ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Jul; 20 (7): 655 - 658
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

Pharmacokinetics of 2-hydroxyflutamide, a major metabolite of flutamide, in normal and CCl_4-poisoned rats

XU Chang-Jiang, LI Duan[1]
(*Department of Pharmacology, School of Pharmacy, Shanghai Medical University, Shanghai 200032, China*)

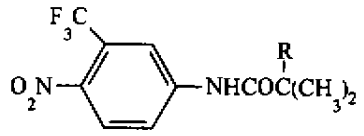
KEY WORDS 2-hydroxyflutamide; pharmacokinetics; carbon tetrachloride poisoning; high pressure liquid chromatography

ABSTRACT

AIM: To study the pharmacokinetics of 2-hydroxyflutamide (HF), a major active metabolite of flutamide (Flu), in normal and CCl_4-poisoned rats. **METHODS**: Normal and CCl_4-poisoned rats were given ig HF 25 mg · kg^{-1}. HF concentrations of plasma were determined by HPLC with YWG C 18 column, Flu was used as an internal standard. The mobile phase was composed of methanol : water = 3 : 2 (vol), and absorbance was measured at λ 295 nm. **RESULTS**: HF elimination was inhibited in CCl_4-poisoned rats compared with normal rats. K decreased from (0.11 ± 0.05) to (0.05 ± 0.01) h^{-1} ($P < 0.01$), $T_{1/2}$ was prolonged from (6.8 ± 1.9) to (14 ± 4) h ($P < 0.01$), Cl decreased from (0.18 ± 0.06) to (0.12 ± 0.02) L · kg^{-1} · h^{-1} ($P < 0.05$), AUC increased from (149 ± 47) to (226 ± 54) mg · L^{-1} · h ($P < 0.05$). **CONCLUSION**: This HPLC assay was sensitive and precise, and the elimination of HF was inhibited due to CCl_4 poisoning.

INTRODUCTION

Flutamide (Flu) is a nonsteroidal antiandrogen devoid of other hormonal activities, and effective in the treatment of prostate cancer and benign prostatic hyperplasia[1]. Flu is rapidly and completely absorbed, and metabolized widely *in vivo*[2]. 2-Hydroxyflutamide (HF) is the major active metabolite of Flu, and its plasma concentration, elimination half life together with blockade of androgen receptor is greater than that of Flu[2]. The therapeutic effect of Flu depends mainly on HF *in vivo*. There were some studies about the pharmacokinetics of Flu[3-8]. Some pharmacokinetic parameters of HF were obtained when HF was regarded as the metabolite of Flu after Flu dose[4-8], and there was no report about pharmacokinetics studies of HF when it was given directly. We determined Flu and HF simultaneously by HPLC assay in normal and CCl_4-poisoned rats[8]. We studied the pharmacokinetics of HF when HF was given ig in normal and CCl_4-poisoned rats further in this article.



R = H Flutamide
R = OH 2-Hydroxyflutamide

MATERIALS AND METHODS

Reagents HF was synthesized by Prof XIA Peng (Department of Organic Chemistry, School of Pharmacy, Shanghai Medical University). Flu was made by Hongqi Pharmaceutical Factory of Shanghai Medical University, and used as the internal standard. Suspension of HF was prepared in 0.5 % sodium carboxymethyl cellulose at the concentration of 5.0 g · L^{-1}. Methanol was HPLC reagent. Cyclohexane, acetic ether, and CCl_4 were of AR.

Standard solution Stock solution of HF or Flu was prepared in methanol at the concentration of 1.0 g · L^{-1}, and stored below 4 ℃.

Rats Sprague-Dawley rats (Grade Ⅱ, ♂, 230 - 260 g, Certificate № 02-22-2 conferred by Shanghai Medical Experimental Animal Management

[1] Correspondence to Prof LI Duan. Phn 86-21-6404-1900, ext 2556. Fax 86-21-6403-7260. E-mail dli@shmu.edu.cn
Received 1998-10-23 Accepted 1998-01-29

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Jul; 20 (7)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Committee) were purchased from Experimental Animal Center of Shanghai Medical University. Rats were injected sc CCl_4 1.5 mL·kg^{-1} to produce liver poisoned model. Serum alanine aminotransferase (AlaAT), total bilirubin (Bil), and albumin (Alb) were determined at 24, 48, and 72 h[8]. CCl_4-poisoned rats were used at 24 h after sc CCl_4 in following experiments.

Medication and sampling Rats were given ig HF 25 mg·kg^{-1}. Blood samples were collected at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, and 48 h *via* caudal vein heparinized, and plasma was separated and stored below −20 ℃. Plasma 0.1 mL with internal standard Flu (10 mg·L^{-1}, 1.0 μg in methanol 0.1 mL) and 1 mL pH 7.6 phosphate buffer solution (0.01 mol·L^{-1}) was extracted with the mixture of cyclohexane:acetic ether = 4:1 (vol) 3 mL twice, after centrifugation (1 500 × *g*, 15 min), and the solvent was removed under a nitrogen flow at 40 ℃. The residue was dissolved in 50 μL of methanol, and 10 μL was injected for HPLC.

HPLC assay Waters HPLC system consisted of 510 pump, 486 UV detector, U6k injector (Millipore Corporation, USA). HPLC column: YWG C 18, 10 μm, 3.9 mm × 300 mm. An equilibrating model recorder XWT-104 was the product of Shanghai Dahua Instrument Factory. Mobile phase was a mixture of methanol: water = 3 : 2 (vol). Chromatography assay was performed at room temperature using a flow rate of 1 mL·min^{-1} which produced a back pressure of 2000 PSI. Absorbance was measured at 295 nm with 0.05 absorbance units of full scale (AUFS).

Pharmacokinetics evaluation The pharmacokinetic parameters of HF was calculated and analyzed with PK-GRAPH software (provided by Shanghai Second Medical University). Comparison of pharmacokinetic parameters between normal and CCl_4-poisoned rats was carried out by *F*-test with NDST software.

RESULTS

Chromatography There were no endogenous interferences at the retention time (*Tr*) of HF and Flu from the biological drug-free control. The *Tr* of HF and Flu were 5.8 and 8.6 min, respectively. The minimal detection concentration of HF was 0.1 mg·L^{-1} (Fig 1).

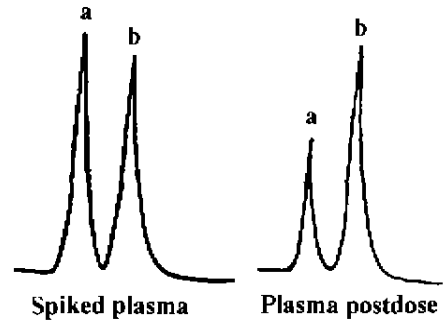


Fig 1. Chromatograms of HF (a, 5.8 min) and Flu (b, 8.6 min) in rats plasma.

Calibration and quality control The standard curve showed a good linearity over a range of 0.2 −20 mg·L^{-1} for HF ($\bar{Y} = 0.7205X - 0.0184$, $r = 0.9997$). The mean recoveries of HF for the method were 94 % ± 4 % at 1.0 mg·L^{-1}, 98 % ± 7 % at 5 mg·L^{-1}, and 96 % ± 5 % at 20 mg·L^{-1} ($n = 5$). The intra and inter-day accuracy was determined by calculating the relative standard deviation (RSD) at 3 control concentrations of HF, and RSD % averaged 4.45 % and 6.69 %, respectively. The detection limit of HF was 2 ng at signal-to-noise ratio of 2.

Pharmacokinetics The curve for HF after ig in rats was fitted with 1-compartment model. HF elimination was inhibited in CCl_4-poisoned rats compared with that in normal rats. *K* decreased from (0.11 ± 0.03) to (0.05 ± 0.01) h^{-1} ($P < 0.01$), $T_{1/2}$ was prolonged from (6.8 ± 1.9) to (14 ± 4) h ($P < 0.01$) (Tab 1, Fig 2).

Tab 1. Pharmacokinetics of HF in normal and CCl_4-poisoned rats. $n = 5$ rats. $\bar{x} \pm s$.
$^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* normal.

Parameter	Normal	Poisoned
K_a/h^{-1}	0.40 ± 0.12	0.24 ± 0.16^b
K/h^{-1}	0.11 ± 0.03	0.05 ± 0.01^c
$T_{1/2}$/h	6.8 ± 1.9	14 ± 4^c
$V_d \cdot F^{-1}$/L·kg^{-1}	2.1 ± 0.3	2.7 ± 0.8^a
$Cl \cdot F^{-1}$/L·kg^{-1}·h^{-1}	0.18 ± 0.06	0.12 ± 0.02^b
AUC /mg·L^{-1}·h	149 ± 47	226 ± 54^b
T_{max}/h	4.0 ± 0.6	11.6 ± 2.7^c
C_{max}/mg·L^{-1}	9.3 ± 1.9	6.6 ± 2.7^a

DISCUSSION

We used Flu as an internal standard to determine

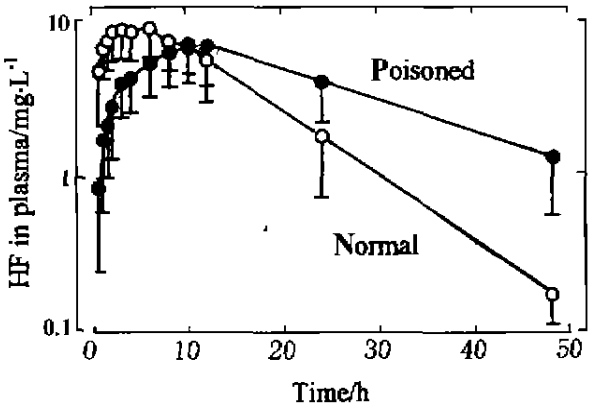


Fig 2. HF concentration in plasma of normal and CCl_4-poisoned rats. $n = 5$ rats. $\bar{x} \pm s$.

the pharmacokinetics of HF by HPLC method when rats were given ig HF, and the method was simple and sensitive. The detection limit of HF was 2 ng. The maximal absorbance wavelength of Flu and HF were 294.4 and 291.8 nm, respectively, and we chose 295 nm as the determination wavelength. There were some HPLC methods for analysis of Flu and/or HF when Flu was administered[5-8]. The plasma sample volume was 2 mL in the first method, and its detection limit of HF was 2 ng[5]. There was pbosphate in the second method, and its detection limit was 6 ng[6]. The detection limit of HF was 4.5 ng in the third method[7]. In our former report, HF detection limit was 2 ng too[8].

The pharmacokinetics of HF in normal rats was provided in our study. The elimination half life of Flu with its active metabolite HF was (1.19 ± 0.29) h and (9.9 ± 0.7) h in normal rats given ig Flu, respectively[8]. If rats were given ig HF directly, its elimination half life was (6.9 ± 1.9) h. There might be a feedback inhibition of HF elimination due to other metabolites while rats were given ig Flu.

Injecting sc CCl_4 could induce cholestasis in rats, and slow down the absorption of drug through gastrointestinal duct[8,9]. Our results indicated that the absorption of HF was slowed down in CCl_4-poisoned rats compared with that in normal rats. The result was in accordance with the absorption of Flu in CCl_4-poisoned rats[8]. C_{max} and T_{max} depend on absorption, distribution, and elimination of drug *in vivo*. In our former report, T_{max} of Flu was delayed in CCl_4-poisoned rats, and C_{max} of Flu was higher than that in normal rats. The absorption and elimination of Flu were both slowed down in CCl_4-poisoned rats, but the distribution volume of Flu decreased significantly in CCl_4-poisoned rats ($P < 0.01$). In this study, T_{max} of HF was delayed in CCl_4-poisoned rats ($P < 0.01$), and C_{max} of HF decreased from (9.3 ± 1.9) mg·L^{-1} to (6.6 ± 2.7) mg·L^{-1} ($P > 0.05$). The absorption and elimination of HF were both slowed down in CCl_4-poisoned rats, but there was no significant difference of HF distribution volume between CCl_4-poisoned rats and normal rats ($P > 0.05$). Liver is a major organ of drug metabolism. The activities of metabolizing enzymes would be decreased on account of CCl_4-poisoned liver[9]. The result that HF elimination was significantly inhibited in CCl_4-poisoned rats suggested that liver should be the major organ of HF metabolism.

REFERENCES

1 Narayan P, Trachtenberg J, Lepor H, Debruyne FMJ, Tewari A, Stone N, *et al*. A dose-response study of the effect of flutamide on benign prostatic hyperplasia: result of a multicenter study. Urology 1996; 47: 497 – 504.

2 Katcher B, Buxbaum S. Disposition of a new, nonsteroid, antiandrogen, α,α,α-trifluoro-2-methyl-4′-nitro-*m*-propionotoluidide (flutamide), in men following a single oral 200 mg dose.
Clin Endocrinol Metab 1975; 41: 373 – 9.

3 Radwanski E, Perentesis G, Symchowicz S, Zampaglione N. Single and multiple dose pharmacokinetic evaluation of flutamide in normal geriatric volunteers. J Clin Pharmacol 1989; 29: 554 – 8.

4 Schulz M, Schmoldt A, Donn F, Becker H. The pharmacokinetics of flutamide and its major metabolites after a single oral dose and during chronic treatment. Eur J Clin Pharmacol 1988; 34: 633 – 7.

5 Asade RH, Prizont L, Muino JP, Tessler J. Steady-state hydroxyflutamide plasma levels after the administration of two dosage forms of flutamide. Cancer Chemother Pharmacol 1991; 27: 401 – 5.

6 Belanger A, Giasson M, Couture J, Dupont A, Cusan L, Labrie F. Plasma levels of hydroxy-flutamide in patients with prostatic cancer receiving the combined hormonal therapy: an LHRH agonist and flutamide. Prostate 1988; 12: 79 – 84.

7 *Farthing D, Sica D, Fakhry I, Walters DL, Cefali EA*, Allan G. Determination of flutamide and hydroxyflutamide in dog plasma by a sensitive high performance liquid chromatography method utilizing mid-bore chromatography. Biomed Chromatogr 1994; 8: 251 – 4.

8 Xu CJ, Li D. Pharmacokinetics of flutamide and its

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Jul; 20 (7)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

metabolite 2-hydroxyflutamide in normal and hepatic injury rats. Acta Pharmacol Sin 1998; 19: 39-43.
9 Howden CW, Birnie GG, Brodie MJ. Drug metabolism in liver disease. Pharmacol Ther 1989; 40: 439-74.

氟他胺的主要代谢产物 2-羟基氟他胺在正常及 CCl_4 中毒大鼠的药物动力学

许长江，李 端（上海医科大学药学院药理学教研室，上海 200032，中国）

关键词 2-羟基氟他胺；药物动力学；四氯化碳中毒；高压液相色谱法

目的：研究氟他胺的一个主要活性代谢产物 2-羟基氟他胺(HF)在正常及 CCl_4 中毒大鼠的药物动力学。**方法**：正常及 CCl_4 中毒大鼠灌胃 HF 25 mg·kg^{-1}。采用高压液相色谱法测定 HF 浓度，以氟他胺为内标，色谱柱填料为 YWG C18，流动相为甲醇:水 = 3:2（体积比），检测波长为 295 nm。**结果**：与正常大鼠比较，HF 在 CCl_4 中毒大鼠的消除明显受到抑制。K 由 (0.11 ± 0.05) h^{-1} 减小到 (0.05 ± 0.01) h^{-1} ($P < 0.01$)，$T_{1/2}$ 由 (6.8 ± 1.9) h 延长到 (14 ± 4) h ($P < 0.01$)，Cl 由 (0.18 ± 0.06) L·kg^{-1}·h^{-1} 下降到 (0.12 ± 0.02) L·kg^{-1}·h^{-1} ($P < 0.05$)，AUC 由 (149 ± 47) mg·L^{-1}·h 增加到 (226 ± 54) mg·L^{-1}·h ($P < 0.05$)。**结论**：该高压液相色谱法灵敏、准确。在 CCl_4 中毒大鼠，HF 的消除明显受到抑制。

（责任编辑 刘俊娥）

《现代药理实验方法》出版

中国药理学会理事长、中国医学科学院药物研究所张均田教授主编的《现代药理实验方法》已由北京医科大学中国协和医科大学联合出版社于 1998 年 10 月出版，全书 380 多万字，分上下册、29 篇、158 章，并附有近千幅插图。

此书以介绍现代药理学的新方法和新技术为主旨，融分子生物学、生物化学、生理学、细胞生物学、免疫学、毒理学、药物化学、计算机及现代仪器分析技术于一体，按照不同类型（整体、离体、组织培养），不同层次（整体、组织、细胞、亚细胞和分子水平）分章分节述说。

编者和作者是活跃在各专业领域里的有实践经验的科学工作者，他们之中有老一辈科学家、年富力强的中年学者以及有才华的青年科学工作者和博士生。美国、加拿大、澳大利亚、德国和瑞典的一些科学家和华裔学者亦欣然命笔，加入了编著者的行列。

本书题材广泛，内容新颖，能够满足药理学及其他相关学科基础研究工作者的共同需要，是药理学及其他学科科研人员、高等医药院校教师、高年级学生、硕士生、博士生的重要参考工具书。

本书定价每套 298 元，欢迎读者购买。

联系地址：100050 北京市 中国医学科学院药物研究所 新楼 3-27 室 屈志炜 电话：010-6316-5181