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Pharmacokinetics of flutamide and its metabolite 2-hydroxyflutamide in normal and hepatic injury rats

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

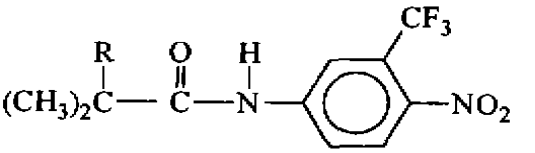
KEY WORDS flutamide; 2-hydroxyflutamide; pharmacokinetics; liver; carbon tetrachloride poisoning; high pressure liquid chromatography

AIM: To develop a new HPLC assay to study the pharmacokinetics of flutamide (Flu) and its active metabolite 2-hydroxyflutamide (HF) in rats. **METHODS**: Normal or hepatic injury rats were given ig Flu 50 $mg \cdot kg^{-1}$. Reverse phase HPLC was developed with a μ-Bondapak C 18 column. Internal standard was methyltestosterone. The mobile phase was a mixture of methanol : acetonitrile : water : diethyl ether = 40:20:35:1 (vol). Absorbance was measured at λ_{234} nm. **RESULTS**: The pharmacokinetic parameters of Flu were as follows: in normal rats, $K = 0.62 \pm 0.16\ h^{-1}$, $Cl = 6.0 \pm 1.0\ L \cdot kg^{-1} \cdot h^{-1}$, $AUC = 8.6 \pm 1.3\ mg \cdot L^{-1} \cdot h$, $C_{max} = 2.4 \pm 0.7\ mg \cdot L^{-1}$; in hepatic injury rats, $K = 0.16 \pm 0.03\ h^{-1}$, $Cl = 0.63 \pm 0.29\ L \cdot kg^{-1} \cdot h^{-1}$, $AUC = 100 \pm 44\ mg \cdot L^{-1} \cdot h$, $C_{max} = 6.7 \pm 2.8\ mg \cdot L^{-1}$. The pharmacokinetic parameters of HF were as follows: in normal rats, $K_{(m)} = 0.07 \pm 0.01\ h^{-1}$, $AUC = 219 \pm 22\ mg \cdot L^{-1} \cdot h$, $C_{max} = 8.6 \pm 0.6\ mg \cdot L^{-1}$; in hepatic injury rats, $K_{(m)} = 0.05 \pm 0.01\ h^{-1}$, $AUC = 170 \pm 42\ mg \cdot L^{-1} \cdot h$, $C_{max} = 3.8 \pm 0.8\ mg \cdot L^{-1}$. There were significant differences between the parameters of normal and hepatic injury rats ($P < 0.01$) except AUC of HF ($P > 0.05$). **CONCLUSION**: This HPLC assay was sensitive and precise, and the elimination of Flu and HF was inhibited significantly due to hepatic injury.

Flutamide (Flu) is a nonsteroid antiandrogen drug employed in the treatment of prostate cancer, and it is rapidly and completely absorbed and excreted mainly through the kidney. At least 11 metabolites are present. The major metabolite 2-hydroxyflutamide (HF) blocks androgen recepors greater than Flu. The elimination half-time of HF is greater than Flu, too[1]. The therapeutic effect of Flu is largely due to HF *in vivo*, so it is important to mornitor the plasma levels of Flu and HF simultaneously.

Several methods for the determination of Flu and HF have been reported. They have certain limitations, such as complex detection equipment employing radioactivity and electron capture detection[2,3], a large sample volume (2 mL)[4], lack of an internal standard[3-6], or only for determination of HF[4,5]. Accordingly, we intended to develop a simple and sensitive HPLC method to determine Flu and HF simultaneously.

Flu is primarily metabolized via hepatic metabolism. Hepatic injury is associated with decreases in metabolizing enzymes and cytochrome P450 contents[7]. The clearance of Flu might be prolonged due to hepatic injury. This study was designed to compare the pharmacokinetics of Flu and its active metabolite HF, and study the excretion in urine during hepatic injury.



R = H Flutamide
OH 2-Hydroxyflutamide

MATERIALS AND METHODS

Reagents Flu (yellow crystal, purity > 99 %, mp 113 - 114.5 ℃) was made by Hongqi Pharmaceutical Factory of Shanghai Medical University. Suspension of Flu was prepared in 0.5 % sodium carboxymethyl cellulose at the concentration of 5.0 $g \cdot L^{-1}$. HF was synthesized by Prof XIA Peng (Department of Organic Chemistry, School of Pharmacy, Shanghai Medical University). Methanol and acetonitrile were HPLC reagents. Cyclohexane, diethyl ether, and CCl_4 were of AR. Methyltestosterone was used as an internal standard, and purchased from Sigma.

[1] Correspondence to Prof LI Duan. Phn 86-21-6404-1900, ext 2558. Fax 86-21-6403-7260. E-mail dli@shmu.edu.cn
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Standard solution Stock solution of Flu or HF was prepared in methanol at the concentration of 1.0 $g \cdot L^{-1}$, and stored below 4 ℃.

Rats Sprague-Dawley rats (conventional grade, ♂, n = 25, 240 – 270 g. Certificate No 02-22-11 conferred by Shanghai Medical Experimental Animal Management Committee) were purchased from Experimental Animal Center of Shanghai Medical University. Rats were injected sc CCl_4 1.5 $mL \cdot kg^{-1}$. Serum alanine aminotransferase (AlaAT), total bilirubin (Bil), and albumin (Alb) were determined at 24, 48, and 72 h[8] (Tab 1). CCl_4 poisoning rats were used 24 h after sc CCl_4 in following experiments.

Tab 1. Serum biochemistry after CCl_4 poisoning. $n = 5$ **rats,** $x \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* **normal.**

Time/h	AlaAT/U	Bil/mg·L^{-1}	Alb/g·L^{-1}
0	28 ± 11	7.4 ± 2.5	23 4 ± 1.7
24	440 ± 248[c]	168 ± 121[c]	23.0 ± 2.0[a]
48	1 000 ± 600[c]	78 ± 49[c]	25.2 ± 1.9[a]
72	455 ± 145[c]	19.2 ± 6.2[c]	23.4 ± 2.1[a]

Medication and sampling Rats were given ig Flu 50 $mg \cdot kg^{-1}$. 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, plasma was separated. Urine samples were collected by individual metabolic cage. Plasma 0.1 mL or urine 0.5 mL with internal standard methyltestosterone (15 $mg \cdot L^{-1}$, 0.75 μg in methanol 0.05 mL) and 1 mL pH 7.6 phosphate buffer solution (0.01 $mol \cdot L^{-1}$) was extracted with 3 mL of organic solution (cyclohexane : diethyl ether = 9 : 1, vol/vol) twice, after centrifugation (2000 × g, 15 min), the solvent was evaporated at 50 ℃ under a nitrogen flow. The residue was dissolved in 50 μL of methanol, and 10 μL was injected for HPLC.

HPLC Waters HPLC system consisted of 510 pump, 486 UV detector, U6k injector (Millipore Corporation, USA). HPLC column: μ-Bondapak C 18, 10 μm, 3.9 mm × 300 mm (USA). An equilibrating model recorder XWT-104 was the product of Shanghai Dahua Instrument Factory. Mobile phase was a mixture of methanol : acetonitrile : water : diethyl ether = 40 : 20 : 35 : 1 (vol), prepared and degassed daily. Chromatography assay was performed at room temperature (20 ℃) using a flow rate of 1 $mL \cdot min^{-1}$ which produced a back pressure of 2000 PSI. Absorbance was measured at 234 nm with 0.03 absorbance units of full scale (AUFS).

Quantitation and linearity The standard curves showed a good linearity over a range of 0.1 – 20 $mg \cdot L^{-1}$ for Flu (plasma $\hat{Y} = -0.054 + 1.41\ X$, $r = 0.9989$; urine $\hat{Y} = -0.0078 + 1.11\ X$, $r = 0.9993$) and HF (plasma $\hat{Y} = -0.0065 + 1.11\ X$, $r = 0.9998$; urine $\hat{Y} = -0.019 + 1.20\ X$, $r = 0.9994$).

Recovery Extracting recovery for the method was determined by evaluating the peak height ratio of extracted plasma to unextracted methanol standards by adding 0.1, 0.5, or 2.0 μg Flu and HF to 0.1 mL blank plasma or 0.5 mL blank urine (Tab 2).

Tab 2. Extracting recovery of Flu and HF in plasma or urine. $n = 4$ **experiments,** $x \pm s$.

Added/ mg·L^{-1}	Plasma/ %		Urine · %	
	Flu	HF	Flu	HF
1	94 ± 7	93 ± 9	89 ± 8	88 ± 7
5	95 ± 4	96.0 ± 2.0	90 ± 7	89 ± 8
20	98 ± 5	96 ± 10	94 ± 5	92 ± 6

The accuracy and precision of the method were determined by calculating the coefficiency of variation (CV) at each control concentration of either Flu or HF (Tab 3).

Tab 3. Combined intra and inter-day accuracy and precision. $n = 5$ **experiments,** $x \pm s$.

Drug	/mg·L^{-1}	Intra-day /mg·L^{-1}	CV %	Inter-day /mg·L^{-1}	CV %
Flu	1.0	1.04 ± 0.01	6.46	1.04 ± 0.05	5.02
	5 0	5.03 ± 0.07	1.39	5.01 ± 0.11	2.15
	20 0	20.1 ± 0.8	4.09	20 1 ± 1.0	5.14
HF	1.0	0.96 ± 0.06	5.71	1 00 ± 0 07	6.70
	5.0	5.03 ± 0.11	2 22	5.01 ± 0.12	2.21
	20.0	19.9 ± 1.2	6.14	20.5 ± 0.8	3 82

Pharmacokinetics The pharmacokinetic parameters of Flu and its metabolite HF were calculated according to the method[9]. The data were analyzed with a PK-GRAPH software (provided by Department of Pharmacology, Shanghai Second Medical University) on a 586 personal computer to determine the compartment model and the pharmacokinetic parameters. Comparison of pharmacokinetic parameters between groups was carried out with F-test.

RESULTS

Chromatography There were no endogenous interferences at the retention time (T_r) of Flu, HF, and methyltestosterone from the biological drug-free control (Fig 1). The T_r of HF, Flu, and methyltestosterone were 4.5, 5.5, and 6.6 min, respectively. The minimal detection concentrations of Flu and HF were 0.05 and 0.1 mg·L^{-1}, respectively, at signal-to-noise ratio of 2. The detection limits of Flu and HF were 1 and 2 ng, respectively.

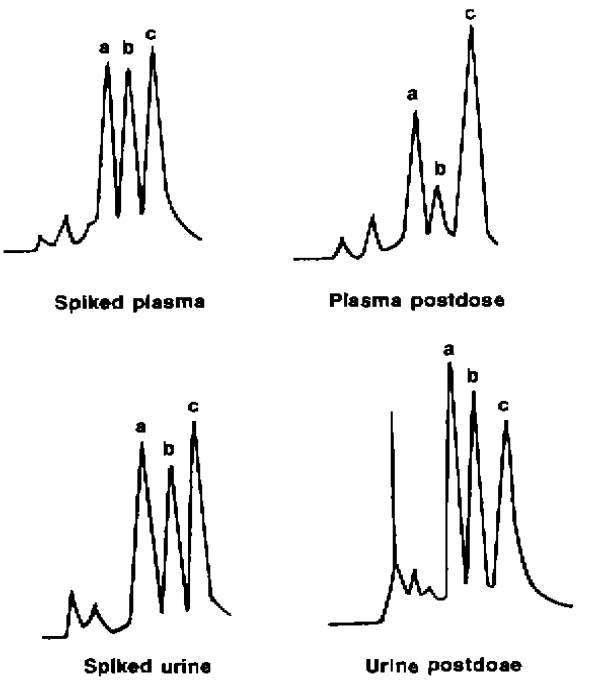

Fig 1. Chromatograms of HF (a), Flu (b), and methyltestosterone (c).

Pharmacokinetics The curve for Flu after ig in rats was fitted with 1-compartment model. Flu elimination was inhibited due to hepatic injury in rats, K decreased from 0.62 ± 0.16 to 0.16 ± 0.03 h^{-1} ($P < 0.01$), $T_{\frac{1}{2}}$ was prolonged from 1.19 ± 0.29 to 4.4 ± 0.8 h ($P < 0.01$), clearance decreased from 6.0 ± 1.0 to 0.63 ± 0.29 L·kg^{-1}·h^{-1} ($P < 0.01$). The AUC of Flu was higher in rats with hepatic injury (100 ± 44 mg·L^{-1}·h) than that in normal rats (8.6 ± 1.3 mg·L^{-1}·h) ($P < 0.01$). The V_d for Flu was lower in rats with hepatic injury (3.9 ± 1.8 L·kg^{-1}) than that in normal rats (10.2 ± 2.4 L·kg^{-1}) ($P < 0.01$).

$K_{(m)}$ is the constant of elimination rate of metabolite. $K_{(m)}$ of HF were 0.07 ± 0.01 h^{-1} in normal rats and 0.05 ± 0.01 h^{-1} in hepatic injury rats ($P < 0.01$). $T_{\frac{1}{2}}$ was prolonged from 9.9 ± 0.7 to 14.8 ± 1.9 h due to hepatic injury ($P < 0.01$). AUC of HF tended to be lower in rats with hepatic injury ($P > 0.05$). The elimination of HF was inhibited, too. The elimination of HF was fitted with elimination rate limited (ERL) model, and K was greater than $K_{(m)}$ (Fig 2, Tab 4).

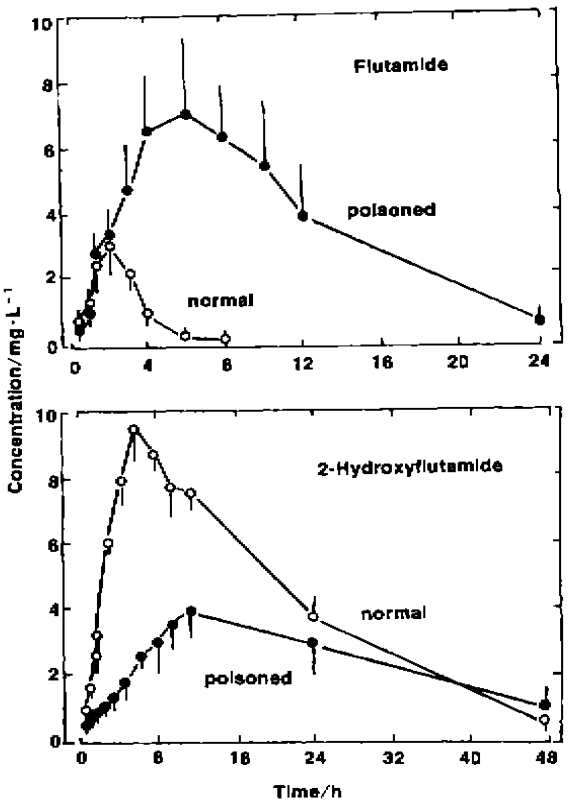

Fig 2. Flu and HF concentrations of plasma in normal and CCl_4-poisoned rats. $n = 5$ rats, $\bar{x} \pm s$.

Excretion in urine Percentage of cumulative excretion of Flu in urine in normal and hepatic injury rats at the dosage of 50 mg·kg^{-1} were 0.078 % ± 0.023 % and 0.078 % ± 0.027 %, respectively ($n = 5$ rats, $P > 0.05$); but there was difference in % of cumulative urinary excretion of HF between normal and hepatic injury rats: 0.067 % ± 0.024 % *vs* 0.11 % ± 0.023 % ($n = 5$ rats, $P < 0.05$).

DISCUSSION

We developed a simple and sensitive procedure which could be employed for the analysis of Flu and

Tab 4. Pharmacokinetics of Flu and HF in normal and CCl_4-poisoned rats. $n = 5$ rats, $\bar{x} \pm s$. $^cP < 0.01$ *vs* normal of Flu, $^dP > 0.05$, $^fP < 0.01$ *vs* normal of HF.

Parameter	Flutamide		2-Hydroxyflutamide	
	Normal	Poisoned	Normal	Poisoned
K_a/h^{-1}	1.03 ± 0.24	0.24 ± 0.08[c]		
K/h^{-1}	0.62 ± 0.16	0.16 ± 0.03[c]		
$K_{(m)}/h^{-1}$			0.07 ± 0.01	0.05 ± 0.01[f]
$T_{\frac{1}{2}}/h$	1.19 ± 0.29	4.4 ± 0.8[c]	9.9 ± 0.7	14.8 ± 1.9[f]
$V_d/L \cdot kg^{-1}$	10.2 ± 2.4	3.9 ± 1.8[c]		
$Cl \cdot F^{-1}/L \cdot kg^{-1} \cdot h^{-1}$	6.0 ± 1.0	0.63 ± 0.29[c]		
$AUC/mg \cdot L^{-1} \cdot h$	8.6 ± 1.3	100 ± 44[c]	219 ± 22	170 ± 42[d]
T_{max}/h	1.61 ± 0.27	5.8 ± 1.5[c]	8.4 ± 0.4	15.8 ± 2.0[f]
$C_{max}/mg \cdot L^{-1}$	2.4 ± 0.7	6.7 ± 2.8[c]	8.6 ± 0.6	3.8 ± 0.8[f]

HF simultaneously. The three-dimentional structures of Flu and HF were similar to testosterone, so we used methyltestosterone as the internal standard for HPLC assay. The maximum absorbance wavelengths of Flu, HF, and methyltestosterone were 227, 226, and 241 nm, respectively. They all had evident absorbance at 234 nm. The detection limits of Flu and HF were 1 and 2 ng, respectively. The mobile phase had no inorganic salt, and its pH approximated to 5.6. There were three HPLC methods for analysis of Flu or HF[4-6]. They were all lack of internal standard. There was only one method for analysis of Flu and HF simultaneously[6]. Its detection limits of Flu and HF were 1.5 and 4.5 ng, respectively. The pH of mobile phase was 2.9, it would threat the column life. Its extraction procedure was simpler than ours. The other two methods were only used to determine HF. There was phosphate in the second method, and its detection limit was 6 ng[5]. The plasma sample volume was 2 mL in the third method, and its detection limit was 2 ng[4]. There was one gas – liquid chromatographic method[2] using an internal standard to determine Flu and HF, but it was limited by employing ^{63}Ni electron capture detector. The internal standard was 4-nitro-3-trifluoromethyl-hexananilide, which was difficult to obtain.

$$A \xrightarrow{K_m = f_m \cdot K} A_{(m)} \xrightarrow{K_{(m)}} A_{e(m)}$$

K_m is the constant of formation rate of metabolite, and f_m is fraction of metabolite formation. $f_m = 1$ is the prerequisite for the following equation:

$$C_{(m)} = \frac{K \cdot c}{K_{(m)} - K} (e^{-Kt} - e^{-K_{(m)}t})$$

While K is greater than $K_{(m)}$, after enough time, e^{-Kt} tends to zero, so we can calculate $K_{(m)}$[9].

Our data showed the elimination of active metabolite HF was fitted with ERL model, $K > K_{(m)}$ both in normal and hepatic injury rats. $K_{(m)}$ and $T_{\frac{1}{2}}$ of HF were 0.07 ± 0.01 h^{-1} and 9.9 ± 0.7 h in normal rats, respectively. If rats were given ig HF 50 $mg \cdot kg^{-1}$ directly, its K and $T_{\frac{1}{2}}$ were 0.14 ± 0.02 h^{-1} and 4.9 ± 0.7 h, respectively (unpublished data). Our results suggested there might be feedback inhibition of HF elimination due to other metabolites while the rats were given Flu.

The decreases in V_d and clearance of Flu resulted in statistically significant changes in K and $T_{\frac{1}{2}}$ in rats with hepatic injury. These results reflected a slower metabolic clearance of Flu with hepatic injury in rats, resulting in a greatly reduced first-pass effect. The AUC of the metabolite HF showed a lower trend in rats with hepatic injury ($P > 0.05$).

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氟他胺及其代谢产物 2-羟基氟他胺在正常及肝损伤大鼠的药物动力学

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 氟他胺；2-羟基氟他胺；药物动力学；肝；四氯化碳中毒；高压液相色谱法

目的：建立一新的高压液相色谱法用来研究氟他胺(Flu)及其活性代谢产物 2-羟基氟他胺(HF)的药物动力学. **方法**：正常及肝损伤大鼠灌胃 Flu 50 $mg \cdot kg^{-1}$. 采用反相高压液相色谱法，以甲基睾丸素为内标，流动相为甲醇：乙腈：水：乙醚 = 40:20:35:1（体积比），检测波长为 234 nm. **结果**：Flu 的 K 与 Cl 分别由 $0.62 \pm 0.16\ h^{-1}$ 及 $6.0 \pm 1.0\ L \cdot kg^{-1} \cdot h^{-1}$ 减小到 $0.16 \pm 0.03\ h^{-1}$ 及 $0.63 \pm 0.29\ L \cdot kg^{-1} \cdot h^{-1}$（$P < 0.01$），AUC 与 C_{max} 分别由 $8.6 \pm 1.3\ mg \cdot L^{-1} \cdot h$ 及 $2.4 \pm 0.7\ mg \cdot L^{-1}$ 增加到 $100 \pm 44\ mg \cdot kg^{-1} \cdot h$ 及 $6.7 \pm 2.8\ mg \cdot L^{-1}$（$P < 0.01$）. HF 的 $K_{(m)}$ 由 $0.07 \pm 0.01\ h^{-1}$ 减小到 $0.05 \pm 0.01\ h^{-1}$（$P < 0.01$）. **结论**：在肝损伤大鼠，Flu 与 HF 消除受到显著抑制.