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Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 417–422
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Pharmacokinetics and metabolism of 1,5-dicaffeoylquinic acid in rats following a single intravenous administration

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Received 19 April 2005; received in revised form 6 June 2005; accepted 15 June 2005
Available online 6 September 2005

Abstract

1,5-Dicaffeoylquinic acid (1,5-DCQA) is a potentially important HIV-1 integrase inhibitor widely distributed in many plants. To characterize the pharmacokinetic and metabolic properties of 1,5-DCQA in rats following single intravenous administration (160 mg/kg), the plasma concentrations of 1,5-DCQA were measured by high-performance liquid chromatography (HPLC) and the metabolites formed in urine were identified by liquid chromatography–mass spectrometry (LC–MS) in parallel to diode-array detection (DAD). The results showed that the concentrations of 1,5-DCQA in plasma declined rapidly in a biphasic manner with a mean terminal half-life ($t_{1/2}$) of 1.40 h. The mean clearance (CL) and the apparent volume of distribution (Vd_B) of 1,5-DCQA were 0.44 l/h/kg and 0.89 l/kg, respectively. A total of 15 metabolites in rat urine were identified, including four isomeric *O*-mono-methylated (M1–M4), six isomeric *O*-di-methylated (M5–M10), one isomeric *O*-mono-methyl-glucuronidated (M11) and four isomeric *O*-di-methyl-glucuronidated (M12–M15) metabolites. The *O*-methylation positions of three important metabolites (M1, M2 and M5) were determined (3″-, 3′-, and 3′,3″-) by comparing with synthesized standards. These results suggested that the disappearance of 1,5-DCQA from plasma was rapid, and that its quick urinary excretion and extensive metabolism, including methylation and glucuronidation, were two factors causing its rapid elimination from the circulation.

Keywords: 1,5-Dicaffeoylquinic acid; Pharmacokinetics; Metabolism; High-performance liquid chromatography; Liquid chromatography–mass spectrometry

1. Introduction

Dicaffeoylquinic acids (DCQAs) are a class of natural polyphenolic compounds widely distributed in many plants [1–5]. Structurally, they are characterized by two caffeic acid molecules connected to one quinic acid molecule through ester bonds.

Current pharmacological agents for human immunodeficiency virus (HIV) infection include drugs targeted against HIV reverse transcriptase and HIV protease. The DCQAs are a potentially important class of HIV inhibitors and act at a site distinct from that of the current HIV therapeutic agents. Their action target is on the integrase [6–10] that is an essential enzyme mediating integration of the HIV genome into the host chromosomes. As important lead compounds for HIV drug discovery, the DCQAs have drawn more and more attention in the development of the therapy of HIV infection in the past 10 years.

Drug metabolism plays an important role throughout the drug discovery and development process. Therefore, it is important to identify the circulating metabolites of drug after administration. Recently the metabolism of 1,5-dicaffeoylquinic acid (1,5-DCQA, Fig. 1) in rats after oral dosing was studied. The results showed that 1,5-DCQA could be efficiently *O*-methylated and glucuronidated with the formation of a large number of metabolites in blood, bile and urine, and that the enzymes involved were COMT (catechol-*O*-methyltransferase) and UGTs (UDP-glucuronosyltransferases) in both small intestine and liver

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0731-7085/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.jpba.2005.06.037

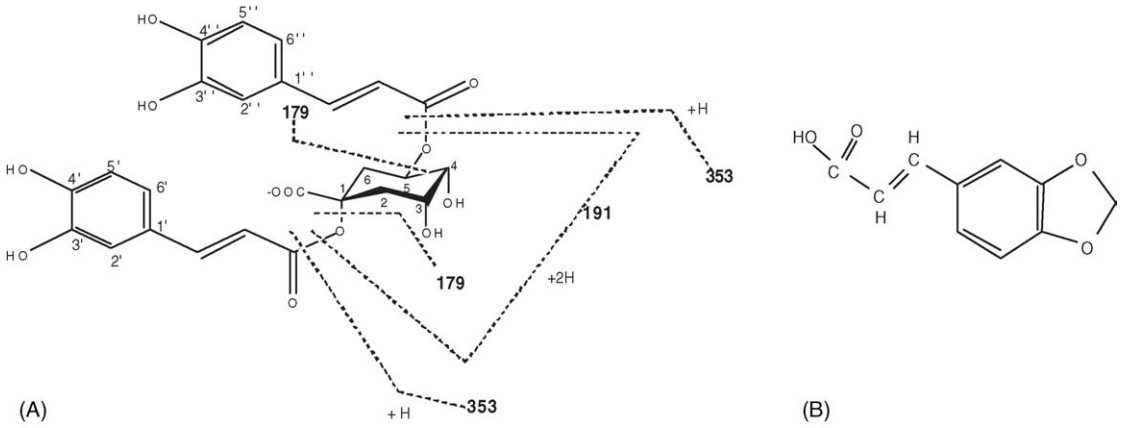

Fig. 1. Chemical structures of 1,5-DCQA (A) and methylenecaffeic acid (B) (internal standard).

[11]. COMT, using *S*-adenosyl-L-methionine as the methyl donor, plays an important role in the metabolic *O*-methylation of catechol-containing compounds, including endobiotic and xenobiotic catechols [12].

By far, the metabolic fate of 1,5-DCQA in rats after intravenous injection has not been investigated. In addition, up to now no study is available on the pharmacokinetic behavior of the DCQAs. If any, only Takenaka et al. reported that no parent compound could be detected in plasma when 3,5-dicaffeoylquinic acid (3,5-DCQA) was orally administered to rats, and if by intravenous injection 90% of it disappeared from plasma within 30 min [13]. The purpose of the present study was to explore the pharmacokinetic and metabolic behavior of 1,5-DCQA in rats after intravenous administration, which might help to provide an insight into the pharmacokinetic properties of other DCQAs.

2. Experimental

2.1. Chemicals

1,5-DCQA, methylenecaffeic acid (internal standard) and the synthesized standards of M1, M2 and M5 were provided by Beijing Institute of Radiation Medicine (Beijing, China) and authenticated at the National Center of Biomedical Analysis (Beijing, China). Acetonitrile was HPLC grade. All other chemicals were analytical grade and used without further purification.

2.2. HPLC analysis

The Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) consisted of a quaternary pump, an autosampler and a mobile phase vacuum degassing unit. HPLC separation was done by an Agilent XDB-C_{18} column (5 μm, 250 mm × 4.6 mm i.d.). The mobile phase was sodium phosphate buffer (pH 5.0, 52 mM)–acetonitrile (85:15, v/v). The analysis was carried out at a flow rate of 1 ml/min with wavelength of 326 nm and column oven temperature at 30 °C.

2.3. HPLC method validation

Stock solutions of 1,5-DCQA and methylenecaffeic acid (Fig. 1B) were prepared as 5 mg/ml solution in mobile phase and 125 μg/ml solution in water (pH 8.0), respectively, which were stored at 4 °C. The calibration standard samples (0.015, 0.05, 0.2, 0.8, 8, 20 and 40 μg/ml) were prepared by spiking blank plasma with appropriate amounts of the mentioned stock solution. Calibration graphs were constructed by plotting peak–area ratios of 1,5-DCQA to internal standard against nominal concentrations of 1,5-DCQA. The calibration plot was fitted by robust least square fitting using $1/C^2$ weighting. Quality control (QC) samples to determine the precision and accuracy were independently prepared at low (0.015 μg/ml), medium (0.2 and 8 μg/ml) and high (40 μg/ml) concentrations in the same manner. Intra-day precision and accuracy were determined by repeated analyses of QC samples in the same day ($n = 6$), and inter-day precision and accuracy were determined by repeated analyses of QC samples on three consecutive days ($n = 1$ series/day). Relative standard deviations (R.S.D.s) were calculated as the intra- and inter-day precision. The accuracy was defined as the relative error in the calculated value of a standard from that of its true value expressed as a percentage. Samples with the concentrations beyond the higher limit of quantitation were diluted 15 times with blank plasma. The influence of dilution on the quantification of 1,5-DCQA was determined. The calibration curves along with QC samples were prepared separated for each HPLC run.

2.4. LC–MS analysis

Ion trap-based LC–ESI–MS was performed on a Finnigan (Thermo Electron Corporation, Waltham, MA) LCQ system connected to a Finnigan Surveyor HPLC pump equipped with a Finnigan Surveyor DAD. Electrospray and HPLC

separation was accomplished using the settings previously described, where the elution was performed using a gradient from 5% solvent A (CH_3CN) to 75% solvent B (ammonium acetate buffer, 5 mM, pH 5.0) in 30 min and held until 42 min at a flow rate of 0.4 ml/min [11].

2.5. Sample preparation

Plasma or urine sample (200 μl), 15 μl of internal standard solution (125 μg/ml, only for plasma samples) and 5 μl of 7.2N HCl were added into a test tube, followed by vortexing for 20 s. The solution was then extracted with 400 μl of ethyl acetate by vortexing vigorously for 2 min. After centrifugation at 2500 × *g* for 10 min at 4 °C, the supernatant was transferred into an umber colored vial and evaporated to dryness under a nitrogen stream at room temperature. The residue was then reconstituted with 200 μl of the corresponding mobile phase, and an aliquot (20 μl) was injected into the Agilent HPLC system for pharmacokinetic study or into the LC–MS system for metabolic study. In addition, an aliquot of the urine sample prepared was also injected into the Agilent HPLC system using the HPLC separation conditions as described in Section 2.4.

2.6. Animal experiments

Healthy male Wistar rats ($n = 8$, 0.20 ± 0.01 kg) were provided by the Institute of Jingfeng Medical Animal Center (Beijing, China). Prior to the experiment the rats were housed in a temperature-controlled room (22 °C) and maintained in a reverse 12 h light/dark cycle with free access to food and water. In the study day, the rats were all firstly received slight anaesthesia by ether, and then received sterile and fresh solution of 1,5-DCQA dissolved in sodium phosphate buffer (0.1 M, pH 7.2) by lingual vein. In the pharmacokinetic study, blood samples (0.5 ml) were collected via rat suborbital vein ($n = 4$) at 0 (predose), 5, 15, 30 min and 1, 1.5, 2, 4, 6, 9 and 12 h after single intravenous dosing (160 mg/kg), followed by centrifugation at 2000 × *g* for 10 min to prepare the plasma, which were analyzed immediately. For blood volume compensation, 0.5 ml of saline was intraperitoneally injected following each sampling. In the metabolic study, the rats ($n = 4$) were housed in individual metabolism cages during the study period. Urine samples were collected between 0 and 12 h post-single intravenous administration (160 mg/kg). Sample aliquots collected from each animal were pooled and stored at −20 °C until analysis.

2.7. Data analysis

Plasma concentrations were calculated using the Excel program (Microsoft Corp., USA). Pharmacokinetic parameters were determined using non-compartmental methods. All data were averaged and reported as mean ± S.D. The area under the plasma concentration versus time curve up to the last quantifiable time point, AUC_{0-t}, was calculated by the linear trapezoidal rule. The AUC_{0-t} was extrapolated to infinity ($AUC_{0-\infty}$) by adding the quotient of C_t/K_e, where C_t represented the last measurable time concentration and K_e represented the elimination rate constant. K_e was determined by linear regression of the terminal log-linear phase of the concentration versus time curve points (number of points = 5 in all animals). $t_{1/2}$ was calculated as $0.693/K_e$. CL was calculated as the dose divided by the plasma $AUC_{0-\infty}$. The mean residence time (MRT) was calculated from the ratio of total area under the first moment of the drug concentration curve ($AUMC_{0-t}$) to AUC_{0-t}. Vd_B was calculated as clearance/K_e.

3. Results

3.1. HPLC method validation

No interferences from endogenous substances were found in rat plasma. The retention times for 1,5-DCQA and the internal standard were approximately 4.7 and 12.5 min, respectively. The calibration curves showed good linearity over a wide range of 0.015–40 μg/ml with excellent linearity ($r^2 > 0.99$). The lower limit of quantitation was 0.015 μg/ml. The intra- and inter-day R.S.D.s were 4.06–9.61% and 5.85–10.06%, respectively. The intra- and inter-day accuracies were −2.49–6.73% and 3.69–6.00%, respectively. There was no influence of the dilution on the quantification of 1,5-DCQA in samples with high concentrations beyond the higher limit of quantitation.

3.2. Pharmacokinetic study

The mean plasma concentration versus time profile for 1,5-DCQA after single intravenous dose of 1,5-DCQA is presented in Fig. 2, and the pharmacokinetic parameters are summarized in Table 1. 1,5-DCQA was eliminated from

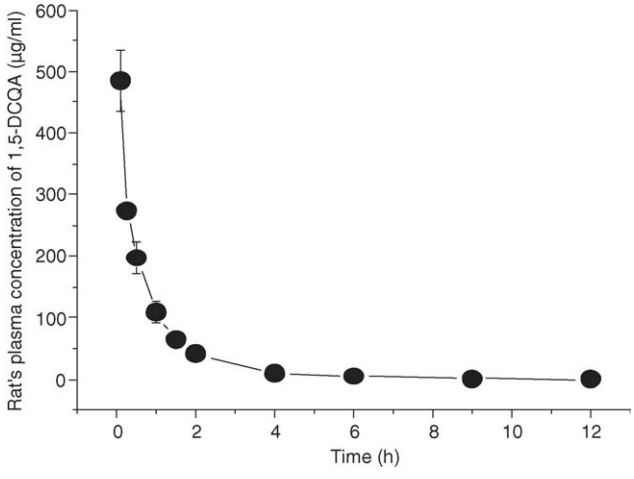

Fig. 2. Mean plasma concentration–time profile of 1,5-DCQA after intravenous administration at a dose of 160 mg/kg ($n = 4$).

Table 1
Summary of the mean (±S.D.) pharmacokinetic parameters of 1,5-DCQA in rat following intravenous administration (160 mg/kg)

Parameters	Values (mean ± S.D.)
$AUC_{0-\infty}$ (mg h/l)	367.80 ± 42.24
K_e (h^{-1})	0.50 ± 0.03
$t_{1/2}$ (h)	1.40 ± 0.09
$MRT_{0-\infty}$ (h)	1.20 ± 0.13
CL (l/h/kg)	0.44 ± 0.06
Vd_B (l/kg)	0.89 ± 0.17

plasma with mean $t_{1/2}$ value of 1.40 h. The mean CL and Vd_B values were 0.44 l/h/kg and 0.89 l/kg, respectively.

3.3. Metabolic study

By comparison with the blank samples, there were 15 components observed in rat urine following intravenous administration (Figs. 3 and 4), which had the typical DAD fingerprints (318–326, 240–252 and 290–300 nm) similar to that of 1,5-DCQA, suggesting that they were its potential metabolites. The retention time of each compound in Fig. 3 was all about 1.4 min later in Fig. 4, and such difference was caused by the different analysis system. Their structures were elucidated by a combination analysis of the MS and MS/MS spectra, chromatographic behavior, as well as mass and chromatographic spectral comparison to several synthesized reference substances. The main characteristic mass fragment ions of 1,5-DCQA and its metabolites are summarized in Table 2.

3.3.1. Parent drug (M0)

Negative mass spectra of M0 showed its molecular ion ($[M-H]^-$) at m/z 515, in agreement with the molecular weight of 1,5-DCQA being 516. Besides, M0 showed four characteristic MS/MS fragment ions: the deprotonated chlorogenic acid (m/z 353, $[M-H-162]^-$, the loss of a caffeic unit), quinic acid (m/z 191), caffeic acid (m/z 179) and m/z 335 ($[353-H_2O]^-$), which were the same as that of the 1,5-DCQA standard. M0 was identified as 1,5-DCQA, and confirmed by a comparison of its retention time with reference substance.

Table 2
Summary of key LC–MS^n data of the metabolites found in rat urine after intravenous dosing (160 mg/kg)

Compound	t_R (min)	MS $[M-H]^-$	MS/MS (fragment ions)
1,5-DCQA (M0)	11.7	515	353, 191, 179, 335
M1, M2	16.4	529	353, 367, 191, 179
M3, M4	17.5	529	367, 353, 191, 179
M5	19.8	543	367, 349, 193
M6	20.9	543	367, 349, 193
M7	22.1	543	349, 367
M8	26.5	543	367, 193, 349
M9	26.9	543	367, 193, 349
M10	27.2	543	349, 367
M11	9.3	705	529, 353, 367
M12	7.9	719	543
M13	11.1	719	543
M14	12.4	719	543
M15	16.3	719	543

Chromatographic and spectroscopic conditions, see Section 2.

3.3.2. Metabolites M5–M10

Metabolites M5–M10 showed the same quasi-molecular ions at m/z 543 ($[M-H]^-$) as well as identical MS/MS fragment ions (full scan) at m/z 367, 349 ($[367-H_2O]^-$) and 193, indicating that they were isomers. Their quasi-molecular ions were 28 Da higher than that of 1,5-DCQA, suggesting that they were the di-methylated metabolites of 1,5-DCQA. Their three MS/MS fragment ions (m/z 367, 349, and 193) were all 14 Da higher than those of the parent compound at m/z 353, 335, and 179, respectively, suggesting that the two methyl groups were on the two respective caffeoyl groups of 1,5-DCQA. M5 was confirmed as 1,5-*O*-diferuoylquinic acid by a comparison of its retention time and mass spectrum with reference substance.

3.3.3. Metabolites M1–M4

Although M1 and M2, like M3 and M4, were not completely separated in Fig. 4, they four showed individual four peaks in Fig. 3, with the retention times of 17.2, 17.7, 18.5 and 18.9 min, respectively. They all exhibited the same quasi-molecular ions at m/z 529 ($[M-H]^-$) as well as identical characteristic MS/MS fragments, indicating that they were isomers. Their MS/MS spectra showed four characteristic

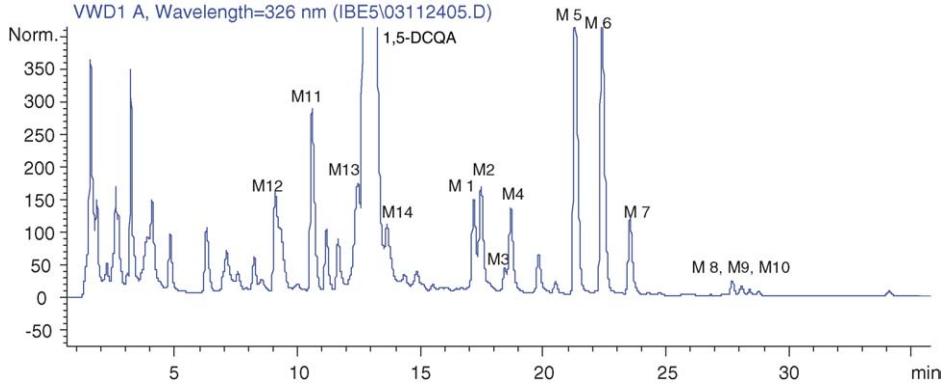

Fig. 3. HPLC–UV chromatography of 1,5-DCQA and its metabolites in rat urine (0–12 h) after 160 mg/kg intravenous dosing.

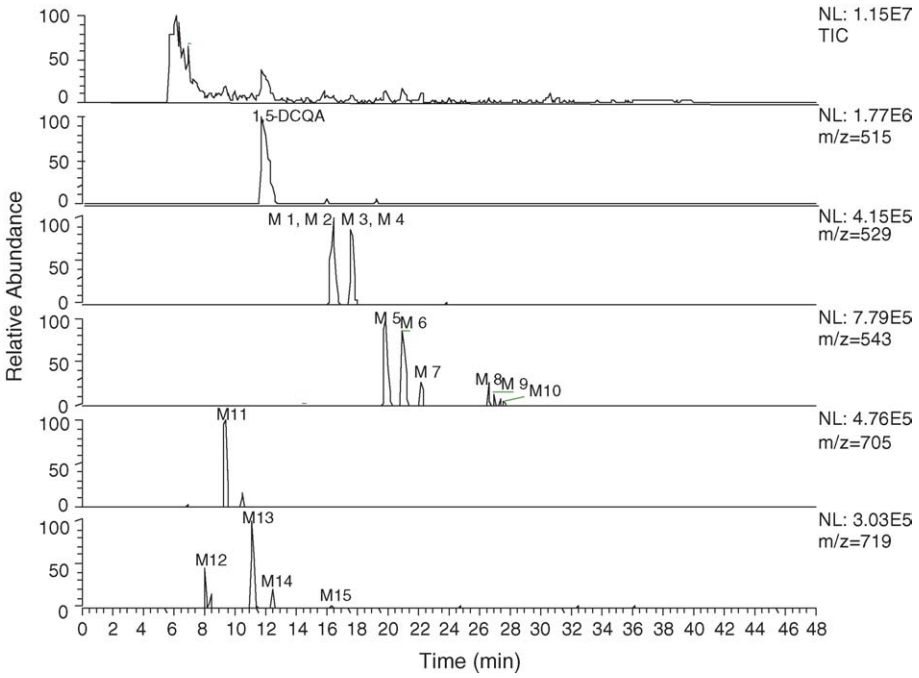

Fig. 4. Total ion current and extracted ion current chromatograms in negative-ion mode of 1,5-DCQA and the major metabolites in rat urine (0–12 h) after 160 mg/kg intravenous dosing.

fragment ions at *m*/*z* 353, 367, 179, and 191, indicating that they were the mono-methylated metabolites of 1,5-DCQA. M1 and M2 were confirmed as 1-caffeoyl-5-feruoylquinic acid and 1-feruoyl-5-caffeoylquinic acid, respectively, by a comparison of their retention times and mass spectra with reference substances.

3.3.4. Metabolite M11

It showed the quasi-molecular ion at *m*/*z* 705 as well as MS/MS fragment ions at 529 ($[M-H-176]^-$), 353, and 367. A loss of 176 Da indicated the moiety might be a glucuronide. It was tentatively identified as the mono-methyl-glucuronide conjugate of 1,5-DCQA.

3.3.5. Metabolites M12–M15

They all showed the same quasi-molecular ions at *m*/*z* 719 ($[M-H]^-$) as well as identical MS/MS fragment ions (full scan), indicating that they were isomers. Their characteristic MS/MS spectra all exhibited fragment ions at *m*/*z* 543 ($[M-H-176]^-$). They were tentatively identified as the di-methyl-glucuronide conjugates of 1,5-DCQA.

4. Discussion

In this study all metabolites identified were methylates and methyl-glucuroconjugates of 1,5-DCQA. By taking into account the respective relative retention times compared with 1,5-DCQA, and by comparing with MS and MS/MS spectra of each product, all metabolites identified in rat urine after intravenous dosing exhibited a perfect coincidence with those observed in rat urine and bile after oral dosing except M1–M4 (the mono-methylated metabolites) [11]. The reason why M1–M4 were observed in rat urine after intravenous administration while not be detected after oral dosing was that mono-methylated metabolites were the primary metabolites of 1,5-DCQA, they could act as substrates and undergo further methylation and/or glucuronidation to form the final metabolites: di-methylated, mono-methyl-glucuronidated and di-methyl-glucuronidated metabolites [11]; and when 1,5-DCQA was intravenously administrated the amount of 1,5-DCQA in the circulation was much larger than that after oral administration. The result suggested that methylation and glucuronidation were two major metabolic pathways of 1,5-DCQA in rats after intravenous dosing, which were also found in rats after oral dosing.

The HPLC method used for the quantitation of 1,5-DCQA has proven to be easy, selective, and precise, which is not only suitable for the pharmacokinetic study of 1,5-DCQA in rats, but also suitable for the study in dogs and monkeys. Because the linear dynamic range was wide (0.015–40 μg/ml), four concentration levels of QC samples were evaluated. The wide linear dynamic range allows for the monitoring of low and high dose administration of 1,5-DCQA within the same assay. After intravenous administration, the plasma concentrations of 1,5-DCQA in rats declined rapidly in a biphasic manner (Fig. 2) with a short $t_{1/2}$ value (1.40 h). The rapid elimination of 1,5-DCQA might be caused by its relative high CL (0.44 l/h/kg) and relative low Vd_B (0.89 l/kg). It had proved that the methylation and glucuronidation of 1,5-DCQA were considerably efficient and rapid (Yang et al., submitted for publication), and in the present study large numbers of methy-

lated and glucuronidated metabolites were found in rat urine (Figs. 3 and 4), suggesting that the extensive metabolism of 1,5-DCQA was one factor leading to its relative high CL value. Furthermore, large amount of 1,5-DCQA excreted in rat urine (Fig. 3) suggested that its excretion in native form might also be a factor leading to the relative high CL. As for the low Vd_B value, it might be caused by a high binding of 1,5-DCQA to rat plasma (unpublished results).

Takenaka et al. reported that 90% of 3,5-DCQA disappeared from plasma within 30 min when it was intravenously administered to Sprague–Dawley rats at a dose of 10 mg/kg [13]. Although the data obtained in the present study showed that the disappearance of 1,5-DCQA in Wistar rat plasma was rapid (about 60% disappeared within 1 h), the rate was apparently slower than that of 3,5-DCQA. This difference might be attributable to the different rat strain used, the different dose administered or other reasons.

Renal and biliary excretion had proven to be two important elimination routes for 1,5-DCQA and its metabolites, including M5–M15, in rats following oral dosing [11]. It seemed that the biliary excretion, besides renal excretion, should also play an important role in the elimination of M5–M15 in rats following intravenous dosing. If it were the case, the glucuronidated metabolites, with increased aqueous solubility in comparison with their substrates, excreted in the bile could be hydrolyzed by the β-glucuronidase in rat gut and the resulting substrates reabsorbed, entering into an entero-hepatic cycle. Additionally, the methylated metabolites excreted in the bile might also enter into the entero-hepatic recycling by direct absorption.

Plumb et al. reported that the ester bond of chlorogenic acid, a dietary polyphenol with the similar structure to 1,5-DCQA, could not be affected by enzyme(s) in human liver, small intestine or plasma, while it could be hydrolyzed by human faecal microflora with the formation of caffeic acid [14]. In the present study some low molecular weight compounds were observed in rat urine after dosing. Although they were also detected in blank samples, their amounts in the blank were apparent lower. They might come from the absorption of the degraded products of 1,5-DCQA catalyzed by the microflora in rat colon. As for why these compounds were also present in blank samples probably because the food for rats was not semi-purified [15]. The postulation and the result required further investigation.

5. Conclusions

This paper reported for the first time that when 1,5-DCQA was intravenously administrated, it disappeared rapidly from plasma, and at the same time large numbers of its methylated and methyl-glucuronidated metabolites were observed in rat urine besides 1,5-DCQA itself. It suggested that quick urinary excretion and extensive metabolism of 1,5-DCQA, including methylation and glucuronidation, were two factors causing its rapid elimination from the circulation. The information gained from this study could be very useful for the further pharmacokinetic studies of other DCQAs.

Acknowledgements

The project supported by the 863 Program (No. 2003AA2Z347B), the National Natural Science Foundation of China (No. 30371669), and the Key Scientific Technological project of Bingjing (No. Z000410504131).

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