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Short communication

Pharmacokinetic study of ellagic acid in rat after oral administration of pomegranate leaf extract

Fan Lei [a], Dong-Ming Xing [b], Lan Xiang [b], Yu-Nan Zhao [a], Wei Wang [b], Lu-Jun Zhang [b], Li-Jun Du [b,*]

[a] *Institute of Medicinal Plant, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100094, China*
[b] *Laboratory of Pharmaceutical Sciences, Department of Biological Science and Biotechnology, Tsinghua University, Beijing 100084, China*

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Abstract

Quantification of ellagic acid, the principal bioactive component of pomegranate leaf extract, in rats plasma following oral administration of pomegranate leaf extract was achieved by using a high-performance liquid chromatographic method. The calibration curve for ellagic acid was linear ($r^2 = 0.9998$) ver the concentration range 0.026–1.3 μg/ml. The intra- and inter-day assays of ellagic acid from rat plasma were less than 6.52% at concentration range from 26 to 1300 ng/ml and good overall recoveries (94.5–102.4%) were found on same concentrations. The concentration–time profile was fitted with an open two-compartment system with lag time and its max concentration of ellagic acid in plasma was 213 ng/ml only 0.55 h after oral administration extract 0.8 g/kg. The pharmacokinetic profile indicates that ellagic acid has poor absorption and rapid elimination after oral administration pomegranate leaf extract, and part of it was absorbed from stomach.

Keywords: Ellagic acid; Pharmacokinetics

1. Introduction

Pomegranate (*Punica granatum*) a traditional Chinese medicine used as antibacterial, anti-inflammatory and hemostasis agent, is rich of phenolic compounds. Extracts from many parts of this plant such as juices, seed oil and peel have been reported to exhibit strong antioxidant activity [1,2]. The extract of pomegranate leaves had been shown free radical scavenging activity and antioxidant effect in vitro. By the bioassay-guided isolation, ellagic acid (EA) has been led as the main active compound of the extract. The phenolic nature of EA makes itself a powerful antioxidant [3,4] (structure shown in Fig. 1). It has been reported that EA can inhibit the mutagenicity and carcinogenicity in a wide range of mutagents especially chemical agents such as aflatoxin B, benzo(a)pyrene(B(a)P),4,4′-dinitro-2-biphenylamine, 1,3-dinitropyrene and nitro compounds in vivo and in vitro [5–7]. But its anti-tumorigenic activity had been evaluated in several in vivo model systems often with conflicting results [8,9]. Reasons for the inability of EA to tumors are still unknown, but it seemed possible that a sufficiently high concentration of EA

* Corresponding author. Tel.: +86-10-6277-3630; fax: +86-10-6277-3630.
E-mail address: pharm@mail.tsinghua.edu.cn (L.-J. Du).

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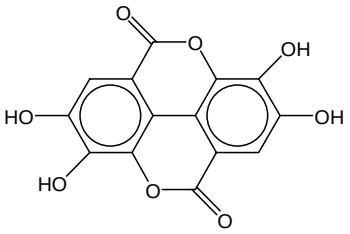

Fig. 1. Structure of ellagic acid.

is not present in plasma or target cells after oral administration.

The pharmacokinetics of EA by oral administration has not been adequately investigated. Although Doyle and Griffiths [10] have isolated several metabolites of EA from blood, bile, urine and feces, they have not detected EA in plasma, and suggested that EA would be metabolized by intestinal micro-organisms under the condition of oral administration. Teel and Martin [11] have found that the levels of ^{3}H-ellagic acid were highest in blood 30 min, in urine and bile 120 min and in liver, lung and kidney 15 min after oral administration to mouse, but still can not point out the distribution and elimination of EA. Smart et al. [12] have reported that very low level of EA (<1 nmol/g) in blood, lung or liver was detected after oral administration. EA was rapidly eliminated and almost 90% was eliminated from blood within 15 min after i.v. administration.

The present study described the pharmacokinetic properties of EA in rat after oral administrated of pomegranate leaf extract.

2. Experimental

2.1. Chemicals and reagents

The reference standard of EA (purification was 98%) was kindly provided by Dr. Lan Xiang. The pomegranate leaf extract was extracted by our laboratory, with match number: 020915 (containing EA 10.66%). Phosphoric acid and potassium dihydrogen phosphate were analytical grade. Methanol and acetonitrile (HPLC grade) were purchased from Baker (J.T. Baker, USA). Double-distilled water was used for all preparations.

2.2. Animals

Male Wistar rats (180–220 g), been obtained from Laboratory Animal Institute of Chinese Academy of Medical Sciences, were kept in an environmentally controlled breeding room (temperature: 25 ± 2 °C, humidity: 60 ± 5%, 12 h dark/light cycle) for 1 week before being used for experiments. They were fed standard laboratory chow with water ad libitum. All rats were fasted overnight before the experiment. All animal treatments were according to the recommendation of the Regulations for the Administration of Affairs Concerning Experimental Animals.

2.3. HPLC system

The HPLC system consisted of a 515 HPLC pump (Waters, USA), a Rheodyne 7725i manual injector (Waters, USA), a 996 Photodiode Array Detector (Waters, USA) and a Millennium ^{32}Chromatogram Working Station. A Hypersil C_{18} column, 4.6 × 150 mm, 5 μm (Da-Lian elite, China) was used. The mobile phase consisted of amethanol −0.2% phosphoric acid water solution (25:55, v/v). It was filter through a 0.22 μm millipore filter and degassed prior to use. The flow rate was 0.8 ml/min. Detection was performed at a wavelength of 254 nm under a constant temperature (40 ± 1 °C).

2.4. Blood sampling

For metabolism studies, serial blood samples were collected at pro-dose, 0.25, 0.5, 1.0, 1.5, 2, 4, 6, 8, 12 and 24 h after oral uptake of a single 0.8 g/kg body mass dose of the extract (containing 85.3 mg/kg EA) in an aqueous solution. Each sample was immediately transferred to a heparinized glass tube and centrifuged at 1500 × *g* for 15 min at 8–10 °C. The plasma was then transferred to another glass tube and stored at −20 °C until analyzed.

For the absorbing area studies under ether anaesthesia, five rats were ligated the end of stomach sphincter, the passage at pylorus that opens into duodenum to prevent drug solution from entering the duodenum. Animals were oral administrated with extract

0.8 g/kg when they regained consciousness. Blood samples were obtained 15 min after gavaging. Plasma samples were separated and stored at −20 °C until analyzed.

2.5. Preparation of plasma samples

A 1.0 ml portion of rat plasma was adjusted to pH 2.5 with 0.3 ml of 1 M potassium dihydrogen phosphate solution and 30 μl 50% phosphoric acid. Then each sample was vortex-mixed with 5 ml acetonitrile for 1 min, centrifuged at 3000 × *g* for 15 min at 8–10 °C. The supernatant liquor was gently evaporated to dryness in a water bath at 37 °C. The residue was dissolved in 0.1 ml methanol. Each proceeded sample was stored at 0–4 °C until further analysis. The same sample handling process was used for recovery and precision determinations in plasma.

2.6. Calibration curve

A calibration curve was performed by the analysis of various concentrations (0.026, 0.065, 0.13, 0.65, 1.3 μg/ml) of EA spiked in rat plasma. The concentration was determined from the peak area by using the equation for linear regression obtained from the calibration curve.

2.7. Precision and recovery

Recoveries from rat plasma were determined by adding EA at concentrations of 0.026, 0.065, 0.13 and 1.3 μg/ml and the precision (intra- and inter-day) of the method was calculated at the same four concentrations. After analyzed plasma sample, the resulting peak area was compared with the standard of EA carried in methanol to provide the recovery values. The intra-day variance was determined by assayed the spiked samples on the same day and inter-day variance was assayed over four consecutive days. Coefficients of variation (C.V.) were calculated from these values.

2.8. Pharmacokinetic analysis

Data were expressed as mean ± S.D. All statistical analyses were performed using Microsoft Excel 2002. The pharmacokinetic parameters were calculated by the software 3p87 (the Practical Pharmacokinetic Program 1987), suggested by China Pharmacological Society.

3. Results

3.1. HPLC chromatograms

Under the condition described above, the HPLC chromatograms of blank plasma, plasma spiked with EA (1.3 μg/ml) and the plasma obtained 30 min after oral administration of the extract were shown in Fig. 2. The retention times of EA in the extract and EA standard were 17.834 and 17.730 min, respectively. No interfering peak was observed at the retention time of EA be detected (Fig. 2).

3.2. Calibration curve

The calibration curve for EA was linear (r^2 = 0.9998) over the concentration range from 0.026 to 1.3 μg/ml. A regression equation for the line was $y = 1.01E^{-6} \times -0.0024$ (*x*: area under the curve, *y*: concentration of EA in plasma).

3.3. Recovery and precision

The recoveries of EA from rat plasma were 91.2, 106.6, 105.7 and 100.5% for the concentrations of 0.026, 0.065, 0.13 and 1.3 μg/ml, respectively (Table 1). The reproducibility of the method was defined by examining both intra- and inter-day variance. The C.V. values of intra- and inter-day assay were 1.79, 1.30, 0.69 and 0.54% and 2.68, 3.35, 4.60 and

Table 1
Recovery of the ellagic acid[a]

Spiked concentration (μg/ml)	Measured concentration (μg/ml)	Recovery (%)	C.V. (%)
0.026	0.022 ± 0.0005	94.5	4.87
0.065	0.069 ± 0.001	102.4	1.80
0.13	0.137 ± 0.003	101.9	4.22
1.3	1.306 ± 0.057	106.3	4.50

[a] Each value represents the mean ± S.D. ($n = 3$).

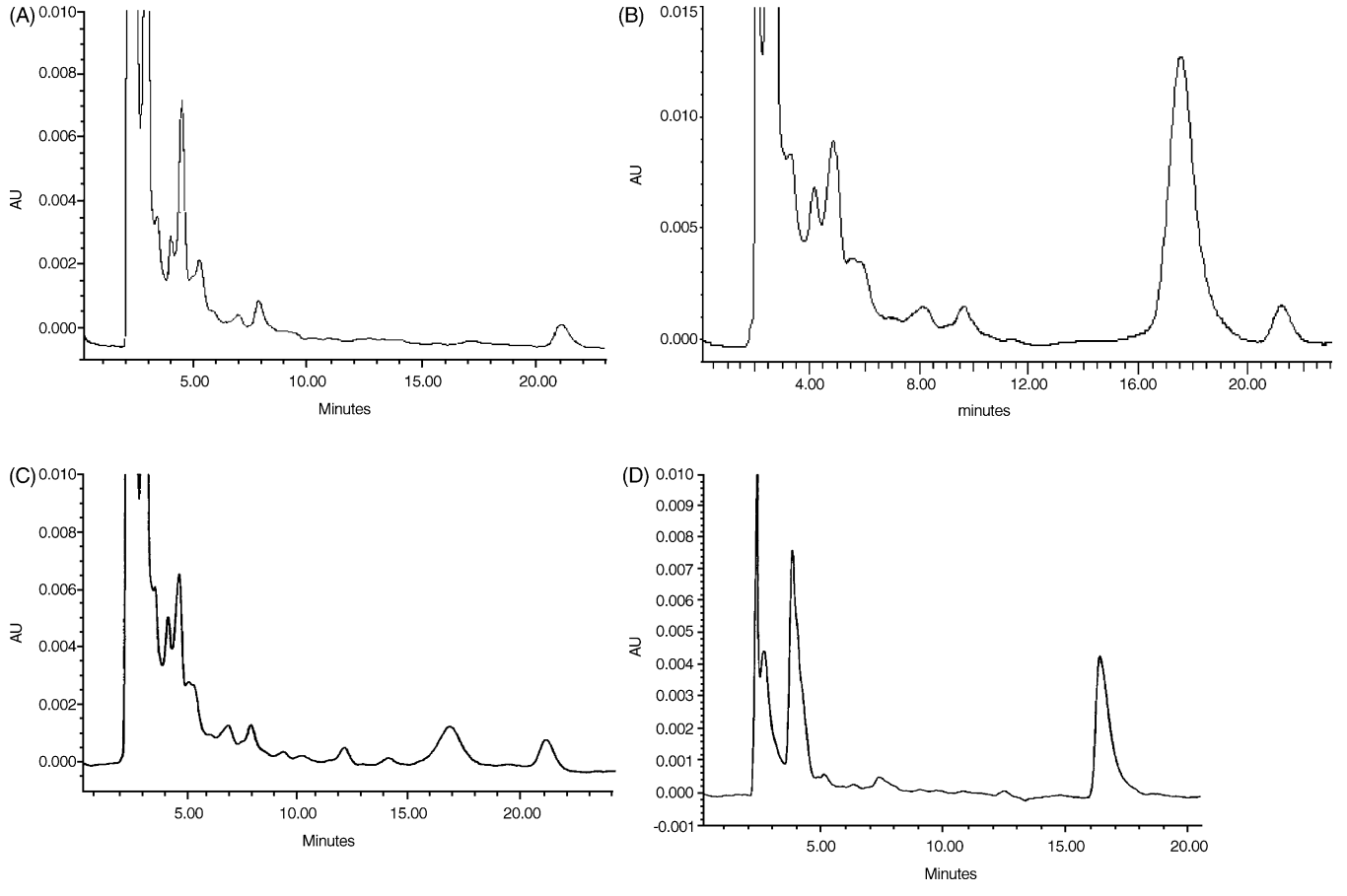

Fig. 2. Chromatograms of EA in (A) blank plasma, (B) blank plasma spiked with EA (1.3 μg/ml), (C) plasma sample obtained 30 min after oral administration of pomegranate leaf extract and (D) the pomegranate leaf extract.

Table 2
Validation of the intra- and inter-day assay

	Spiked concentration (μg/ml)	Measured concentration (μg/ml)[a]	Accuracy (%)	C.V. (%)
Intra-day ($n = 3$)	0.026	0.024 ± 0.0004	92.5	1.79
	0.065	0.067 ± 0.001	102.9	1.30
	0.130	0.137 ± 0.001	105.7	0.691
	1.300	1.371 ± 0.007	105.5	0.541
Inter-day ($n = 4$)	0.026	0.025 ± 0.001	91.2	2.68
	0.065	0.067 ± 0.002	106.5	3.35
	0.130	0.133 ± 0.006	105.7	4.59
	1.300	1.382 ± 0.090	100.5	6.52

[a] Each value represents the mean ± S.D.

6.52% at concentrations of 0.026, 0.065, 0.13 and 1.3 μg/ml, respectively (Table 2).

3.4. Pharmacokinetics and Kinetic analysis

The plasma concentration–time profile of EA in rats ($n = 5$) was presented in Fig. 3. The concentration was below the quantitative limit (<0.026 μg/ml) 12 h after oral administration of leaf extract (containing 85.3 mg/kg EA) with maximum plasma concentration (203 ng/ml) at 0.54 h post dosing. The plasma level of EA declined with α and β half-lives ($t_{1/2}$) of 0.77 and 5.0 h. All the pharmacokinetic parameters of EA were listed in Table 3.

The concentration of EA in rats underwent the ligating operation, was 0.083 ± 0.028 μg/ml 15 min after oral administration the extract.

4. Discussion

In this study, pomegranate leaf extract was orally administrated to rats. The results, as derived from upper studies, suggested that the pharmacokinetics of unbound EA appeared to best fit the kinetics of a two-compartment model with first-order absorption and lag time with rapid distribution phases (half-life was 0.77 h) in rat blood. Following oral administration of the extract, the blood level of EA was increase rapidly and the max concentration reached 203 ng/ml 0.54 h after the dose. This may contribute to the extract absorbed directly from the stomach and most of EA was absorbed from stomach according to the data of the absorption area research. Although the elimination of EA was not rapid ($t_{1/2\beta} = 5$ h), the concentration was decreased rapidly and half of it was out of blood 40 min after oral administration the extract. Rapid absorption, distribution and elimination were the characteristics of EA after oral administration the extract.

The disposition described here agreed with reports of Smart et al. [12] and Teel and Martin [11], suggested that the poor absorption and rapid distribution

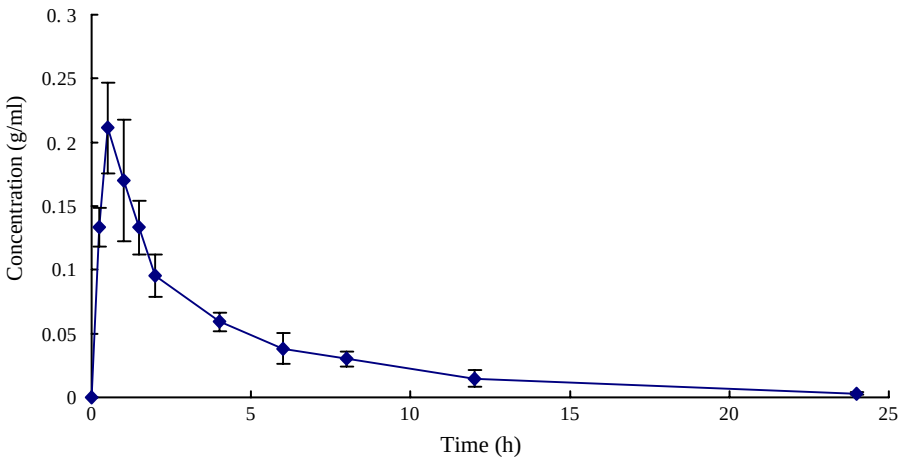

Fig. 3. Plasma concentration–time curve of EA in rats after oral administration of pomegranate leaf extract (at a dose containing 85.3 μg/kg EA). Each point and bar represents the mean ± S.D. ($n = 5$).

Table 3
Pharmacokinetic parameters of EA in rat plasma after oral administration of pomegranate leaf extract at a dose containing 85.3 mg/kg EA ($n = 5$)

Parameter	Estimate (mean ± S.D.)	Parameter	Estimate (mean ± S.D.)
A (μg/ml)	0.267 ± 0.121	$t_{1/2\alpha}$ (h)	0.770 ± 0.153
α (h^{-1})	1.011 ± 0.199	$t_{1/2\beta}$ (h)	5.000 ± 2.730
B (μg/ml)	0.073 ± 0.012	$t_{1/2Ka}$ (h)	0.140 ± 0.056
β (h^{-1})	0.112 ± 0.008	K_{21} (h^{-1})	0.357 ± 0.089
Ka (h^{-1})	5.905 ± 3.257	K_{10} (h^{-1})	0.329 ± 0.093
AUC (μg h/ml)	0.838 ± 0.071	K_{12} (h^{-1})	0.437 ± 0.114
CL(s) (l/(h kg))	102.32 ± 8.80	Lag time (h)	0.125 ± 0.074
C_{max} (μg/ml)	0.203 ± 0.047	T (peak) (h)	0.541 ± 0.061
V_d (l/kg)	334.07 ± 115.74		

V_d: volume of distribution.

of EA may prevent tissues from attaining and maintaining sufficiently high concentration for effectively as an in vivo antioxidant and anti-mutgenicity agent. In this study, the max concentration of EA in plasma by oral administration pomegranate leaf extract was much higher than the concentration of EA by oral administration it. Three possible reasons may be responsible for this special high level of EA but the real reasons still need further investigation. First, the crude extract contained many other components besides EA and some of them might facilitate EA absorption from the stomach and the intestine such as changing intestine pH to inhibit EA ionization, because EA was a weak acid which was ionized at physiological pH. Second, some of components might inhibit the intestinal micro-organisms from metabolizing EA. Third, there might be some of the EA derivative in the crude extract which changed to EA by metabolism in vivo.

By oral administration pomegranate leaf extract, the levels of EA in plasma was detectable and much higher than the concentration after oral administration EA according to other studies [10,12]. Further studies are necessary to fully discover the reason. Since EA may be a potentially useful anti-carcinogen in human being, how to effectively increase its bioavailability, enhance its concentration in blood or target tissues and decrease its elimination will be the goal of future projects.

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