ELSEVIER

Journal of Chromatography B, 691 (1997) 212–216

JOURNAL OF CHROMATOGRAPHY B

Short communication

High-performance liquid chromatographic determination of the enantiomers of the benzoquinolinone LY191704, a human type 1 5α-reductase inhibitor, in plasma

Nagy A. Farid*, Nikola L. Coleman

Division of Drug Metabolism and Disposition, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285, USA

Received 3 May 1996; revised 29 July 1996; accepted 5 August 1996

Abstract

A stereoselective reversed-phase liquid chromatographic method for the determination of compounds LY300502 and LY300503 (enantiomers of LY191704) in rat and dog plasma was developed. The assay involved extraction of the compounds using a strong cation-exchange solid-phase extraction column, from which the compounds are eluted with 1% of 1 *M* HCl in methanol. The enantiomers were separated on a Daicel Chiralcel OD-R column. The mobile phase consisted of water–acetonitrile–methanol (50:40:10, v/v) at a flow-rate of 0.3 ml/min. UV detection was achieved at 220 nm. The disposition of the enantiomers of LY191704 in rats and dogs was found to be stereoselective and species specific.

Keywords: LY191704; LY300502; Benzoquinolinones; 5α-Reductase inhibitor

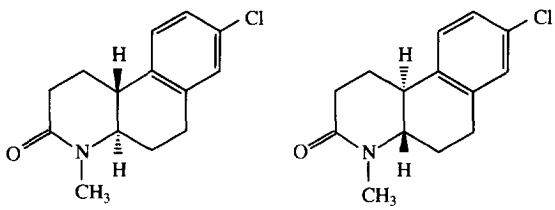
1. Introduction

Dihydrotestosterone is an etiological factor in the development of prostate cancer, benign prostate hyperplasia, and dermatological conditions such as acne and androgenic alopecia. Dihydrotestosterone is produced in vivo from testosterone through enzymatic reduction catalyzed by 5α-reductase. A series of benzoquinolinone inhibitors of human steroid 5α-reductase have been evaluated in a collaborative effort [1]. The results contributed to the identification of two isozymes of human 5α-reductase, designated types 1 and 2 [2,3]. Human type 1 5α-reductase predominates in skin, prostate epithelia and, to a lesser extent, in prostate fibromuscular stroma. Because of their selectivity as inhibitors of type 1 5α-reductase, the benzoquinolinones offer an excellent tool in helping to understand the role of human type 1 5α-reductase and the potential therapeutic uses of such a compound in a variety of endocrine disorders.

LY191704 (**I**), (4α*R*,*S*, 10β*R*,*S*)-4-methyl-8-chloro-*trans*-1,2,3,4,4α,5,6,10β-octahydrobenzo-[*f*]-quinolin-3-one is the racemic mixture of the compounds LY300502 (**II**), the (4α*R*, 10β*R*) enantiomer, and LY300503 (**III**), the (4α*S*, 10β*S*) enantiomer (Fig. 1). Compound **I** was identified as a potent and selective inhibitor of human type 1 5α-reductase [4]. The pharmacological activity of **I** has been recently reported [5]. Previous studies have demonstrated that the inhibitory effect of the enantiomers on human type 1 5α-reductase were essentially equivalent [6]. The pharmacokinetics and disposition of **I** in rats and dogs, and of the individual enantiomers in rats, have

*Corresponding author.

PII S0378-4347(96)00383-0



II III

Fig. 1. Structures of LY300502 (**II**) and LY300503 (**III**).

been recently reported [7]. These studies suggested differences in the disposition of compounds **II** and **III** in rats. The aim of the present work was to develop an enantioselective HPLC method for the determination of the enantiomeric ratio of compounds **II** and **III** in rat and dog plasma samples, following the administration of **I**, to assist in elucidation of the behavior of enantiomers in vivo.

2. Experimental

2.1. Chemicals and reagents

Compounds **I**, **II** and **III** were synthesized at Lilly Research Laboratories (Indianapolis, IN, USA). The enantiomeric purity of each enantiomer was 98% or higher. HPLC grade solvents were used and all other reagents and chemicals were of the highest purity.

2.2. Chromatography

The chromatographic (HPLC) system consisted of a Waters 510 HPLC pump and a WISP 712 autoinjector (Waters, Milford, MA, USA). The enantiomers LY300502 and compound 300503 were separated at ambient temperature utilizing a Daicel Chiralcel OD-R column, 250×4.6 mm I.D. (Daicel Technologies, Exton, PA, USA). The mobile phase consisted of water–acetonitrile–methanol (50:40:10, v/v) at a flow-rate of 0.3 ml/min. An ABS Model 785A UV detector (Applied Biosystems, Ramsey, NJ, USA) set at 220 nm, was used.

2.3. Preparation of standards and plasma samples

Separate stock standard solutions of compounds **II** and **III** were prepared in methanol to contain 150 μg/ml. From these stock solutions, five standards were prepared in plasma to contain a total (**II** plus **III**) of 5 μg/ml, where the ratios of **II** to **III** were 0.24, 0.50, 0.97, 2.78 and 4.68. The exact ratios were determined based on the weight of each enantiomer and its enantiomeric purity. Two control samples were prepared to contain a total (**II** plus **III**) of 150 ng/ml plasma at the **II** to **III** ratios of 0.40 and 2.0, and two others at the same ratios but where the total (**II** plus **III**) concentration was 5 μg/ml.

2.4. Samples extraction and preparation

Plasma sample or standard, 300 μl, was added to a 1-ml SCX solid-phase extraction cartridge (Analytichem Bond Elut SCX column, Cat # 1210-2013, Varian, Harbor City, CA, USA), which had been activated with 2 ml methanol then washed with 2 ml water. The reservoir was filled with water, and vacuum was applied to load the contents of the plasma sample on the column. The extraction cartridge was rinsed with 1 ml water, followed by 1 ml 20% methanol in water, and the rinses were discarded. The compounds of interest were eluted with 3 ml 1% 1 *M* HCl in methanol. The eluate was evaporated to dryness under nitrogen at 40°C. The residue was reconstituted in 300 μl of the mobile phase and 175 μl was injected onto the HPLC system.

3. Results and discussion

Under the chromatographic analysis conditions detailed above, compounds **III** and **II** had retention times of 36.8 and 38.7 min, respectively. The selectivity (α) and resolution of the two peaks were determined to be 1.08 and 1.00, respectively. There were no interferences in the chromatograms from endogenous plasma components. Attempts to reduce the chromatographic run time (by increasing the concentration of the organic modifier) resulted in loss of resolution between the compounds of interest. The determination of **II** to **III** weight ratio was calculated from the linear regression line of the actual weight ratios of the standards vs. the ratio of their peak heights (**II**/**III**) under the chromatographic conditions listed above.

Table 1
Overall recovery of compounds **II** and **III** from spiked plasma samples

Sample concentration (μg/ml)	*n*	**II**/**III** ratio	Recovery (mean±S.D.) (%)	
			II	**III**
Rat plasma				
4.96	6	0.384	95.4±7.9	90.6±7.4
4.79	6	1.778	94.2±6.2	92.2±6.1
0.149	3	0.384	89.2±3.1	96.3±4.5
0.144	3	1.778	84.2±4.1	93.8±8.0
Dog plasma				
4.96	3	0.384	87.5±6.6	82.1±5.8
4.79	3	1.778	88.6±3.5	88.7±3.2

3.1. Recovery

The recovery of known concentrations of **II** and **III** from spiked plasma samples was obtained by comparison of the peak height of each compound following extraction to that of an absolute standard chromatographed under the same conditions. (The absolute standard was a solution of the compounds in the mobile phase at concentrations similar to those in the plasma sample but not subjected to the extraction steps). The recovery data were obtained at two concentration levels of **II** plus **III** (between 0.14 and 5 μg/ml), and at **II** to **III** ratios of 0.384 and 1.778. The recovery of each compound from rat and dog plasma ranged from 82 to 96% (Table 1).

3.2. Accuracy, inter and intra-day precision

The accuracy, intra- and inter-day precision data for the determination of the ratio of **II** to **III** in rat plasma samples are presented in Table 2. The assay validation data also included the determination of the accuracy and intra-day precision data for the analysis of dog plasma samples for one day (Table 2). The data indicated that the accuracy of the determinations ranged from 88 to 114% in rat and dog plasma samples, and that the inter-day coefficient of variation ranged from 0.37 to 4.60% in rat plasma samples.

3.3. Linearity and limits of detection and quantitation

The correlation coefficient determined for the standard curves was 0.999 or greater. Linearity was established in the weight ratio range of 0.24 to 4.68 (**II**/**III**). Since this was an assay for ratio determi-

Table 2
Accuracy, intra- and inter-day precision of determination of the **II** to **III** weight ratio in spiked rat and dog plasma samples (n=3/day)

Day	Total concentration of enantiomers (μg/ml)	**II**/**III** ratio	Found ratio (mean±S.D.)	Inter-day (mean±S.D.)
Rat plasma				
1	5.32	1.788	1.805±0.003	
2			1.803±0.001	
3			1.816±0.005	1.808±0.007
1	5.51	0.384	0.405±0.001	
2			0.406±0.003	
3			0.405±0.000	0.405±0.002
1	0.160	1.778	1.921±0.045	
2			1.751±0.064	
3			1.843±0.052	1.835±0.084
1	0.165	0.384	0.438±0.004	
2			0.400±0.005	
3			0.421±0.008	0.421±0.018
Dog plasma				
	5.32	1.788	1.576±0.004	
	5.51	0.384	0.374±0.002	
	0.160	1.778	1.556±0.022	
	0.165	0.384	0.365±0.009	

nation, no attempt was made to establish a lower limit of detection. However, assay of the validation samples demonstrated that the ratio of **II/III** could be accurately determined in plasma samples containing 160 ng/ml total enantiomers weight.

3.4. Application

Rats and dogs were administered **I** daily at 10, 30, or 100 mg/kg for one month [7]. Plasma samples were obtained from the animals following the first and last doses and analyzed for the ratio of **II** to **III**. In dog plasma, the concentration of **II** equalled that of **III**, at all dose levels, following the first dose and did not change from unity upon daily dosing with **I** for one month (Fig. 2). In rat plasma samples, the ratio of **II** to **III** ranged from 1.6 to 2.0 following the first dose, and from 1.6 to 2.4 following the last dose (Fig. 2). The results clearly showed that **III** was subjected to a greater first pass metabolism in rats. In

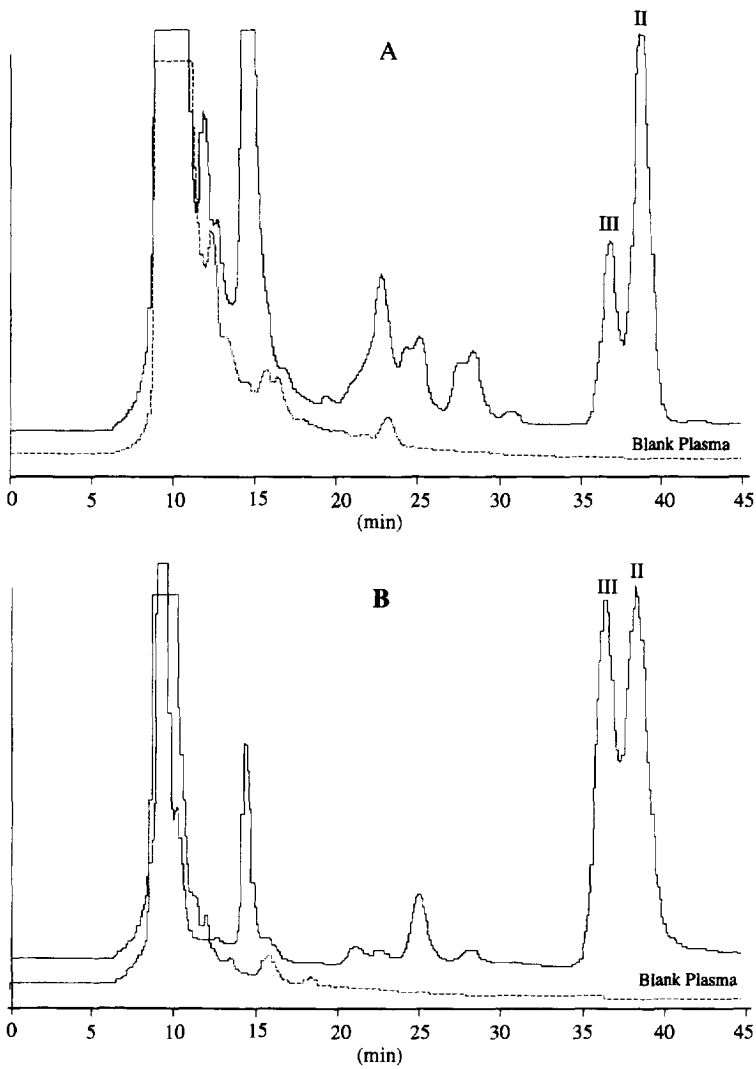


Fig. 2. HPLC chromatograms of the separation of the enantiomers **II** and **III** after extraction from plasma samples. (A) Rat plasma obtained 1 h after the last dose following daily administration of 100 mg **I**/kg for one month. (B) Dog plasma obtained 1 h after the first 100 mg **I**/kg dose.

addition, the increase in the ratio of **II** to **III** upon daily administration of **I** to rats for one month was particularly noticeable at the 30 and 100 mg/kg doses, suggesting that the enantiomer **III** induced its own metabolism [7]. The data demonstrated that the preferential metabolism of the enantiomers of **I** was species specific.

4. Conclusions

The present method was developed for the determination of the ratio of the enantiomers **II** and **III** in plasma samples. High-performance liquid chromatographic determination of the enantiomers proved to be a powerful tool in elucidation of the behavior of each enantiomer in different species. This information was an important factor in leading to the selection of **II** for further development.

References

[1] K.S. Hirsch, C.D. Jones, J.E. Audia, S. Andersson, L. McQuaid, N.B. Stamm, B.L. Neubauer, P. Pennington, R.E. Toomey and D.W. Russell, Proc. Natl. Acad. Sci. USA, 90 (1993) 5277.

[2] S. Andersson and D.W. Russell, Proc. Natl. Acad. Sci. USA, 87 (1990) 3640.

[3] S. Andersson, D.M. Berman, E.P. Jenkins and D.W. Russell, Nature, 354 (1991) 159.

[4] C.D. Jones, J.E. Audia, D.E. Lawhorn, L.A. McQuaid, B.L. Neubauer, A.J. Pike, P.A. Pennington, N.B. Stamm, R.E. Toomey and K.S. Hirsch, J. Med. Chem., 36 (1993) 421.

[5] B.L. Neubauer, N.A. Farid, H.R. Gray, C.W. Hanke, K.S. Hirsch, K.C. Hsiao, C.D. Jones, D.E. Lawhorn, L. McQuaid, R.E. Toomey, K. Valia and J.E. Audia, Drugs Future, 20 (1995) 144.

[6] B.L. Neubauer, personal communication (1995).

[7] N.A. Farid, K.M. Schreiner, N.L. Coleman, R.B.L. van Lier and S.A. Wrighton, Drug Metab. Dispos., in press.