 ± 0.20 µg/ml.

Following oral administration of quercetin 4 g to 4 subjects (50 to 65 mg per kg with individual body weights between 61 and 80 kg) no measurable plasma concentration could be found at any time. This implied that, if any quercetin at all were present in plasma, the concentration was below the sensitivity of the method, which was 0.1µg/ml.

2. Urinary Excretion of Quercetin and Its Metabolite

The cumulative urinary excretion of quercetin and its conjugated metabolite after intravenous administration is shown in Fig. 3. Three quarters of the total amount of quercetin and its meta-

Table 1. Plasma concentrations (µg/ml) of quercetin following intravenous administration of 100 mg

Subject	Time (min) 5	10	20	40	80	160	320	540
1	3.3	2.5	1.8	1.2	0.94	0.64	-	
2	3.0	1.2	0.9	0.5	0.22	0.15	0.08	-
3	2.1	1.5	0.8	ND[a]	0.54	0.36	-	-
4	5.1	3.1	2.1	1.3	0.64	0.35	0.09	-
5	5.0	1.8	1.2	0.6	0.47	0.36	0.22	0.11
6	3.8	2.0	1.0	0.4	0.21	0.15	0.08	-
Mean	3.7	2.0	1.3	0.8	0.50	0.33	0.12	0.11
SE	0.4	0.3	0.2	0.2	0.10	0.07	0.03	-

[a] = not determined

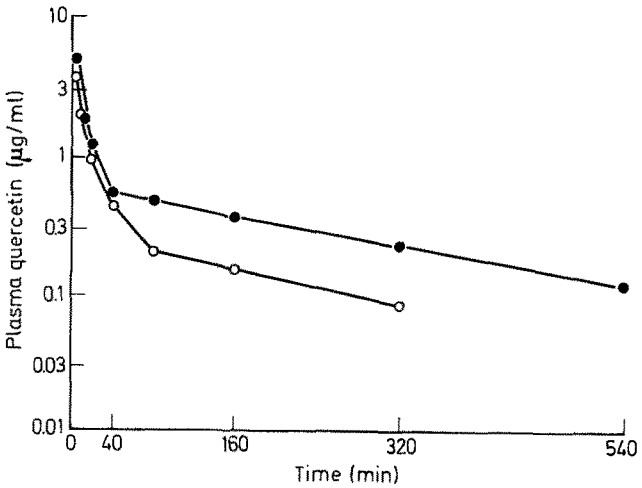

Fig. 2. Log quercetin plasma concentration versus time in subjects 5 (●——●) and 6 (o——o) following intravenous administration of 100 mg

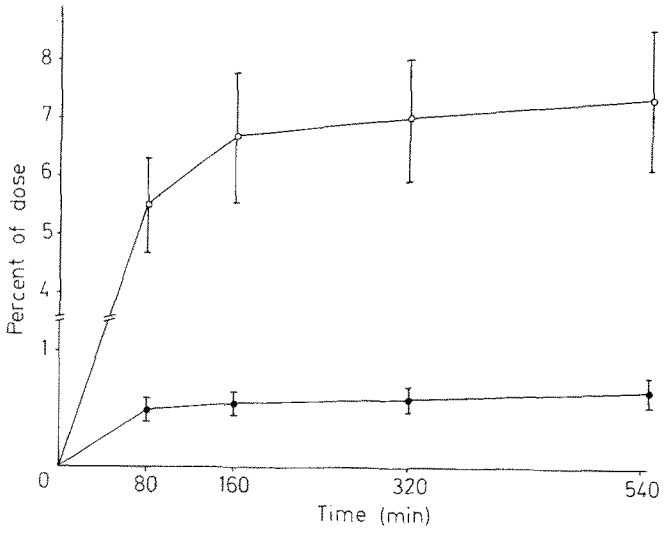

Fig. 3. Cumulative urinary excretion of unchanged quercetin (●——●) and its conjugated metabolite (o——o) versus time

Table 2. Pharmacokinetic parameters describing the disposition of quercetin following intravenous administration of a 100 mg dose

Subject	A (μg/ml)	α (h^{-1})	B (μg/ml)	β (h^{-1})	t 1/2 α (min)	t 1/2 β (h)	k_{el} (h^{-1})	V_d (l/kg)	Cl_{tot} (ml·min^{-1})	Cl_{ren} (ml·min^{-1})
1	2.85	5.407	1.41	0.299	7.7	2.3	0.813	0.32	310	2.8
2	2.23	2.982	0.28	0.237	14.0	2.9	1.330	0.47	841	9.4
3	2.32	6.761	0.84	0.319	6.2	2.2	1.062	0.42	562	7.1
4	4.37	3.857	1.26	0.491	10.8	1.4	1.524	0.28	437	0.9
5	5.93	7.308	0.68	0.257	5.7	2.7	1.912	0.23	465	3.8
6	4.24	4.801	0.29	0.241	8.7	2.9	2.171	0.31	807	3.7
Mean	3.66	5.186	0.79	0.307	8.8	2.4	1.469	0.34	571	4.6
SE	0.54	0.619	0.18	0.036	1.2	0.2	0.191	0.03	79	1.2

bolite excreted in 9 hours were already found in the first collection period (0-80 min). The excretion of either compound could only be followed for 9 hours, as nothing was detected in the 9 to 24 h collection period. The percentage of quercetin recovered unchanged in urine amounted to 0.65 ± 0.14% (mean ± SE), or 624 ± 136 μg. Thus, it showed great variation and individual values ranged between 168 and 1565 μg. 7.44 ± 1.20% of the dose administered was excreted as conjugated quercetin in 9 hours.

After oral administration of quercetin 4 g to 4 volunteers, no quercetin or quercetin conjugate was detected in the urine of any subject at any time after ingestion.

3. Recovery from Faeces

Analysis of faeces for quercetin for 72 hours following oral administration of a single dose of 4 g showed that 53 ± 5% of the dose was recovered. Examination of the next stool passed after 72 hours revealed no (subject 3) or only traces (subject 1) of additional quercetin.

4. Protein Binding

Protein binding as measured by the gel filtration technique, exceeded 98% in four experiments. The sensitivity of the method did not permit measurement of concentrations of less than 0.1 μg/ml, so that binding of more than 98% could not be accurately defined.

5. Kinetic Data

Pharmacokinetic data calculated from plasma levels and urinary excretion after intravenous administration of quercetin 100 mg are listed in Table 2. In all subjects in the study, the plasma level-time curve could be fitted by a two-exponential equation of the form:

$$c = Ae^{-\alpha t} + Be^{-\beta t}$$

where A and B are the y-intercepts of the semilogarithmic regression lines of the first and second slopes at zero time, and α and β are the rapid and slow rate constants of plasma elimination, respectively. The curves seem to be consistent with an open two-compartment model (14).

The early rapid decay with a mean half life of 9 min most likely represented the distribution phase of the drug in the body. The second part of the curve, although in some cases only followed for 160 or 320 min, appeared to characterize the overall elimination of quercetin with a mean half life of 2.4 hours. With the exception of one subject (no 4), there was little variation in the half life (range 2.2 to 2.8 hours).

The volume of distribution for a two-compartment open model can be calculated from the equation

$$Vd = \frac{D}{AUC \times k_{el}}$$

(15), where D is the dose entering the body, AUC is the area under the plasma level-time curve calculated for $t = \infty$, and k_{el} is the overall elimination rate constant, which is not identical with the elimination rate constant β of the second slope, but is calculated by

$$k_{el} = \frac{A + B}{\frac{A}{\alpha} + \frac{B}{\beta}}$$

The absolute volume of distribution was 24.4 ± 3.3 l, and the relative V_d was 0.34 ± 0.03 l/kg.

The total body plasma clearance of quercetin in normal subjects, calculated by $Cl_{tot} = V_d + k_{el}$, was high and corresponded to a mean of 571 ml/min (Table 2). Renal clearance, by Wagner's method (16), was 4.6 ± 1.2 ml/min. The difference between total body clearance and renal clearance can be regarded as the metabolic clearance, provided there was no route of elimination other than metabolism and urinary excretion.

DISCUSSION

The present study in 6 volunteers shows that disappearance of quercetin from plasma after a single intravenous dose was apparently biexponential with a mean terminal half life of 2.4 hours. Although an additional deep compartment cannot be conclusively excluded, its existence seems unlikely, since, at least in one subject (no 4), the plasma level-time curve was followed for 9 hours without evidence of a third slope. The method and dose used in this study mean that multiple dosing would be necessary for absolute proof of this statement. No relationship between plasma concentration and pharmacological effect has ever been established for flavonoids, so it is not known if plasma levels below 0.5 µg/ml, as found, for example, 80 min after an 100 mg dose would still be effective.

The decline of the plasma level curve after the distribution phase seemed to be due mainly to metabolism of the drug, less than 1% of the dose administered being excreted unchanged in urine in most subjects (Table 2). Biliary excretion has been shown to be an additional major route of elimination for several flavonoids (2, 17, 18). For example, in the biliary cannulated rat, 33-44% of an intravenous dose of catechin was recovered in the bile in 24 hours (18), whereas rutin, a glycoside of quercetin, was not detectable at all in bile after parenteral or oral administration. The role of biliary excretion in the elimination of quercetin still needs to be determined.

Results obtained in the present study demonstrated conjugation as one route of metabolism of quercetin in man, the amount of the conjugate in urine being 7.4% in 9 hours. It should be emphasized that conjugates are still the only flavonoid metabolites that have been isolated, the origin of which can clearly be attributed to biotransformation in tissues. Other reported metabolites of flavonoids are all ring fission products, which are now known to be mainly, if not wholly, breakdown products of intestinal microflora (2-4, 6, 19, 20). If biliary excretion of quercetin occurred to a significant extent, intestinal formation of ring fission products might also occur after intravenous administration, leading to possible reabsorption and urinary excretion of the metabolites. Further studies of the metabolism of quercetin in man are needed to show whether any of the metabolites are pharmacologically active.

When Clark and MacKay in 1950 (9) were unable to detect any quercetin or rutin in urine after their oral administration, the findings were interpreted as due to use of a rather insensitive and unspecific method of analysis. The question of enteral absorption of flavonoids in man has never been seriously examined since, with the exception of one study on urine analyses for catechin by Das (8). Most of the animal experiments and many clinical studies on the effectiveness of flavonoids have been performed with parenterally administered compounds, and the fact that flavonoids are almost exclusively prescribed as oral preparations has always been neglected.

The results of the present study clearly demonstrate that unchanged quercetin does not reach the general circulation at all, or only in negligible amounts, in man. The sensitivity of the method used would permit measurement of quantities of more than 200 µg in a 24 hour urine sample, which is equal to 0.005% of an oral dose of 4 g. Since 0.5% of intravenously administered quercetin was excreted unchanged, absorption of 1% or more of the oral dose should still have resulted in measurable urine concentrations of quercetin, assuming identical metabolism after either route of administration. It should not be doubted that intestinal breakdown products of quercetin may be absorbed and excreted as such or in a further metabolized form in urine. There is also a possibility of a substantial first-pass effect leading to metabolism of quercetin in the gut wall during absorption of the drug, or during its first passage through the liver. If the resulting metabolites were quercetin conjugates, it should still have been possible to detect at least some of them in the hydrolyzed urine after an oral dose of 4 g of the parent compound.

Preliminary results with other flavonoids indicate that they are present

in urine, at least in some subjects, following oral dosing. Hydroxyethylrutosides are absorbed better, probably due to their better solubility characteristics in water and organic solvents (21).

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