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RESEARCH PAPER

Disposition of the Flavonoid Quercetin in Rats After Single Intravenous and Oral Doses

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ABSTRACT

The pharmacokinetic and mean time tissue distribution parameters, after a single 50-mg/kg dose of quercetin administered as intravenous bolus, oral solution, and oral suspension, were determined using rat as an animal model. Following intravenous administration, the elimination rate constant and the elimination half-life were found to be 0.0062 min^{-1} and 111 min, respectively. Examining the mean time tissue distribution parameters reflected a strong binding affinity of the drug molecules to both plasma and tissue proteins. In addition, the low permeability rate of drug molecules in the peripheral system was demonstrated. Following the oral administration of the drug, the extent of absorption was greater from solution than from suspension. Moreover, the solution showed a shorter T_{max} and a higher C_{max} than suspension. The absolute bioavailability for the solution was 0.275 and that for suspension was 0.162. The mean residence time (MRT) and the mean absorption time (MAT) were higher for suspension, reflecting the need for dissolving the drug in order to be absorbed. The mean (in-vivo) dissolution time ($MDT_{in\text{-}vivo}$) was 34.5 min. Thus, an oral quercetin formulation that can readily form a drug solution in the gastrointestinal tract may enhance the absorption of the drug.

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1. INTRODUCTION

Flavonoids are polyphenolic compounds widely distributed in plants and thus present in vegetables normally consumed by humans. There is a growing interest in the potential beneficial effects on health of these nonnutritional components. Flavonoids have multiple chemical and biological actions, including antioxidant,[1–3] chilation,[4] anticarcinogenic,[5] and secretory activities.[6,7] Furthermore, some flavonoids may act as antiviral, antitumor, anti-inflammatory, and antiallergenic agents.[8–10] One flavonoid, quercetin, is of particular interest because of its significant quantitative presence in human foods. The highest concentrations of quercetin occur in apples, onions, and tea.[11,12] Quercetin has been used with therapeutic intent to treat inflammation, allergy, bee sting, ulcer,[13] and varicosity.[14] Studies have been reported that quercetin exerts powerful antiproliferative activity on tumor cells of human breast,[15] leukemia,[16] gastrointestinal,[17] and ovary[18] at micromolar concentrations. All these reports suggest that quercetin could be a compound with potential medical applications.

Essential for the ability of quercetin to exert its action, in vivo, is the extent of its absorption after ingestion and its ability to be distributed in various body tissues. The absorption of quercetin constitutes an area of research that still not fully investigated. In one study, absorption of quercetin, in man, was determined to be about 24% of the orally administered drug solution.[19] This is in contrast to the observation of poor absorption (less than 1%) in human volunteers administered 4 g of the drug in the form of capsules.[20] As the drug has poor water solubility,[21] these conflicted data may result from the differences in the dissolution characteristics of the given dosage forms.

The objective of this study was to evaluate the bioavailabilities of quercetin formulated as solution and suspension dosage forms. In this regard, the intravenous (I.V.) administration of the drug was essential and permitted the determination of its pharmacokinetic parameters and tissue distribution characteristics. Rats were utilized as an animal model because they have been frequently used in previous dietary, carcinogenic, and other studies.[22–24] The determination of drug plasma levels was carried out using a sensitive high-performance liquid chromatography (HPLC) assay method developed in our laboratory.[25]

2. MATERIALS AND METHODS

2.1. Materials

Quercetin dihydrate (Winlab, Middlessex, UK), HPLC grade kaempferol (Fluka BioChemika, Switzerland), HPLC grade methanol, HPLC grade acetonitrile, glacial acetic acid, perchloric acid, polyethylene glycol 200 (PEG 200), dimethyl sulfoxide, ethanol, glycerin, and sodium carboxymethylcellulose (Na CMC) were obtained from BDH Chemicals Ltd., Poole, UK. Water was a Milli-Q quality.

A volume of 0.1 mL of 35% perchloric acid was added to 10 mL of methanol to prepare acidified methanol used for deproteinization of plasma.

2.2. Quercetin Formulations

A weighed amount of 400 mg quercetin dihydrate was dissolved in 10 mL of a solvent mixture consisting of 30% v/v dimethyl sulfoxide and 70% v/v PEG 200. A volume of 0.35 mL of the prepared solution, containing 12.5 mg quercetin, was injected intravenously to each rat. Control experiment was carried out by injecting an equal volume of drug-free solvent mixture.

A weighed amount of 840 mg quercetin dihydrate was levigated, in a porcelain mortar, with 1 mL of glycerin. The resulting slurry was further triturated and gradually diluted by the addition of the suspending agent (0.67% w/v Na CMC aqueous solution). The volume was completed to 30 mL with the suspending agent. Each rat was orally administered 0.5 mL of the freshly prepared suspension via a stainless steel buccogastric tube. Control experiment was conducted.

A solvent system consisting of 10% v/v ethanol and 90% v/v PEG 200 was used to dissolve the drug. A weighed amount of 390 mg quercetin dihydrate was dissolved in 25 mL of the solvent system. Each rat was administered 0.9 mL of the solution via a stainless steel buccogastric tube. Control experiment was also performed.

2.3. Animals and Drug Administration

Male Sprague–Dawley rats weighing 250 g were used. The animals were maintained under conditions of constant temperature, humidity, and light cycle and fed a standard diet. The rats were fasted overnight before the experiment, and continued fasting 6 h postdose, but with free access to water. At the time of administration, the rat was anesthetized lightly with ether. At each sampling time, the rat was reanesthetized and the required amount of blood was withdrawn by means of a cardiac puncture. The blood was collected into a heparinized glass tube. The rat was then sacrificed. Six rats were used at each time point. Plasma was separated, after centrifugation of blood samples for 10 min at 3000 rpm (Mistral 1000, MSE, UK), and stored at -20°C pending analysis.

2.4. Analytical Method

Quercetin plasma levels were determined by the reverse phase HPLC method described in our previous report.[25] Briefly, a high-performance liquid chromatograph (Shimadzu, Koyato, Japan) equipped with LC-10A pump connected with a stainless steel Novopak C18 column (average particle size of 4 μm, 3.9 mm i.d. × 150 mm), a guard column of the same packing material, Rheodyne injector (California, USA) fitted with 100-μL loop, and 481 UV/VIS variable wavelength detector (Waters, Milford, MA, USA) was used. A mixture of acetonitrile and 5% acetic acid (30:70 v/v) was used as the mobile phase. The mixture was filtered through a 0.22-μm membrane (Millipore, Bedford, MA, USA) under vacuum, then degassed by flushing with nitrogen for 5 min. The mobile phase was pumped isocratically at a flow rate of 1.0 mL/min at 25°C. The chromatograms were recorded and integrated at a chart speed of 0.25 cm/min. The effluent was monitored at 375 nm using an attenuation level of 8.

Plasma aliquot of 200 μL was mixed with 20 μL of the internal standard (methanolic solution of kaempferol 130 μg/mL) in a vortex mixer for 15 s. Precipitation of plasma proteins was accomplished by the addition of 400 μL of acidified methanol, shaken on a vortex mixer for 5 min, and centrifuged at 12,000 rpm for 5 min. A volume of 100 μL of the supernatant was immediately injected into the system. The average drug/internal standard peak area ratio, of two injections from each plasma sample, was determined. The concentration of the drug was calculated from a preconstructed calibration curve that was linear in the range of 0.06–15 μg/mL. Plasma samples that were found to contain higher concentrations of the drug were appropriately diluted with blank plasma.

2.5. Pharmacokinetic Analysis

Pharmacokinetic parameters for quercetin were determined from the plasma concentration–time data. Following I.V. administration, the drug plasma levels were fitted to a two-compartment open model. The parameters derived from the model (A, α, B, and β) were calculated.

The plasma–concentration time data collected after oral administration of the drug were used to calculate the following parameters:

1. The maximum plasma concentration (C_{max}) and the time to reach this maximum (T_{max}) were obtained directly from the concentration–time profile.
2. The area under the plasma concentration–time curve up to the last time (t_{last}) showing a measurable concentration (C_{last}) of the analyte (AUC_t) was determined using the linear trapezoidal rule.
3. The apparent elimination rate constant was calculated using the technique of the least-squares regression of the data for the last three points of the plasma concentration–time curve.
4. The $AUC_{0-\infty}$ was determined by adding the quotient of C_{last} and the appropriate k_{el} to the corresponding AUC_t. The sampling period covered, on average, more than 80% of the total AUC.
5. The area under the first moment curve ($AUMC_t$) was estimated according to the linear trapezoidal rule and extrapolated to infinity using the following equation:

$$AUMC_{\infty} = AUMC_t + t_{last} \cdot C_{last}/k_{el} + C_{last}/(k_{el})^2$$

The mean residence time (MRT) was calculated from the area under the moment curve divided by the area under the curve. The mean absorption time (MAT) was calculated as the difference between MRTpo and $MRT_{I.V.}$, where MRTpo is the mean residence time after oral administration, and $MRT_{I.V.}$ is the mean residence time after I.V. administration. The mean (in-vivo) dissolution time ($MDT_{in\text{-}vivo}$) was

calculated as the difference between MAT_{SUSP} and MAT_{SOL}.[26,27]

The total body clearance (Cl/F) and the apparent volume of distribution at steady state (Vd_{ss}/F) were calculated using the following equation[28]:

$$Cl/F = dose/AUC_{\infty}$$

$$Vd_{ss}/F = (dose \cdot AUMC_{\infty})/(AUC_{\infty})^2$$

3. RESULTS AND DISCUSSION

3.1. Intravenous Administration

Quercetin was administered intravenously as a solution using a solvent system consisting of PEG 200:DMSO (70:30 v/v). This solvent mixture allowed the preparation of a concentrated solution with a minimum precipitation of drug after injection. In addition, using a low percentage of DMSO minimized its hemolytic and hypotensive effects. Therefore, the injected rats showed full activity and no mortality for over a 24-h period postinjection. Moreover, minimum hemolytic product peaks appeared in the HPLC trace, allowing a better quantitation of the drug without a real need to perform sample purification as it was reported.[21]

Plasma concentration–time data are listed in Table 1. Each time-point data represents the average obtained from six rats. The data were fitted to a two-compartment model and the calculated pharmacokinetic parameters are given in Table 2. After injection of the drug, there was a rapid decline in the plasma level over the first 45 min (distribution phase),

Table 1. Quercetin plasma concentration–time data collected after I.V., oral solution, and oral suspension administration of 50 mg/kg dose to rats.

	Plasma concentration[a] (μg/mL)		
Time (min)	Intravenous injection	Oral solution	Oral suspension
5.0	47.88 ± 28.19 (16.14–70.54)	NS	NS
10.0	39.51 ± 28.19 (32.23–50.76)	NS	NS
15.0	25.31 ± 8.37 (17.54–38.16)	0.39 ± 0.06 (0.32–0.46)	NS
20.0	NS	0.45 ± 0.07 (0.33–0.53)	NS
30.0	7.60 ± 4.53 (3.98–16.12)	0.59 ± 0.08 (0.44–0.72)	0.095 ± 0.05 (ND–0.99)
45.0	1.84 ± 0.34 (1.5–2.19)	0.76 ± 0.09 (0.62–0.93)	0.140 ± 0.07 (ND–0.15)
60.0	0.34 ± 0.07 (0.29–0.49)	0.92 ± 0.13 (0.79–1.21)	0.31 ± 0.14 (0.16–0.53)
90.0	0.31 ± 0.02 (0.29–0.33)	0.99 ± 0.17 (0.79–1.35)	0.44 ± 0.21 (0.17–0.72)
120.0	0.24 ± 0.09 (0.14–0.36)	1.04 ± 0.23 (0.80–1.45)	0.55 ± 0.24 (0.20–0.94)
180.0	0.15 ± 0.04 (0.10–0.16)	0.89 ± 0.14 (0.76–1.1)	0.61 ± 0.23 (0.25–1.01)
240.0	0.12 ± 0.03 (0.09–0.17)	0.59 ± 0.11 (0.43–0.75)	0.47 ± 0.08 (0.20–0.75)
300.0	0.09 ± 0.02 (0.08–0.11)	0.39 ± 0.09 (0.30–0.46)	0.30 ± 0.18 (0.14–0.6)
360.0	NS	0.26 ± 0.06 (0.18–0.35)	0.20 ± 0.16 (0.09–0.31)

ND = Not detected.
NS = No sample was collected.
[a]Each value represents the mean ± SD of six rats (range is given in parentheses).

Table 2. Pharmacokinetic parameters of quercetin after intravenous administration of 50 mg/kg dose to rats.

Parameter	Value	Parameter	Value
A (μg/mL)	79.51	Vd_{ss} (mL/kg)	1155.5
α (min^{-1})	0.0848	MRTB (min)	23.53
B (μg/mL)	0.502	MRTC (min)	12.72
β (min^{-1})	0.00624	MRTP (min)	10.81
$AUC_{0-\infty}$ (μg min/mL)	1,018	IMRTP (min)	164.65
$AUMC_{0-\infty}$ (μg min^2/mL)	23,949	MTTP (min)	153.84
Cl (mL/min/kg)	49.12	Cl_d (mL/min/kg)	3.45

followed by a second and less rapidly declining phase (elimination phase). The plasma levels achieved 5 min after injection were in the range of 16.14 to 70.54 μg/mL, with plasma levels above 0.1 μg/mL being maintained up to 6 h.

The mean time (MT) parameters dealing with the tissue distribution of quercetin were calculated from the I.V. data as described.[29] The so-called "peripheral bioavailability" of the drug may be evaluated by examining the various mean time parameters in terms of the tendency of the drug molecules to distribute into and remain in the peripheral system. The mean residence time of drug in the body (MRTB = 23.53 min) is the sum of the mean residence time in the peripheral tissue (MRTP = 10.81 min) and the mean residence time in the central compartment or circulation (MRTC = 12.72 min). The calculated parameters indicate that quercetin molecules tend to spend slightly greater time (54% of its MRTB) in the central compartment than in the peripheral system. This finding may reflect equal binding affinities of the drug molecules to both plasma and tissue proteins. It was reported that about 98% of the circulating flavonol is bound to plasma proteins.[20,23] The mean transient time of drug molecules in the peripheral tissue (MTTP = 153.84 min) is the mean time it takes drug molecules to return to the general systemic circulation (central compartment) subsequent to entering the peripheral tissue. The high value of MTTP for quercetin, relative to other drugs,[30] therefore, possibly reflects the strong tissue binding of the drug. The intrinsic mean residence time in the peripheral tissue (IMRTP = 164.65 min) describes the average total time molecules which have distributed in the tissue spend in the peripheral tissue before being eliminated, centrally or peripherally, from the body. As expected, IMRTP is larger than MTTP. Finally, the probability of distribution, which is defined as the probability that a drug molecule which arrives in the central compartment will become distributed in the peripheral tissue, was found to be very low (0.066), which may imply a low relative permeability rate for the drug molecules in the peripheral system. In conclusion, the examining of the various peripheral MT parameters indicates strong plasma and tissue protein binding of the drug molecules. In addition, once the drug molecules have been distributed in the peripheral tissue, they will be retained and experience great difficulty in returning back to the central compartment due to low permeability rate.

3.2. Oral Administration

Quercetin was given orally in two different formulations, namely, solution and suspension. Assuming that no precipitation occurred, after the administration of the solution, the dissolution step, prerequired for drug absorption, may be bypassed. Hence, the differences in the bioavailability that might appear in the plasma levels could be attributed to the differences in the dissolution characteristics of the two dosage forms. Figure 1 shows the mean plasma drug concentration vs. time profiles after the oral administration of 50 mg/kg dose either in solution or in suspension. The mean pharmacokinetic parameters following these administrations are listed in Table 3. As it was decided not to collect more than one blood sample from each rat in order to keep the hemodynamics of the animal unaffected, no statistical analysis for the pharmacokinetic parameters could be performed.

A peak concentration was reached in 120 min for the solution, while it took 180 min for the suspension, indicating faster absorption of quercetin from solution. However, the value of the ratio of $C_{max}/AUC_{0-\infty}$ was found to be the same for the two oral dosage forms.

The variability, in terms of the standard deviation, from the mean was much less in the solution.

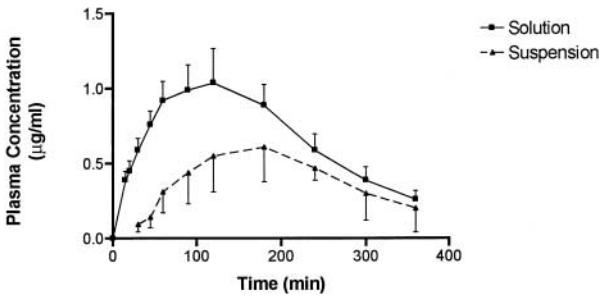

Figure 1. Mean plasma–time profile of quercetin (± SD) following the oral administration of 50 mg/kg of drug solution or suspension to six rats.

Table 3. Pharmacokinetic parameters of quercetin following oral administration of 50 mg/kg dose to rats as solution or suspension.

Parameter	Solution	Suspension
C_{max} (μg/mL)	1.04	0.61
T_{max} (min)	120	180
AUC_t (μg min/mL)	243.4	138.3
$AUC_{0-\infty}$ (μg min/mL)	280	165.2
$AUMC_{0-\infty}$ (μg min^2/mL)	56,770	39,150
C_{max}/ $AUC_{0-\infty}$ (min^{-1})	0.0037	0.0037
K_{el} (min^{-1})	0.0070	0.0073
$t_{½\beta}$ (min)	99	95
MRT (min)	202.8	237
MAT (min)	179.3	213.5
F (absolute)	0.275	0.162
Cl/F (mL/min/kg)	178.6	302.7
Vd_{ss}/F (liter/kg)	36.2	71.7

The results also show that the $AUC_{0-\infty}$ (expressing the magnitude of absorption) is greater for the solution. The absolute bioavailability (F absolute) for the solution was 0.275, whereas that of the suspension was 0.162. The relative bioavailability of the suspension formulation to that of the solution was found to be about 59%.

The mean residence times, $MRT_{I.V.}$, MRT_{SUSP}, and MRT_{SOL}, were found to be 23.5, 237, and 203 min, respectively. The delayed absorption of quercetin following the administration of suspension (longer T_{max}) might explain the increase in the value of MRT_{SUSP} compared to that of MRT_{SOL}. This claim is supported by the fact that the calculated elimination rate constants for the three administrations remain almost unaltered. This was further reflected in the MAT parameters; the MAT_{SUSP} and MAT_{SOL} were found to be 213.5 and 179 min, respectively. The MAT_{SUP} is about 1.2 times greater than that of solution, which demonstrates a longer period for absorption of quercetin from suspension. The mean (in-vivo) dissolution time, $MDT_{in\text{-}vivo}$, was found to be 34.5 min.

Based on the obtained results, it is believed that enhancing the dissolution characteristics of quercetin, in order to form a nonprecipitatable solution having a relatively high concentration, can exert a significant influence on both bioavailability and clinical effects of the drug.

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