ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jun; 23 (6): 551 - 555
http://www.chinaphar.com

Pharmacokinetics of leflunomide in Chinese healthy volunteers

LI Jun[1], YAO Hong-Wei, JIN Yong, ZHANG Yun-Fang, LI Chang-Yu, LI Yuan-Hai, XU Shu-Yun
(*Institute of Clinical Pharmacology, Anhui Medical University, Hefei 230032, China*)

KEY WORDS leflunomide; pharmacokinetics; high pressure liquid chromatography

ABSTRACT

AIM: To study the pharmacokinetics of leflunomide in Chinese healthy volunteers. **METHODS**: A single (20, 40, and 60 mg) and 30-d-repeated (20 mg/d) oral doses of leflunomide were performed on 18 (12 males and 6 females) and 6 (4 males and 2 females) Chinese healthy volunteers respectively. A_{771726}, the active metabolite of leflunomide, in serum was determined by high performance liquid chromatography (HPLC). Data were analyzed by a 3p97 program on a Legend computer. **RESULTS**: Serum concentration curves of A_{771726} in single and repeated oral administration of leflunomide conformed to one compartment model of the first order absorption. Leflunomide was absorbed rapidly with $T_{1/2,ka}$ of between 1.15 h and 2.23 h in single oral administration. The major pharmacokinetic parameters of A_{771726} in 20, 40, and 60 mg groups were $T_{1/2,ke}$(h): 211 ± 18, 170 ± 24, 252 ± 26; T_{max}(h): 13 ± 12, 13 ± 4, 9 ± 5; C_{max}(mg/L): 2.0 ± 0.5, 5.2 ± 0.6, 6.7 ± 1.5; AUC ($mg \cdot h \cdot L^{-1}$): 647 ± 137, 1344 ± 191, 2555 ± 907, respectively. In repeated oral administration, steady state concentration was achieved within 30 d. The mean trough concentration was between 32.0 mg/L and 39.7 mg/L. The C_{max}, T_{max}, AUC_{0-24} were (41.5 ± 2.4) mg/L, (307 ± 75) h, and (22099 ± 1234) $mg \cdot h \cdot L^{-1}$, respectively. **CONCLUSION**: The absorption of leflunomide was rapid, and its elimination was slow after oral administration. The pharmacokinetic results showed that it exhibited first order kinetic characteristics.

INTRODUCTION

Leflunomide was a novel isoxazole drug with immunomodulatory and antiproliferative properties that was effective in experimental models of autoimmune diseases and in allo- or xenotransplantation[1,2]. The antiinflammatory and immunomodulatory characteristics of leflunomide were first described using two rodent models of autoimmune diseases, adjuvant arthritis, and allergic encephalomyelitis[3]. Since those studies, additional reports have confirmed that leflunomide is effective in controlling a number of autoimmune diseases in rodents, including systemic lupus erythematosus, proteoglycan-induced polyarthritis, organ-specific diseases, and autoimmune uveitis[4-6]. Based on those observations, the effects of leflunomide have been tested in patients with rheumatoid arthritis and no clinically significant toxicity was detected at doses of which clinical and immunological improvements had been detected[3].

Despite these encouraging results, the action mode of this immunomodulator was not fully understood. But there was strong evidence that it had effects on *de novo* pyrimidine biosynthsis, specifically dehydroorotate dehydrogenase (DHODH), and inhibited certain tyrosine kinase responsible for signal transduction[7].

Leflunomide itself was a prodrug. It was well absorbed in animals and human, but it was metabolized quickly into an active metabolite, A_{771726}, *in vivo*[8-11]. In phase Ⅱ clinical trials, leflunomide showed high tolerability and efficacy in patients with advanced rheumatoid arthritis[2,12]. The purpose of the study reported here was to determine the clinical pharmacokinetic profile of leflunomide through determination of A_{771726}, leflunomide's active metabolite, and consequently to provide dosage recommendations for phase Ⅱ trials.

MATERIALS AND METHODS

Drugs and reagents Leflunomide (Batch No. 970901, 10 mg/tablet) and A_{771726} standard (Batch No. 970925, purity > 99.5 %) were obtained from Cinkate Corp, USA; acetonitrile, ethyl acetate, acetate acid, sodium acetate, and ethanol were of analytical grade.

[1] Correspondence to Prof LI Jun.
Phn/Fax 86-551-284-0542. E-mail amuicplj@mail.hf.ah.cn
Received 2001-07-30 Accepted 2002-02-25

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jun; 23 (6)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Apparatus A Shimadzu LC-6 high performance liquid chromatography equipment consisted of LC-6A pumps, SCL-6A system controller, SPD-6AV ultraviolet spetrophotometric detector, ODS-C18 column (150 mm × 4.6 mm) (Shimadzu Corp, Japan), and V4.0+ software on a Legend computer.

Subjects Twenty-four healthy volunteers (males 16, females 8, age 20.8 a ± 0.9 a, weight 60 kg ± 6 kg, height 169 cm ± 7 cm) were involved in the study. All subjects passed complete physical examination, revealing no clinically relevant hepatic, renal, cardiac, hematologic, or other diseases, and all had no other drugs two weeks before and during the study. A standardized meal was served. Cigarettes and alcohol were restrained. In accordance with the Declaration of Helsinki, all volunteers gave their written consent to their participation in the study, and the protocol was approved by an Institutional Review Board of Anhui Medical University.

Single oral administration Eighteen healthy volunteers were stratified randomly into three groups. Each group, which consisted of 4 males and 2 females, received single oral leflunomide 20, 40, or 60 mg together with 200 mL water respectively after overnight fast. Blood samples were drawn predose and 1, 2, 4, 8, 12, 18, 24, 48, 96 h and 8, 13, 20, 28 d postdose. After centrifugation, the serum was isolated and stored at −20 ℃ untill analysis.

Repeated oral administration Six healthy volunteers including 4 males and 2 females were given 2 tablets total containing 20 mg of leflunomide together with 200 mL water once daily for 30 consecutive days. Blood samples were taken predose from 1 to 30 d and 1, 2, 4, 8, 12, 16, 20, and 24 h postdose on d 30.

During the whole study course, subjects were instructed by the investigator to report immediately the occurrence of any adverse events.

Analytical method The concentration of A_{771726}, active metabolite of leflunomide, in serum was analyzed by high performance liquid chromatography. The mobile phase components were sodium acetate-acetic acid (0.01 mol/L) and acetonitrile (67:33, v/v, pH 4.6). Ultraviolet wavelength was 290 nm. The flow-rate was 1 mL/min.

The peak areas of the A_{771726} were plotted versus the concentrations of the A_{771726}. Values of unknown serum drug concentrations were determined from calibration curve.

Human serum standard solutions A_{771726} was dissolved in acetonitrile and distilled water (1:1, v/v), then different amount of this solutions was added into blank 5-mL glass tubes and evaporated to dryness by nitrogen. The residues were dissolved with 0.2 mL human serum to make A_{771726} of 0.195, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, and 25 mg/L, respectively, as serum standard solutions.

Serum extraction procedure Serum sample 200 μL, ethanol 200 μL, distilled water 200 μL, and ethyl acetate 4 mL were vortex-mixed in a 10-mL glass tube for 1 min. After centrifugation at 700 × g for 20 min, the organic phase was aspirated. The remainder was extracted by addition of 4 mL ethyl acetate again as described above. The two organic phases were mixed and evaporated to dryness under a gentle stream of nitrogen at 35 ℃ in a water bath. The residues were reconstituted with 200 μL of ethanol, and 50 μL of samples were injected into the chromatography.

Pharmacokinetic calculations Pharmacokinetic parameters were calculated by 3p97 program. Maximum drug serum concentrations (C_{max}) and time of their occurrence (T_{max}) were taken directly from the observed data.

RESULTS

Method validation In the conditions as described above, no significant interfering peaks were observed. The retention time of A_{771726} was 6.4 min (Fig 1). The calibration curve of A_{771726} in serum was in good linearity over the concentration range of 0.195 – 25 mg/L, and the coefficient of correlation was 0.9996. The regression equation was $A = 2140.3 + 46753C$ (A: peak areas, C: serum concentrations). The limit of detection was 0.195 mg/L, and the average recovery was (89.4 ± 2.2) % – (95.3 ± 3.7) % over the range of 0.195 – 25 mg/L (Tab 1). The within-day and between-day reproducibility was shown in Tab 2, and the coefficient of variation was all below 10 %.

Tab 1. Average recovery of A_{771726}. $n = 5$. $\bar{x} \pm s$.

Concentration /mg·L^{-1}	Peak area		Recovery /%
	Serum sample	Standard	
0.195	8 554 ± 485	8 984 ± 507	95 ± 4
3.125	160 838 ± 8 732	172 179 ± 5 792	93 ± 4
12.50	590 719 ± 17 858	638 898 ± 12 544	92.4 ± 1.3
50	2 489 146 ± 19 841	2 785 768 ± 90 271	89.4 ± 2.6

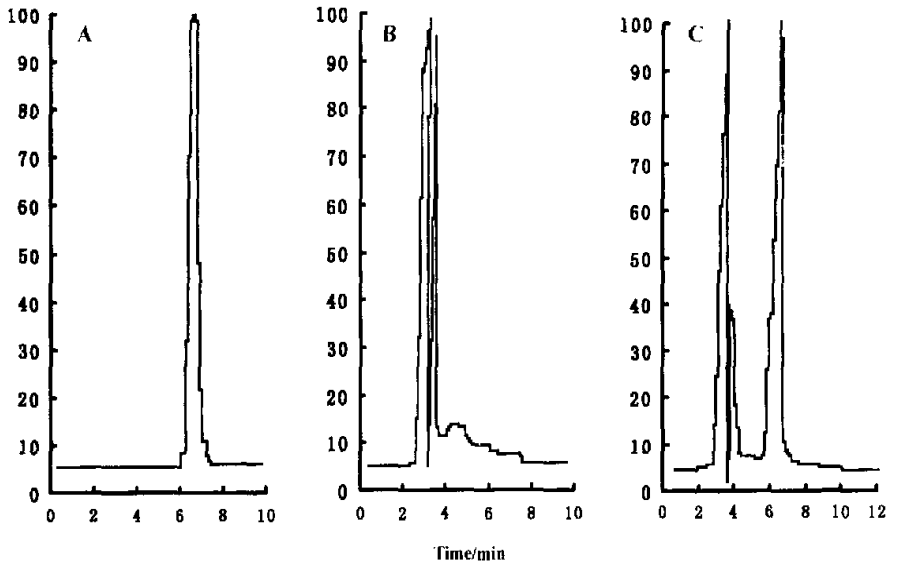

Fig 1. Separation of A_{771726}. A: A_{771726} standard. B: Blank serum. C: A_{771726} in serum.

Tab 2. Within-day and between-day reproducibility of A_{771726}.

Concentration/ mg·L^{-1}	Coefficients of variation/ % Within-day	Between-day
0.195	6.3	7.3
3.125	5.0	6.4
12.50	5.1	7.3
50	4.8	7.3

Single oral administration Data of drug serum concentration of different times in three groups (20, 40, and 60 mg) were shown in Tab 3.

Pharmacokinetic parameters were calculated by 3p97 program, and C_{max} and T_{max} were taken directly from the above data. Drug serum concentration-time curves of 20, 40, and 60 mg groups conformed to one compartment model of the first order absorption. Pharmacokinetic parameters of three dose groups were shown in Tab 4.

Repeated oral administration Data of drug serum concentration of 28, 29, and 30 d were shown in Tab 5. Those suggested drug serum concentrations in six healthy volunteers reach steady state level after oral administration of leflunomide 20 mg once daily for consecutive 30 d. Mean trough concentrations were between 32.0 mg/L and 39.7 mg/L. Within a period of oral administration on d 30, data of drug serum concentration of different times were shown in Fig 2. Drug

Tab 3. A_{771726} serum concentration-time data following single oral administration of leflunomide 20, 40, and 60 mg in 18 volunteers. $n=6$. $\bar{x} \pm s$.

Time/h	Serum concentration/mg·L^{-1} 20 mg	40 mg	60 mg
1	0.9±0.4	1.6±0.4	2.3±0.8
2	1.1±0.4	3.0±1.0	5.2±1.4
4	1.7±0.6	3.9±0.4	5.9±1.5
8	2.3±0.5	4.3±0.4	7.3±1.7
12	1.8±0.6	4.6±1.4	7±4
18	1.8±0.5	5.8±0.7	5.1±1.4
24	2.1±0.4	4.4±0.8	6.0±2.3
48	1.8±0.5	5.5±0.8	7.8±2.5
96	1.9±0.6	5.1±0.8	7.9±1.9
8 d	1.3±0.3	3.2±0.5	5.0±1.1
13 d	1.3±0.4	2.3±0.4	3.8±1.1
20 d	0.58±0.23	1.5±0.5	2.3±0.7
28 d	0.202±0.023	0.29±0.10	0.9±0.4

serum concentration-time curves conformed to one compartment model of the first order absorption. Main pharmacokinetic parameters were shown in Tab 6.

Adverse reaction In the whole study, either the single or in the repeated oral administration of leflunomide, no adverse reactions were observed. We had only found that WBC of one female volunteer significantly lowered than normal level on d 28 in repeated oral administration, but it restored to normal range in 2 weeks after stopping administration.

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jun; 23 (6)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Tab 4. Pharmacokinetic parameters following oral administration of leflunomide 20, 40, and 60 mg in 18 volunteers. $n=6$. $\bar{x} \pm s$.

Parameters	20 mg	40 mg	60 mg
k_a/h^{-1}	0.6 ± 0.3	0.38 ± 0.17	1.2 ± 1.4
k_e/h^{-1}	0.0034 ± 0.0003	0.0042 ± 0.0005	0.0033 ± 0.0003
$T_{1/2,ka}/h$	2.2 ± 2.8	2 ± 8	1.2 ± 0.8
$T_{1/2,ke}/h$	211 ± 18	170 ± 24	252 ± 26
T_{max}/h	13 ± 12	13 ± 4	9 ± 5
$C_{max}/mg \cdot L^{-1}$	2.0 ± 0.5	5.2 ± 0.6	6.7 ± 1.5
$AUC/mg \cdot h \cdot L^{-1}$	647 ± 137	1344 ± 191	2555 ± 907
Cl/f ($L \cdot h^{-1}$)	0.032 ± 0.007	0.030 ± 0.004	0.026 ± 0.009
V/F ($L \cdot h^{-1}$)	0.41 ± 0.10	0.30 ± 0.04	0.39 ± 0.10

Tab 5. Trough concentration of A_{771726} following oral administration of leflunomide daily for consecutive 30 d in 6 volunteers.

Subjects	Serum concentration/$mg \cdot L^{-1}$ d 28	d 29	d 30	$\bar{x} \pm s$
1	36.83	36.20	36.35	36.5 ± 0.3
2	39.31	39.24	40.26	39.7 ± 0.8
3	37.41	35.33	34.67	36.4 ± 1.5
4	31.30	31.25	33.47	32.0 ± 1.8
5	32.74	33.10	36.15	34.0 ± 1.9
6	36.95	37.32	37.16	37.14 ± 0.19
$\bar{x} \pm s$	36 ± 3	35.4 ± 2.9	36.4 ± 2.4	

Tab 6. Main pharmacokinetic parameters after oral administration of leflunomide 20 mg once daily for 30 d in 6 volunteers.

Parameters	$\bar{x} \pm s$
$C_{max}/mg \cdot L^{-1}$	41.5 ± 2.4
T_{max}/h	307 ± 75
$AUC_{0-24}/mg \cdot h \cdot L^{-1}$	22099 ± 1234
$C_{av}/mg \cdot L^{-1}$	38.4 ± 2.1
DF/%	14 ± 6

C_{av}: average concentration; DF: peak-trough fluctuation

DISCUSSION

Leflunomide has demonstrated clinical efficacy in rheumatoid arthritis. After oral administration, the highly non-polar leflunomide was metabolized completely during first pass, and 99 % of its primary, pharmacologically active metabolite, A_{771726}, was bound to plasma proteins, primarily albumin. The pharmacokinetics of

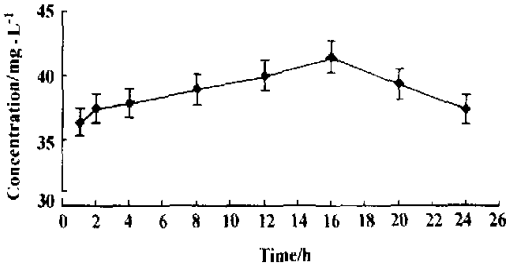

Fig 2. A_{771726} serum concentration at different time in six volunteers. $\bar{x} \pm s$.

A_{771726} appeared to be linear over the dosage range of 5 mg to 25 mg daily in 6 month phase Ⅱ trials. Mean plasma half-life was 360 – 432 h, with total plasma clearance of 0.30 $mL \cdot kg \cdot h^{-1}$. The less non-polar A_{771726} was cleared *via* several metabolic pathways, including biliary and urinary excretion[12].

Our results indicated drug serum concentration curves, either in the single or repeated oral administration of leflunomide, conformed to one compartment model of the first order absorption. Moreover, leflunomide was metabolized into A_{771726} rapidly, and A_{771726} was eliminated slowly, and serum concentration of A_{771726} increased porportionally with dosage range 20 to 60 mg, which conformed to foreign data[13].

This investigation indicated that repeated oral administration of leflunomide 20 mg once daily for consecutive 30 d, the serum concentration neared steady level, mean trough concentrations were between 32.0 mg/L and 39.7 mg/L. All these observations were consistent with that the effective therapeutic dose of leflunomide treating rheumatoid arthritis was between 11 and 25 mg with oral administration. The effective concentration of A_{771726} was 13 mg/L above. The mean

steady concentration was between 30 mg/L and 40 mg/L, which could produce stable therapeutic effect. All of those suggested oral administration of leflunomide 20 mg once daily would produce clinical therapeutic effect in rheumatoid arthritis, and provided dosage recommendations for phase Ⅱ trials.

On the basis of results of adverse reaction, although no adverse reactions were observed, we should heed adverse events, especially changes of blood cells.

REFERENCES

1 Herrmann ML, Schleyerbach R, Kirschbaum BJ. Leflunomide: an immunomodulatory drug for the treatment of rheumatoid arthritis and other autoimmune diseases. Immunopharmacology 2000; 47: 273-89.
2 Schattenkirchner M. The use of leflunomide in the treatment of rheumatoid arthritis: an experimental and clinical review. Immunopharmacology 2000; 47: 291-8.
3 Bartlett RR, Campion G, Musikic P, Schleyerbach R, Zielinski T, Schlorlemmer HU. In: Lewis AJ, Furst DE, editors. Nonsteroidal anti-inflammatory drugs: mechanisms and clinical uses. New York: Marcel dekker; 1994. p 349-52.
4 Pasternak RD, Wadopian NS, Wright RN, Siminoff P, Gylys JA, Buyniski J. Disease modifying activity of HWA486 in rat adjuvant arthritis. Agents Actions 1986; 21: 241-3.
5 Popvic S, Bartlett RR. Disease modifying activity of HWA486 on the development of SLE in MLR/lpr mice. Agents Actions 1986; 19: 313-4.
6 Smith LL, Glaser RL, Miller ST, Cohen GM. Efficacy of novel immunomodulators leflunomide and rapamycin in autoimmune uveitis. FASEB J 1992; 21: 284-9.
7 Davis JP, Cain GA, Pitts WJ, Magolda RL, Copeland RA. The immunosuppressive metabolite of leflunomide is a potential inhibitor of human dihydroorotate dehydrogenase. Biochemistry 1996; 35: 1270-3.
8 Dias VC, Lucien J, LeGatt DF, Yatscoff RW. Measurement of the active leflunomide metabolite (A_{771726}) by reverse phase high performance liquid chromatography. Ther Drug Monit 1995; 17: 84-8.
9 Lucien J, Dias VC, LeGatt DF, Yatscoff RW. Blood distribution and single-dose pharmacokinetics of leflunomide. Ther Drug Monit 1995; 17: 454-9.
10 Silva HT, Shorthouse R, Morris RE. Single- and multiple-dose pharmacokinetics and pharmacodynamics of leflunomide's active metabolite A_{771726} in normal Lewis rats. Transplant Proc 1996; 28: 3092-4.
11 Hsieh GC, Andrews JM, Fey TA, Bretheim PT. Pharmacokinetics of the immunosuppressive and antirheumatic drug, leflunomide, measured by high performance liquid chromatography. FASEB J 1995; 9: A946.
12 Vlastimir M, Zlatko D, Blaz R, Ivo J, Dimitrije M, Jovan D, *et al*. Safety and effectiveness of leflunomide in the treatment of patients with active rheumatoid arthritis. Arthritis Rheum 1995; 38: 1595-603.
13 Weinblatt ME, Kremer JM, Coblyn JS, Maier AL, Helfgott SM, Morrell M, *et al*. Pharmacokinetics, safety, and efficacy of combination treatment with methotrexate and leflunomide in patients with active rheumatoid arthritis. Arthritis Rheum 1999; 42: 1322-8.

来氟米特在中国健康志愿者的药代动力学

李 俊[1]，姚宏伟，金 涌，张运芳，李常玉，李元海，徐叔云 （安徽医科大学临床药理研究所，合肥 230032，中国）

关键词 来氟米特；药物动力学；高压液相色谱法

目的：研究来氟米特在中国健康志愿者的药代动力学。**方法**：18名健康志愿者随机分为三组，分别单剂量口服来氟米特(20, 40, 60 mg)；6名健康志愿者多剂量口服来氟米特20 mg/d，连续30 d。高效液相色谱法检测来氟米特活性代谢物(A_{771726})。**结果**：A_{771726}的血药浓度变化符合一级吸收的一房室模型。单剂量口服来氟米特(20, 40, 60 mg)的主要药动学参数为：$T_{1/2,ke}$(h)：211±18，170±24，252±26；T_{max}(h)：13±12，13±4，9±5；C_{max}(mg/L)：2.0±0.5，5.2±0.6，6.7±1.5；AUC ($mg \cdot h \cdot L^{-1}$)：647±137，1344±191，2555±907。口服来氟米特20 $mg \cdot d^{-1}$，连续30 d，血药浓度达到稳态水平。平均谷浓度为32.01-39.72 mg/L。C_{max}，T_{max}和AUC_{0-24}分别为(41.5±2.4) mg/L，(307±75) h和(22099±1234) $mg \cdot h \cdot L^{-1}$。**结论**：本品口服吸收快，消除慢。口服20 $mg \cdot d^{-1}$，连续30 d，血药浓度达到稳态水平。在所试剂量范围内，A_{771726}的血药浓度变化符合一级吸收的一房室模型。

（责任编辑 吕 静）