Cancer Chemother Pharmacol (2003) 51: 519–524
DOI 10.1007/s00280-003-0603-2

ORIGINAL ARTICLE

Ivo P. Nnane · Vincent C. O. Njar · Angela M. H. Brodie

Pharmacokinetics of novel inhibitors of androgen synthesis after intravenous administration in mice

Received: 22 October 2002 / Accepted: 13 February 2003 / Published online: 25 April 2003

Abstract *Purpose*: The pharmacokinetics of several new androgen synthesis inhibitors were investigated after intravenous administration in mice. The inhibitors were: 3β-hydroxy-17-(1*H*-imidazol-1-yl)androsta-5,16-diene (VN/85-1), 3β-hydroxy-17-(1*H*-1,2,3-triazol-1-yl) androsta-5,16-diene (VN/87-1), 17-(1*H*-imidazol-1-yl) androsta-4,16-diene-3-one (VN/108-1) and 17-(5′-isoxazol-yl)androsta-4,16-dien-3-one (L-39). *Methods*: Male Balb/c mice were injected with VN/85-1, VN/87-1, VN/108-1 or L-39 at 10, 25 and 50 mg/kg doses. Blood was collected at various times after drug administration via the eye orbit. The concentrations of VN/85-1, VN/87-1, VN/108-1 or L-39 in plasma were analyzed by a reversed-phase HPLC method with UV detection. *Results*: The plasma levels of VN/85-1, VN/87-1, VN/108-1 and L-39 declined biexponentially with terminal elimination half-lives ranging from 0.88 to 1.77 h. The terminal half-lives for VN/87-1, VN/85-1 and VN/108-1 were similar. However, the terminal half-life for L-39 was significantly longer than those for VN/87-1, VN/85-1 and VN/108-1. The systemic clearance values for the steroids ranged from 0.85 to 10.91 l/h per kg with a rank order of their clearance of L-39 > VN/87-1 > VN/108-1 > VN/85-1. The apparent volumes of distribution at steady state for the steroids ranged from 0.58 to 18.85 l/kg with a rank order of their apparent V_{ss} of L-39 > VN/87-1 > VN/85-1 > VN/108-1. The clearance and apparent V_{ss} for all four compounds were dose-independent following intravenous administration of doses up to 50 mg/kg. *Conclusions*: VN/85-1, VN/87-1, VN/108-1 and L-39 are rapidly cleared from the systemic circulation and display linear pharmacokinetics in mice. The information presented may be used to improve the disposition profiles and activities of the steroidal inhibitors of androgen synthesis in animal models of prostate cancer.

Keywords Pharmacokinetics · Androgen synthesis inhibitors · Prostate cancer

I. P. Nnane (✉)
Department of Pharmaceutical Sciences,
Temple University School of Pharmacy,
3307 N. Broad Street, Philadelphia,
PA 19140, USA
E-mail: ivo.nnane@temple.edu

V. C. O. Njar · A. M. H. Brodie
Department of Pharmacology and Experimental Therapeutics,
University of Maryland School of Medicine,
Baltimore, MD, USA

Introduction

Prostate cancer remains the second highest cause of cancer-related deaths in males after lung cancer in the Western world. In 1999, the American cancer society estimated that approximately 179,300 new cases were reported and more than 37,000 deaths occurred from the disease in the USA [12]. The disease is more prevalent in older men with more than 75% of the diagnoses being in men over 65 years of age, and it is also important to note there has been a dramatic increase in the incidence of prostate cancer in recent years [28]. Although localized prostate cancer is manageable, 10–50% of localized cases will progress to the metastatic form of the disease, which is difficult to manage. In fact, most patients present with locally extensive or metastatic form of the disease since localized prostate cancer rarely causes symptoms. In the United States, the annual health care cost for the treatment of patients with prostate cancer is estimated to be $4.5 billion. Clearly, prostate cancer presents a major health care problem and new treatment modalities are necessary [3].

Since the discovery of the beneficial effects of castration, androgen ablation has been the first line treatment for all patients with prostate cancer [8, 25]. Androgen ablation can be accomplished by orchidectomy or pharmacotherapeutic means, although most patients opt for chemical castration. Current endocrine therapies such as orchidectomy and luteinizing hormone

releasing hormone (LHRH) agonists/antagonists, which interfere with luteinizing hormone (LH) release at the level of the hypothalamic-pituitary axis, result in reduced testosterone production by the testes and are useful in the early stages of prostate cancer [4, 9]. However, these treatment options fail to interfere with androgen production by the adrenals that may contribute precursor steroids to the prostate [17]. In the testes, 17α-hydroxylase/$C_{17,20}$-lyase is a key enzyme in the biosynthesis of androgens and converts the C_{21} steroid precursors (pregnenolone and progesterone) to the corresponding C_{19} androgens such as testosterone [16]. In the prostate, testosterone is further converted to the more potent androgen, dihydrotestosterone (DHT) by 5α-reductase [1]. Both androgens stimulate prostatic growth although DHT binds to the androgen receptor with higher affinity and plays a much more important role than testosterone in the organogenesis and homeostasis of the prostate [29].

Ketoconazole, an imidazole derivative that inhibits 17α-hydroxylase/$C_{17,20}$-lyase and other steroidogenic enzymes, is used clinically to reduce testosterone biosynthesis for the treatment of patients with advanced prostate cancer [15, 26, 27]. However, ketoconazole is not very potent or specific and has a number of significant side effects. Finasteride, which was recently approved for the treatment of benign prostatic hypertrophy (BPH), is a potent inhibitor of 5α-reductase. However, finasteride is only effective against benign prostatic hypertrophy in patients with minimal disease and although the compound reduces DHT levels, it also increases serum testosterone levels [5, 24]. Antiandrogens such as flutamide, block androgen action at the receptor level but are only partially effective as the disease frequently progresses due to mutations in the androgen receptor [23]. Although a significant majority of prostate cancer patients initially respond to hormonal manipulations, virtually all patients will eventually develop androgen-independent disease [2]. A second line of treatment modalities for advanced prostate cancer is combined androgen blockade (CAB) which is based on depleting or antagonizing both testicular and residual adrenal androgens. Randomized clinical trials comparing CAB, for example orchidectomy plus antiandrogens, to a single therapeutic approach indicate the superiority of CAB, especially in patients with low tumor burden [2]. In addition, the combination of a 17α-hydroxylase/$C_{17,20}$-lyase or 5α-reductase inhibitor with an antiandrogen has also been shown to decrease the concentrations of testosterone in the serum to castrate levels with minimal side effects [7].

Although the success of the standard ablation therapy for combating androgen-independent carcinoma of the prostate has been limited, recent studies with ketoconazole indicate that careful scheduling of treatment can produce prolonged responses in otherwise hormone-refractory patients [15]. This suggests that more potent and selective inhibitors of 17α-hydroxylase/$C_{17,20}$-lyase could be useful agents for treating this disease, even in advanced stages. Clearly, the limitations of the current treatment options, especially for advanced prostate cancer, have fueled the search for other therapeutic options.

We have recently reported the synthesis and evaluation of several steroidal inhibitors of 17α-hydroxylase/$C_{17,20}$-lyase and 5α-reductase [13, 18]. 3β-Hydroxy-17-(1*H*-imidazol-1-yl)androsta-5,16-diene (VN/85-1), 3β-hydroxy-17-(1*H*-1,2,3-triazol-1-yl)androsta-5,16-diene (VN/87-1), 17-(1*H*-imidazol-1-yl)androsta-4,16-diene-3-one (VN/108-1) and 17-(5′-isoxazolyl)androsta-4,16-dien-3-one (L-39) (Fig. 1) have emerged as the most potent inhibitors of these enzymes. Several of the novel steroidal compounds inhibit proliferation of LNCaP cells in vitro and in tumors in male severe combined immunodeficiency (SCID) mice, which are models of human prostate cancer. The novel steroids also manifest antiandrogenic activity in cultures of LNCaP cell lines [10, 11, 14]. In addition, the compounds diminish the levels of circulating testosterone and DHT in male rat tissues [19, 20]. Thus, these compounds could be more effective than current therapies in the treatment of prostate cancer due to their multiple mechanisms of action. The objective of the present study was to evaluate the pharmacokinetic profile of several of the leading steroids following intravenous (i.v.) administration in mice. The information obtained from this investigation will help us to optimize the use of these steroidal inhibitors of androgen synthesis in order to obtain improved disposition profiles and activities in vivo.

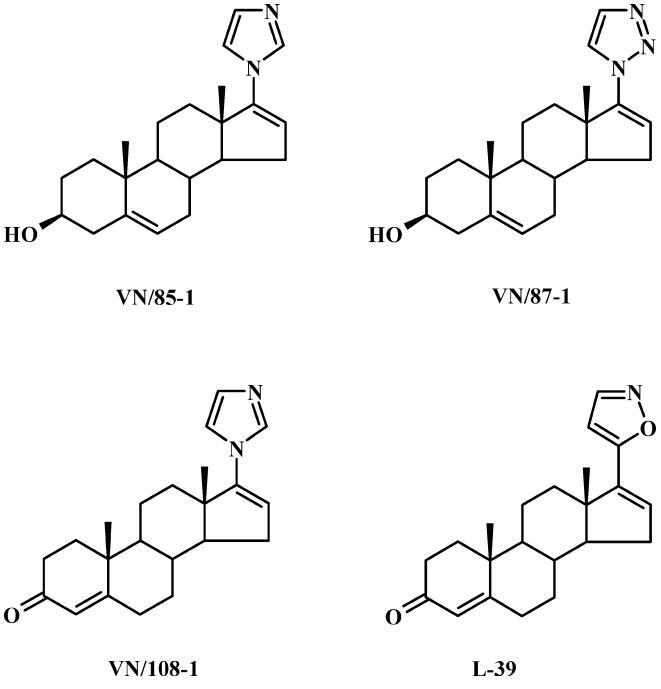

Fig. 1 Chemical structures of 3β-hydroxy-17-(1*H*-imidazol-1-yl)androsta-5,16-diene (VN/85-1), 3β-hydroxy-17-(1*H*-1,2,3-triazol-1-yl) androsta-5,16-diene (VN/87-1), 17-(1*H*-imidazol-1-yl)androsta-4,16-diene-3-one (VN/108-1) and 17-(5′-isoxazolyl)androsta-4,16-dien-3-one (L-39)

Materials and methods

Steroidal inhibitors and reagents

VN/85-1, VN/87-1, VN/108-1 and L-39 and its Δ^5-hydroxy derivative (L-38) were synthesized according to procedures described previously [13, 18]. All other reagents were purchased from Sigma Chemical Company (St. Louis, Mo.).

Animal treatment

Male Balb/c mice (8–10 weeks old, weighing about 20 g) obtained from NCI (Frederick, Md.) were maintained in a controlled environment of about 25°C, 50% relative humidity and a 12-h light/12-h dark cycle, and allowed free access to food and water. The steroids were formulated in 40% β-cyclodextrin in water and a single i.v. bolus dose (10, 25 or 50 mg/kg in 100 µl vehicle) was administered to male mice. Control animals were treated with the vehicle (0.1 ml) alone. Blood (about 0.5 ml) was obtained from two animals per time-point via the eye orbit under halothane anesthesia at 0.0, 0.083, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, and 24 h after administration of the steroids or the vehicle alone. The animals were killed immediately after blood sampling. These experiments were repeated twice for each steroid and for each dose level.

HPLC analysis

Chromatographic separation and quantitation of the steroids and the appropriate internal standards, were achieved by a modified reversed-phase HPLC method on a Waters (Milford, Mass.) Novapak C18 column (3.9×150 mm) protected by a Waters guard cartridge packed with pellicular C18, as described previously [21, 22]. Briefly, the HPLC system used in this study consisted of a Waters solvent delivery system and a Waters 600 controller coupled to a Waters 717^{plus} autosampler and a Waters 996 photodiode array detector operated at 243 nm. The mobile phase used was water/methanol/acetonitrile/acetic acid (30:40:30:0.0001, v/v) at a flow rate of 1.0 ml/min. The HPLC analysis was performed at ambient temperature and data acquisition and management were achieved with a Waters millennium chromatography manager.

Sample preparation

Samples were prepared using a previously described procedure [21, 22] with modification. Briefly, test tubes containing 250 µl mouse plasma, appropriate steroidal compound and the appropriate internal standard, were extracted with diethyl ether (2×2 ml) using a vortex mixer for 1 min and centrifugation at 1500 *g* for 5 min. L-39 (5 µg/ml) was used as the internal standard for the assay of VN/85-1, VN/87-1 and VN/108-1, while L-38 (10 µg/ml) was used as the internal standard for the assay of L-39. The organic layers were evaporated to dryness under a gentle stream of air. The extracts were reconstituted in 250 µl acetonitrile and loaded into a solid-phase Sep-Pak 1-ml C18 cartridge (Waters) prewashed with 1 ml methanol for further purification. The steroids were eluted from the cartridge with 250 µl acetonitrile and the solvent evaporated. The residue was reconstituted in a 50-µl aliquot of the mobile phase and filtered using 0.2-µm Teflon filters before HPLC analysis.

Calibration curves and HPLC assay validation

The calibration curves for each steroid were constructed by spiking various amounts of the steroids into extraction tubes (in duplicate) containing 0.25 ml plasma from untreated animals to give final concentrations of 0.0–100.0 µg/ml. Appropriate blank extraction tubes were also prepared and an aliquot of the internal standard was added to each extraction tube to give a final concentration of 5 µg/ml (L-39) or 10 µg/ml (L-38). The calibration samples were taken through the sample preparation procedure described above. A 10-µl aliquot of the reconstituted extract was injected into the HPLC system and the ratios of the peak area for each analyte to that of the internal standard were plotted against concentrations of appropriate steroidal compound. The precision and accuracy of the assays were determined from a range of known concentrations of each steroidal inhibitor in blank plasma and taken through the extraction and HPLC procedure. These studies were repeated on three separate occasions.

Data analysis

Compartment modeling was performed using WinNonlin (Pharsight Corporation, Mountain View, Calif.). The one- and two-compartment analyses, with various weighting schemes, were evaluated to determine the best model. The goodness of fit was based on random distribution of residuals and the Akaike information criterion. An open two-compartment model best described the plasma level data for each androgen synthesis inhibitor according to the following general expression:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

where C_p is the concentration of each androgen synthesis inhibitor in plasma at any time (t), the coefficients A and B are the extrapolated intercepts of the line of residuals and the linear terminal elimination phase at time zero, respectively. The macroscopic rate constants α and β represent the first-order rate constants for the distribution and the elimination process, respectively. The half-lives for the distribution ($t_{1/2\alpha}$) and elimination ($t_{1/2\beta}$) phases were calculated from the following expressions $0.693/\alpha$ and $0.693/\beta$, respectively.

The clearance (CL), the volume of distribution at steady state (V_{ss}) and the areas under the plasma concentration versus time curves (AUC) were calculated using the following equations:

$$\begin{aligned} CL &= \frac{Dose}{AUC} \\ V_{ss} &= V_p + \frac{k_{12}}{k_{21}} V_p \\ AUC &= \frac{A}{\alpha} + \frac{B}{\beta} \end{aligned}$$

where V_p is the volume of the central compartment, k_{12} is the first-order rate constant for transfer of drug from the central compartment to the tissue compartment, and k_{21} is the first-order rate constant for transfer of drug from the tissue compartment to the central compartment. The data obtained from each experiment were fitted individually and the parameters are reported as means ± SE of the values obtained from separate fits, for each drug and for each dose level. One-way analysis of variance (ANOVA) on SigmaStat for Windows version 1.0 was used to determine the significance of the differences between pharmacokinetic parameters obtained for different treatment groups. The Bonferroni post-hoc test was used for determination of significance. *P*-values < 0.05 were considered statistically significant.

Results

VN/85-1, VN/87-1, VN/108-1 and L-39 were well resolved from the internal standard (L-38) and other endogenous compounds in mouse plasma by reversed-phase HPLC. The peaks were sharp and symmetrical for each androgen synthesis inhibitor and the extraction recoveries for each compound ranged from 90% to 97%. The calibration curves derived for each compound were linear and reproducible and the inter- and intra-

assay variabilities were less than 10%. The limits of quantitation (LOQ) for VN/85-1, VN/87-1, VN/108-1 and L-39 in mouse plasma were 0.20, 0.15, 0.20 and 0.03 μg/ml, respectively. The HPLC assays were fully validated and used to monitor the concentrations of each compound in mouse plasma.

The plasma concentrations of VN/85-1 in mice showed a biexponential decline at each dose level following i.v. administration (Fig. 2). The mean pharmacokinetic parameters of VN/85-1 following an i.v. bolus administration are shown in Table 1. VN/85-1 was rapidly distributed with a mean distribution half-life ($t_{1/2\alpha}$) of about 0.072 h and was eliminated with a mean terminal elimination half-life ($t_{1/2\beta}$) of about 1.24 h (Table 1). The mean terminal half-life obtained at the 10 mg/kg dose was similar to the values obtained at the 25 and 50 mg/kg dose levels ($P < 0.05$). Similarly, the mean total clearances were similar at all dose levels examined ($P < 0.05$). The volume of distribution at steady state (V_{ss}) for VN/85-1 was estimated at about 0.82 l/kg.

After i.v. administration of VN/87-1, the plasma concentrations declined biexponentially with distribution half-lives ranging from 0.065 to 0.074 h, indicating that the compound is rapidly distributed in mice (Fig. 2). The terminal elimination half-lives ranged from 0.97 to 1.04 h, indicating that the compound is rapidly removed from the general circulation (Table 2). The mean total clearances were similar at all dose levels examined ($P < 0.05$).

The plasma concentrations of VN/108-1 in mice also showed a biexponential decline at each dose level following i.v. administration (Fig. 2). The mean pharmacokinetic parameters of VN/108-1 following i.v. bolus administration are shown in Table 3. VN/108-1 was rapidly distributed with a mean distribution half-life ($t_{1/2\alpha}$) of about 0.15 h and was eliminated with terminal elimination half-lives ($t_{1/2\beta}$) ranging from 0.88 to 1.01 h. The mean total clearance of VN/108-1 was about 1.17 l/h per kg and was independent of the dose given ($P < 0.05$).

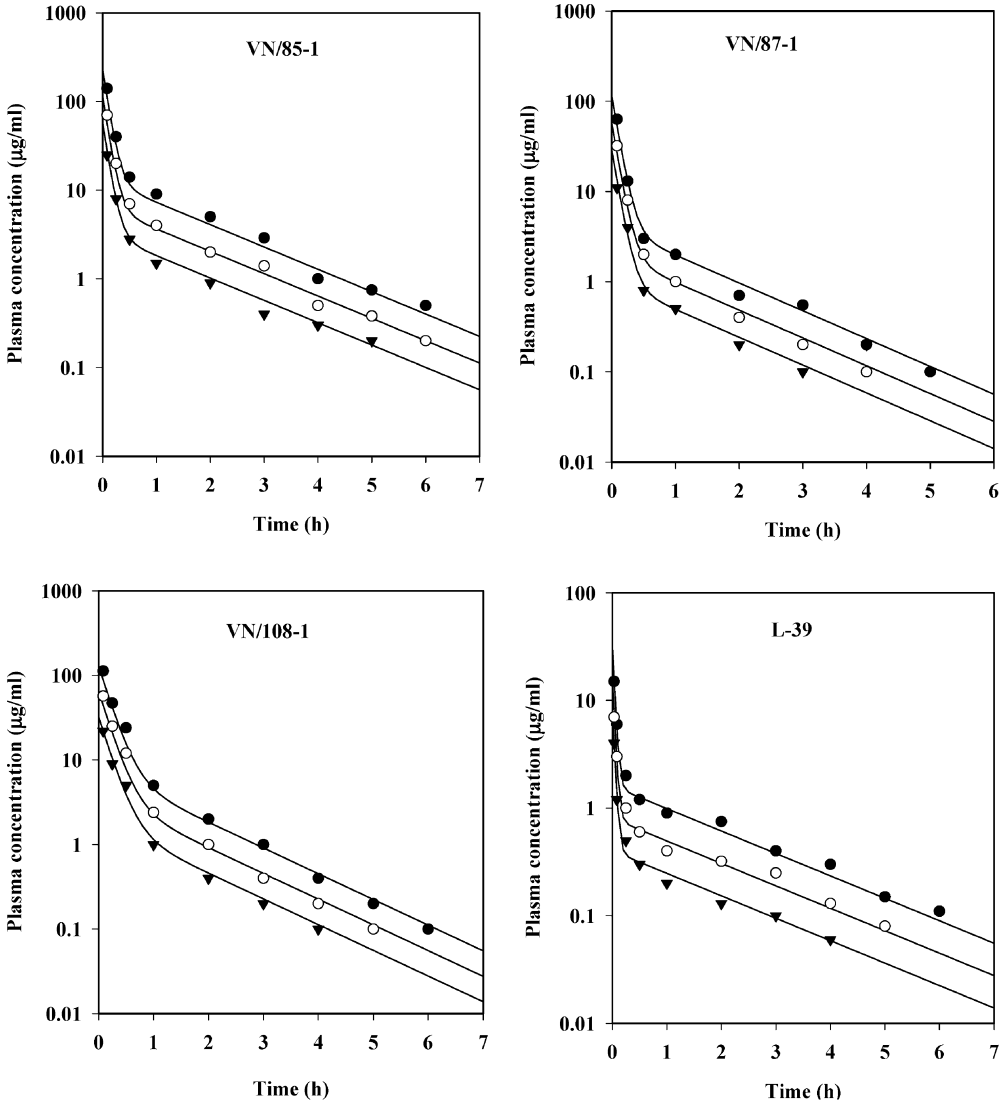

Fig. 2 Plasma concentration versus time curves after i.v. administration of VN/85-1, VN/87-1, VN/108-1 and L-39 in male mice (▼ 10 mg/kg, ○ 25 mg/kg, ● 50 mg/kg). Values represent the mean plasma concentrations from six mice. The standard deviations were 5–10% of the mean values

Table 1 Pharmacokinetic parameters of VN/85-1 in male mice after administration of single intravenous doses. Values are means ± SE, $n = 6$

Parameter	Dose (mg/kg)		
	10	25	50
$t_{1/2\alpha}$ (h)	0.073 ± 0.01	0.073 ± 0.01	0.072 ± 0.01
$t_{1/2\beta}$ (h)	1.24 ± 0.15	1.24 ± 0.13	1.23 ± 0.13
$AUC_{0-\infty}$ (μg·h/ml)	11.62 ± 0.41	29.04 ± 0.90	58.57 ± 1.80
CL (l/h/kg)	0.86 ± 0.03	0.86 ± 0.03	0.85 ± 0.03
V_{ss} (l/kg)	0.82 ± 0.08	0.82 ± 0.07	0.82 ± 0.07

Table 2 Pharmacokinetic parameters of VN/87-1 in male mice after administration of single intravenous doses. Values are means ± SE, $n = 6$

Parameter	Dose (mg/kg)		
	10	25	50
$t_{1/2\alpha}$ (h)	0.071 ± 0.01	0.074 ± 0.01	0.065 ± 0.01
$t_{1/2\beta}$ (h)	0.98 ± 0.33	1.04 ± 0.03	0.97 ± 0.16
$AUC_{0-\infty}$ (μg·h/ml)	3.91 ± 0.22	9.22 ± 0.44	17.99 ± 0.94
CL (l/h/kg)	2.56 ± 0.15	2.71 ± 0.13	2.78 ± 0.07
V_{ss} (l/kg)	1.17 ± 0.33	1.13 ± 0.26	1.13 ± 0.11

Table 3 Pharmacokinetic parameters of VN/108-1in male mice after administration of single intravenous doses. Values are means ± SE, $n = 6$

Parameter	Dose (mg/kg)		
	10	25	50
$t_{1/2\alpha}$ (h)	0.14 ± 0.02	0.15 ± 0.02	0.14 ± 0.02
$t_{1/2\beta}$ (h)	0.88 ± 0.04	1.01 ± 0.04	0.98 ± 0.31
$AUC_{0-\infty}$ (μg·h/ml)	8.54 ± 0.57	21.45 ± 0.25	42.89 ± 2.23
CL (l/h/kg)	1.17 ± 0.07	1.17 ± 0.07	1.16 ± 0.06
V_{ss} (l/kg)	0.58 ± 0.14	0.62 ± 0.13	0.61 ± 0.09

Table 4 Pharmacokinetic parameters of L-39 in male mice after administration of single intravenous doses. Values are means ± SE, $n = 6$

Parameter	Dose (mg/kg)		
	10	25	50
$t_{1/2\alpha}$ (h)	0.027 ± 0.01	0.032 ± 0.01	0.033 ± 0.01
$t_{1/2\beta}$ (h)	1.42 ± 0.03	1.45 ± 0.25	1.77 ± 0.39
$AUC_{0-\infty}$ (μg·h/ml)	0.97 ± 0.24	2.29 ± 0.40	5.20 ± 0.63
CL (l/h/kg)	10.28 ± 1.59	10.91 ± 1.10	9.61 ± 1.17
V_{ss} (l/kg)	14.75 ± 0.61	17.09 ± 4.57	18.85 ± 3.32

After i.v. administration, the plasma concentration of L-39 declined biexponentially with distribution half-lives ranging from of 0.027 to 0.033 h (Fig. 2, Table 4). The mean terminal elimination half-lives ranged from 1.42 to 1.77 h. The mean total clearance of L-39 ranged from 9.61 to 10.91 l/h per kg. The clearance and apparent V_{ss} for L-39 were dose-independent ($P < 0.05$) following i.v. administration of doses up to 50 mg/kg.

Discussion

The results indicate that these novel inhibitors of androgen synthesis exhibit linear pharmacokinetics in mice and suggest that the processes of disposition of these steroids were not saturated over the dose range examined. Some differences were evident between the disposition profiles of these novel inhibitors of androgen synthesis after i.v. administration. The pharmacokinetics of VN/85-1, VN/87-1 and VN/108-1 were characterized by a relatively short half-life and relatively moderate systemic clearance. However, the terminal half-life of L-39 was significantly longer than those of VN/87-1, VN/85-1 and VN/108-1. The systemic clearances of VN/85-1 and VN/108-1 were similar, approximately 2.5-fold less than the systemic clearance of VN/87-1 and about ten-fold less than the systemic clearance for L-39. The relatively high systemic clearance of L-39, which exceeded published values for mouse liver blood flow of 5–6 l/kg per h [6], appears to have been primarily due to its rapid and extensive distribution into tissues. The apparent V_{ss} of VN/85-1 and VN/87-1 were similar, and this may reflect their similar distribution pattern in mice. On the other hand, the apparent V_{ss} of VN/108-1 was significantly lower than those of VN/85-1 and VN/87-1, an indication that its distribution was not as extensive in mice. The apparent V_{ss} of L-39 was considerably higher when compared to the other steroids, and this finding may indicate extensive distribution of L-39 in mice.

The observed differences in the pharmacokinetics of these novel androgen synthesis inhibitors may have been a result of the differences in the chemical properties of the steroids. VN/85-1, VN/87-1 and VN/108-1 are new azolyl steroids in which the azole moiety is attached to the steroid nucleus at C-17 via the nitrogen of the azole [18]. In contrast, L-39 is an isoxazole androstene derivative in which the isoxazole moiety is attached to the steroid nucleus at C-17 via a carbon atom [13]. VN/87-1 (50 mg/kg per day for 28 days) is as effective as castration in its ability to inhibit human LNCaP tumor growth in male SCID mice. The rank order of efficacy of these steroids (50 mg/kg per day for 28 days) in the male SCID mouse model is VN/87-1 > VN/85-1 > VN/108-1 [10]. It is also worthy of note that L-39 (50 mg/kg per day for 28 days) inhibits the growth of human LNCaP tumors in male SCID mice to the same extent as castration [14]. Although the systemic clearance of L-39 was significantly higher than those of VN/85-1, VN/87-1 and VN/108-1, its efficacy in the male SCID mouse model of prostate cancer is similar to that of VN/87-1 and greater than those of VN/85-1 and VN/108-1. Thus, it would appear that the efficacy of these new steroidal compounds to modulate the proliferation of human LNCaP tumors in the male SCID mouse model of prostate cancer is not directly related to the rank order

of their systemic clearances in mice. There are several plausible explanations for this observation. It is possible that VN/87-1 and L-39, the most effective of these steroids in the male SCID mouse model of prostate cancer, are converted to metabolites with antitumor activity [21, 22]. However, the metabolic pathways of these new steroids have not yet been characterized. In addition, the equivalent activity of L-39 to VN/87-1 in vivo following a much lower systemic exposure (AUC) may possibly be due to (a) a greater intrinsic potency at the receptor level, (b) higher L-39 concentrations in the tumors, or (c) differences in protein binding. Additional experiments are being conducted to investigate the tissue and tumor distribution of these androgen synthesis inhibitors using radiolabeled compounds.

VN/85-1, VN/87-1, VN/108-1 and L-39 are rapidly cleared from the systemic circulation in mice, although at different rates. The pharmacokinetic data presented in this report may be used to improve the dosing schedules for these novel compounds in animal models of prostate cancer. Furthermore, the results may be useful in optimizing the structures of these steroidal inhibitors of androgen synthesis in order to obtain improved disposition profiles and increased activities in animal models of prostate cancer.

Acknowledgement This work was supported by a grant-in-aid for research from Temple University.

References

1. Bruchovsky N, Wilson JD (1968) The conversion of testosterone to 5α-androsta-17α-ol-3-one by rat prostate in vivo and in vitro. J Biol Chem 243:2012
2. Chodak GW (1998) Comparing treatments for localized prostate cancer—persisting uncertainty. JAMA 280:618
3. Clark JA, Wray NP, Ashton CM (2001) Living with treatment decisions: regrets and quality of life among men treated for metastatic prostate cancer. J Clin Oncol 19:72
4. Crawford ED, Eisenberger MA, McLeod DG, Spaulding JT, Benson R, Dorr FA, Blumenstein BA, Davies MA, Goodman PJ (1989) A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. N Engl J Med 321:419
5. Cunningham GR, Hirshkowitz M (1995) Inhibition of steroid 5α-reductase with finasteride: sleep related erections, potency and libido in healthy men. J Clin Endocrinol Metab 80:1934
6. Davies B, Morris T (1993) Physiological parameters in laboratory animals and humans. Pharm Res 10:1093
7. Debruyne FM, Dijkman GA (1995) Advances and trends in hormonal therapy for advanced prostate cancer. Eur Urol 28:177
8. Denis LJ (1991) Controversies in the management of localized and metastatic prostate cancer. Eur J Cancer 27:333
9. Foti F, Salerno R, Moneti G, Zoppi S, Fiorelli G, Marinon T, Natali A, Serio M, Martini M, Motta M (1989) Three-month treatment of a long acting gonadotrophin-releasing hormone agonist of patients with benign prostatic hyperplasia: effects of tissue androgen concentration, 5α-reductase activity and androgen receptor content. J Clin Endocrinol Metab 68:461
10. Grigoryev DN, Long BJ, Nnane IP, Njar VCO, Liu Y, Brodie AMH (1999) Effects of new 17α-hydroxylase/$C_{17,20}$-lyase inhibitors on LNCaP prostate cancer cell growth in vitro and in vivo. Br J Cancer 81:622
11. Klus GT, Nakamura T, Li J, Ling Y, Son C, Kemppainen JA, Wilson EM, Brodie AMH (1996) Growth inhibition of human prostate cells in vitro by novel inhibitors of androgen synthesis. Cancer Res 56:4956
12. Landis SH, Murray T, Bolden S, Wingo PA (1999) Cancer statistics. CA Cancer J Clin 49:8
13. Ling Y, Li J, Liu Y, Kato K, Klus GT, Brodie AMH (1997) 17-Imidazolyl, pyrazolyl and isoxazolyl androstene derivatives. Novel steroidal inhibitors of human cytochrome $C_{17,20}$-lyase ($P\text{-}450_{17\alpha}$). J Med Chem 40:3297
14. Long BJ, Grigoryev DN, Nnane IP, Liu Y, Ling YZ, Brodie AMH (2000) Antiandrogenic effects of novel androgen synthesis inhibitors on hormone-dependent prostate cancer. Cancer Res 60:6630
15. Muscato JJ, Ahmann TA, Johnson KM, Wilding W, Monaghan G, Schlossman DM (1994) Optimal dosing of ketoconazole and hydrocortisone leads to long responses in hormone refractory prostate cancer (abstract). Proc Am Assoc Cancer Res 13:22
16. Nakajin S, Hall PF, Onada M (1981) Testicular microsomal P-450 for C_{21} steroid side chain cleavage: spectral and binding studies. J Biol Chem 256:6134
17. Njar VCO, Brodie AMH (1999) Inhibitor of 17α-hydroxylase/$C_{17,20}$-lyase (CYP17): potential agents for the treatment of prostate cancer. Curr Pharm Des 5:163
18. Njar VCO, Kato K, Nnane IP, Grigoryev DN, Long BJ, Brodie AMH (1998) Novel azolyl steroids; potent inhibitors of human cytochrome 17α-hydroxylase/$C_{17,20}$-lyase ($P\text{-}450_{17\alpha}$): potent inhibitors for the treatment of prostate cancer. J Med Chem 41:902
19. Nnane IP, Kato K, Liu Y, Lu Q, Wang X, Ling Y, Brodie AMH (1998) Effects of some novel inhibitors of $C_{17,20}$-lyase and 5α-reductase in vitro and in vivo and their potential role in the treatment of prostate cancer. Cancer Res 58:3826
20. Nnane IP, Njar VCO, Liu Y, Lu Q, Brodie AMH (1999) Effects of novel 17-azolyl compounds on androgen synthesis in vitro and in vivo. J Steroid Biochem Mol Biol 71:145
21. Nnane IP, Long BJ, Ling Y-Z, Grigoryev DN, Brodie AMH (2000) Anti-tumour effects and pharmacokinetic profile of 17-(5′-isoxazolyl)androsta-4,16-dien-3-one (L-39) in mice: an inhibitor of androgen synthesis. Br J Cancer 83:74
22. Nnane IP, Njar VCO, Brodie AMH (2001) Analysis and pharmacokinetic profile of 3β-hydroxy-17-(1*H*-1,2,3-triazol-1-yl)androsta-5,16-diene (VN/87-1), a potent androgen synthesis inhibitor, in mice. J Steroid Biochem Mol Biol 78:141
23. Palmberg C, Koivisto P, Kakkola L, Tammela TL, Kallioniemi OP, Visakorpi T (2000) Androgen receptor gene amplification at primary progression predicts response to combined androgen blockade as second line therapy for advanced prostate cancer. J Urol 164:1992
24. Peters DH, Sorkin M (1993) Finasteride; a review of its potential in the treatment of benign prostatic hyperplasia. Drugs 46:177
25. Rosenberg AG, von Eschenbach AC (1990) Hormonal therapy for prostate cancer. Semin Surg Oncol 6:71
26. Small EJ, Baron AD, Fippin L, Apodaca D (1997) Ketoconazole retains activity in advanced prostate cancer patients with progression despite flutamide withdrawal. J Urol 157:1204
27. Trachtenberg J (1984) Ketoconazole therapy in advanced prostatic cancer. J Urol 132:61
28. Vercelli M, Quaglia A, Marani E, Parodi S (2000) Prostate cancer incidence and mortality trends among elderly and adult Europeans. Crit Rev Oncol Hematol 35:133
29. Wilson JD (1996) Role of dihydrotestosterone in androgen action. Prostate 6:88