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Short Communication

Pharmacokinetics of caffeic acid in rats by a high-performance liquid chromatography method

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Introduction

The secondary metabolism of plants produces many phenolic substances, such as the cinnamic acid group, which is present in almost all animal and human diets.

Caffeic acid (3,4-dihydroxycinnamic acid), the most important of this group, is ingested by humans not only from vegetable foods, but also from coffee products, where it is present as a degradation compound of chlorogenic acid. The presence of a catechol group in the molecule makes it very similar to other endogenous compounds, such as catecholamines.

Some pharmacological properties of this substance have been described previously, for example its action on intestinal motility and biliary secretion [1], capillary permeability [2] and on α-adrenoceptors [3].

However one of its most interesting effects refers to the inhibition of 5- and 12-lipooxygenase activity. Caffeic acid is a non-competitive, but selective and instantaneous inhibitor for this enzyme, and therefore for leukotriene biosynthesis. This effect is due to the catechol group of its molecules, because it has been cited that the active site of 5-lipooxygenase is inactivated by the hydroxyl groups [4]. As a result of its inhibition of enzyme activity, platelet aggregation and thromboxane biosynthesis, caffeic acid could be formulated to treat asthma and allergic-inflammatory diseases [5]. Additionally, Andary *et al.* [6] have confirmed the activity of this phenol acid as a competitive inhibitor of DOPA-decarboxylase, due probably to its structural similarity with levodopa.

In general, a relationship exists between the dose of a drug administered and the pharmacological effect, and subsequently, the plasma levels. For this reason, in this paper the study on the pharmacokinetics of caffeic acid administered as a single intravenous and oral dose has been carried out using HPLC.

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Materials and Methods

I.V. plasma kinetics

Female Sprague–Dawley rats weighing 200–230 g, unfed for 20 h before caffeic acid administration were used. Animal anaesthesia was performed by means of an intraperitoneal injection of a 10% ethylurethane solution (Merck, 1 ml kg^{-1}). The body temperature was maintained constant during the experiments. After heparinisation by a femoral vein canula, caffeic acid was administered. The dose of caffeic acid (Fluka AG) was 40 mg kg^{-1}, in a buffered and isotonic solution (phosphate buffer 0.05 M, pH 7.4). Blood samples of 0.5 ml were collected through a catheter inserted into the right carotid artery at 1, 2, 5, 15, 30, 60, 120 and 180 min. Caffeic acid was not detectable in plasma at 9–10 h. Due to the decrease in hematocrit values, a maximum of 6 blood samples were taken from each animal. After protein precipitation with 3 volumes of methanol, the phenol acid was determined in the supernatant by HPLC with spectrophotometric detection, using *o*-cresol (Merck) as an internal standard.

Oral plasma kinetics

As in the case of the i.v. study, rats were fasted for 20 h before caffeic acid administration. According to the studies of Doluisio [7, 8], there is no significant difference in the absorption process if the fasting period is about 15–20 h, while a longer time provokes physiological and biochemical changes that decrease the absorption ratio. The dose of caffeic acid was 120 mg kg^{-1}, in the same buffered solution. Blood samples were collected at 15 points between 5 and 180 min. These samples were processed in the same way as in i.v. studies and caffeic acid quantified also by HPLC.

Determination by HPLC

Liquid chromatography was carried out using a Perkin–Elmer chromatograph, series 2, equipped with a Data Station Sigma 15, a spectrophotometric detector LC-85B and an oven LC-100. In the literature no method has been cited for caffeic acid determination in biological fluids by HPLC. The method developed in the present work is based on that proposed by Adzet and Puigmaciá [9].

A 5-μm LiChrosorb C_{18} column (150 × 4 mm i.d. Supelco, Bellafonte) was employed with a mobile phase consisting of methanol–water–acetic acid (22.5:75.0:2.5, v/v/v) at 35°C and 1.5 μl min^{-1}, using UV detection at 280 nm. Under these conditions, the retention time of caffeic acid was 3.74 and 8.46 min for *o*-cresol.

I.V. and oral clearance

In order to determine the renal clearance, after a fasting period of 20 h, the animals received caffeic acid at the same dose of that used in plasma kinetics studies. Rats were immediately starved in metabolic cages for 24 h to collect their urine. From the value of plasma half-life calculated previously, 24 h is long enough to ensure the complete excretion of caffeic acid. The extraction of the caffeic acid from the urine was performed according to the method of Booth *et al.* [11], followed by the HPLC assay described above.

Recovery of the HPLC method

Caffeic acid was added at 100 μg ml^{-1} to drug-free plasma and urine, and then analysed by the HPLC method described above, after addition of internal standard. An

aliquot of 3-μl of the supernatant was injected and peak height corresponding to caffeic acid measured. Recovery from plasma was found to be 84% (because of protein binding [10]) and 88% from urine.

Precision and sensitivity

The precision of the HPLC method was evaluated by repeated analysis of plasma and urine standards containing caffeic acid and *o*-cresol concentrations of 50, 100 and 200 μg ml^{-1} ($N = 6$). The sensitivity limit was taken as the lowest concentration that gave a coefficient of variation inferior or equal to 10% for six assays. The limits were around 10 μmol l^{-1} of plasma and 12 μmol l^{-1} of urine.

Results

I.V. plasma kinetics

The assumption was made that the dosage used exploited the linear portion of the curve. A semilogarithmic plot of the results ($N = 6$) (Fig. 1) indicated a biphasic curve, reflected by the correlation ratio after fitting experimental points to 1 or 2 straight lines. Initial estimates of coefficients and exponents of the equation were carried out by the feathering method. These values were inserted in the Extended Least Squares Nonlinear Regression Program in a HP-85 computer.

The equation for the line obtained from this program was:

$$C_t = 292.9 \; x \; e^{-9.00t} + 125.5 \; x \; e^{-0.40t}$$

The Akaike International Criterion (AIC) (correlation value for nonlinear regression) was −21.7421.

From this final equation, the following pharmacokinetic parameters were calculated:

$t_{½}\,\alpha$ = 0.0770 h (distribution half-life).
$t_{½}\,\beta$ = 1.7493 h (elimination half-life).
V_c = 95.60 ml kg^{-1} (central volume of distribution) (D/C_o).
V_d = 288.93 ml kg^{-1} (total volume of distribution) ($D/\beta AUC$).
AUC_0^∞ = 349.42 μg × h ml^{-1} (area-under-the-curve).
Cl_{plasm} = 114.51 ml kg^{-1} h^{-1} (plasma clearance).
K_{12} = 5.22 h^{-1} (transfer microconstant).
K_{21} = 2.98 h^{-1} (return microconstant).
K_{10} = 1.20 h^{-1} (elimination microconstant).

Oral plasma kinetics

It was assumed that the dose given ensured operation on the linear part of the curve. From the plot of the results ($N = 10$) (Fig. 2), it is difficult to predict if caffeic acid follows a single or two-compartment elimination model. The difference between correlation coefficients after fitting the elimination phase to 1 or 2 straight lines is not a discriminative factor, because the mono-exponential treatment considers a great number of degrees of freedom with regard to biexponential one.

Thus, initial estimates of coefficients and exponents of the equations were carried out by the feathering method, and the values inserted in the same regression program mentioned above.

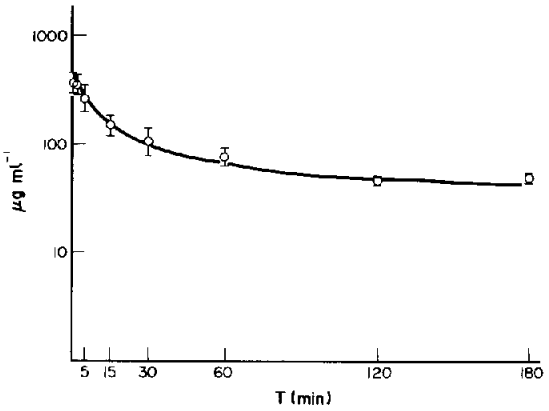

Figure 1
Semilogarithmic plot of plasma levels of caffeic acid after i.v. administration (mean ± SD).

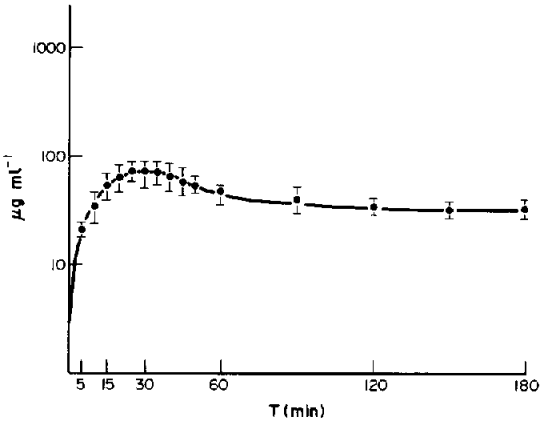

Figure 2
Semilogarithmic plot of plasma levels of caffeic acid in oral administration (mean ± SD), after correction for the zero-time-shift.

The model with the lowest relative standard deviation and the lowest value of AIC [12] was the triexponential model, for which the final equation was:

$$C_t = 373.05 \; x \; e^{-4.80(t-t_0)} + 59.32 \; x \; e^{-0.22(t-t_0)} - 700.40 \; x \; e^{-8.92(t-t_0)}$$

$$AIC = 27.0379.$$

Some pharmacokinetic parameters calculated from this equation and corrected for the value of bioavailability were:

$t_{½}$ a = 0.0777 h (absorption half-life).
$t_{½}$ α = 0.1444 h (distribution half-life).
$t_{½}$ β = 3.1350 h (elimination half-life).
t_0 = 5.40 min (zero-time-shift).

V_c = 116.86 ml kg^{-1} (central volume of distribution).
V_d = 517.65 ml kg^{-1} (total volume of distribution).
AUC_0^∞ = 278.30 μg × h ml^{-1} (area-under-the-curve).
F = 26.54% (Bioavailability)[$(AUC_{or}/AUC_{iv}) \times (D_{iv}/D_{or})$].
Cl_{plasm} = 114.53 ml kg^{-1} h^{-1} (plasma clearance).
K_a = 8.92 h^{-1} (absorption rate constant).
K_{12} = 2.66 h^{-1} (transfer microconstant).
K_{21} = 1.75 h^{-1} (return microconstant).
K_{10} = 0.61 h^{-1} (elimination microconstant).

Renal clearance

After i.v. administration, a urinary recovery of 26.27 ± 9.75% (mean ± SD) (N = 6), and 13.68 ± 4.01% (mean ± SD) (N = 6) after oral ingestion was calculated.

Renal clearance can be calculated as the product of urinary recovery by plasma clearance; i.v. renal clearance results in 30.08 ± 11.17 ml kg^{-1} h^{-1} and oral 15.67 ± 4.60 ml kg^{-1} h^{-1}.

Results

Precision

Caffeic acid and *o*-cresol, at concentrations of 200, 100 and 50 μg/ml^{-1} were added to plasma and urine standards, treated previously as mentioned in Materials and Methods. The results obtained are as follows: plasma, 200 (196.20 ± 10.59); 100 (97.81 ± 6.75); 50 (48.20 ± 3.68) (N = 6); urine, 200 (196.60 ± 10.38); 100 (97.20 ± 6.14); 50 (48.55 ± 3.41) (N = 6).

Reproducibility

Plasma and urine samples spiked with different concentrations of caffeic acid. The relative standard deviation (RSD) for each (in %) is in parentheses: plasma, 200 (5.51); 100 (6.90); 50 (7.63); 25 (8.92); 10 (9.76); 5 (12.21) (N = 6); urine, 200 (5.21); 100 (6.32); 50 (7.03); 20 (8.88); 12 (9.40); 6 (11.70) (N = 6).

Accuracy can only be determined by comparison of calibrations obtained for standards in plasma or urine, compared to calibrations for standards in mobile phase.

Discussion

α and β values diminish by half in oral kinetics, but the ratio α/β (22.70 i.v., 21.71 oral) is almost the same, confirming the two-compartment behaviour of caffeic acid. The values of the microconstants ratio for i.v. injection were: K_{12}/K_{10} = 4.36, K_{12}/K_{21} = 1.75, K_{21}/K_{10} = 2.48; and for oral administration: K_{12}/K_{10} = 4.39, K_{12}/K_{21} = 1.52 and K_{21}/K_{10} = 2.89. These results suppose that phenol acid has some affinity for the peripheral compartment. It is possible to deduce the presence of some accumulation phenomenon, because the K_{12}/K_{21} ratio is close to 1. On this issue, it can be pointed out that the binding ratios of caffeic acid to serum albumin [10] and different tissues [13] are high and of the same order of magnitude.

The values of the K_{21}/K_{10} ratio imply that the return process does not act as a limiting factor of elimination. The smaller result obtained for central volume of distribution in i.v. studies (95.60 ml kg^{-1}) with regard to the standard value (150 ml kg^{-1}) [14], is

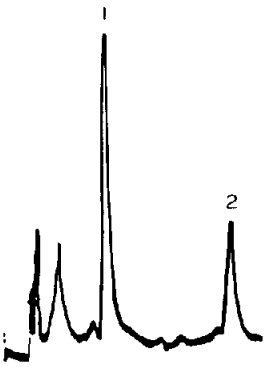

Figure 3
Chromatogram of plasma levels of caffeic acid after i.v. administration in rats. (Sample at 5 min.). ① Caffeic acid. ② *o*-Cresol.

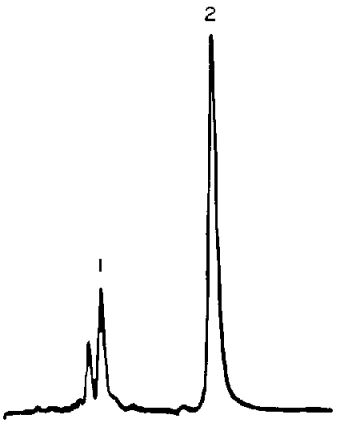

Figure 4
Chromatogram of urine levels of caffeic acid after i.v. administration in rats. ① Caffeic acid. ② *o*-Cresol.

Table 1

t (min)	Caffeic acid concentration (mg ml^{-1}) ± σ_{N-1} in remaining solutions (N = 6). [(D), Duodenum; (S), Stomach]				
	B pH:6.0 (D)	C pH:4.0 (D)	D pH:2.0 (D)	E pH:4.0 (S)	F pH:2.0 (S)
0	0.771 ± 0.072	0.673 ± 0.047	0.392 ± 0.031	0.763 ± 0.042	0.427 ± 0.010
5	0.747 ± 0.092	0.563 ± 0.032	0.270 ± 0.011	0.750 ± 0.021	0.415 ± 0.010
10	0.697 ± 0.141	0.483 ± 0.032	0.237 ± 0.010	0.747 ± 0.015	0.407 ± 0.010
15	0.645 ± 0.163	0.453 ± 0.035	0.230 ± 0.008	0.720 ± 0.001	0.392 ± 0.009
30	0.640 ± 0.099	0.393 ± 0.015	0.207 ± 0.017	0.730 ± 0.014	0.372 ± 0.009
45	0.605 ± 0.148	0.377 ± 0.021	0.185 ± 0.024	0.693 ± 0.015	0.357 ± 0.006
60	0.625 ± 0.021	0.380 ± 0.040	0.172 ± 0.027	0.673 ± 0.015	0.337 ± 0.006
90	0.665 ± 0.078	0.383 ± 0.065	0.162 ± 0.042	0.643 ± 0.015	0.307 ± 0.021
120	0.655 ± 0.149	0.417 ± 0.086	0.187 ± 0.086	0.623 ± 0.029	0.300 ± 0.039
K_a (h^{-1})	0.213 ± 0.056*	0.555 ± 0.123	0.509 ± 0.071	0.107 ± 0.032†	0.227 ± 0.018
r	0.9088	0.9329	0.9715	0.9905	0.8863

Correlation coefficient of log regression. *Not significant versus F and significant ($P < 0.02$) versus C and D. †Significant versus F and C ($P < 0.02$).

probably due to an important binding ratio to plasma proteins. This is easy to explain because the i.v. administration involves a rapid injection (20 s), whereas the oral route is affected by a bioavailability of 26.54%, and involves a slow and gradual incorporation of caffeic acid into general circulation.

According to Sellers and Koch-Weser [15], if the drug is administered by rapid i.v. injection, the plasma concentration near the point of administration is initially very high. Sometimes, it is possible to exceed the binding capacity of plasma proteins, increasing the free fraction of the drug, depending on the distribution and the elimination rate constants.

In other experiments performed previously by the authors [10], it has been found that the binding ratio of caffeic acid to plasma proteins is inversely proportional to phenol acid concentration. Therefore, it is possible that protein binding in relative (but not in absolute) conditions would be greater after oral than after i.v. administration. β values are due to the different V_d values, and this fact produces a large elimination half-life in oral kinetics. However, this elimination rate also depends on the plasma clearance. Although the plasma clearance is the same in both kinetics, the relationship between renal and metabolic clearance is different.

From the pK_a values of the caffeic acid molecule determined in previous experiments [13], and the increase of K_a on decreasing the pH, it is possible to suggest that the main limiting factor of caffeic acid absorption is the pH. At the same value of pH, gastric absorption is lower than intestinal absorption (significant for $P < 0.02$), thus reflecting the different physiological organisation and surface/volume ratio in both zones.

With regard to renal excretion of caffeic acid, in order to establish if there is some reabsorption or tubular secretion phenomenon, the (S − R) (secretion − reabsorption) value has been determined by the equation (16):

$$(S - R) = (Cl_r \times C_p) - (f_f \times GFR \times C_p),$$

where Cl_r is renal clearance, C_p plasma concentration in the middle of the period used to study renal clearance, f_f free fraction and GFR glomerular filtration rate (1 ml min^{-1} 100 g^{-1} in rat).

C_p of caffeic acid is about 1 μg ml^{-1} after i.v. injection and 4.1 μg ml^{-1} after oral administration. At these low concentrations, the binding ratio to plasma proteins is very high (about 95% and 97.5%, respectively) [10], thus the (S − R) value is near to zero, indicating that the glomerular filtration is the main process involved in the renal excretion of caffeic acid.

At the same time, the value of renal clearance is low with regard to plasma clearance, because the protein binding is the limiting factor of glomerular filtration. Finally, plasma clearance is the result of the addition of renal and metabolic clearance. For caffeic acid this extra-renal clearance is 84.4 and 98.9 ml kg^{-1} h^{-1} for i.v. and oral administration respectively.

Hence, the authors consider that the elimination of caffeic acid in rats is carried out essentially by means of metabolic pathways, when the administration is oral.

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