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Pharmacokinetic analysis for the development of new potent anti-HIV-1 agents, the *KR-V* series

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Abstract : The pharmacokinetic properties of *KR-V* compounds, recently developed as new anti-HIV agents, were studied after *i.v.* and *p.o.* administration in rats. The concentrations of the *KR-V* series were determined in rat plasma using an high-performance liquid chromatography (HPLC)-UV detection system. Of the 19 *KR-V* compounds investigated in the present study, only *KR-V* 3, 10, 14, 16 and 18-1 showed oral bioavailability. The plasma concentration-time data could be adequately described by an one-compartment open model. In the *i.v.* kinetic study (10mg/kg), the CLt of *KR-V* 3, 10, 14 and 16 (> 4L/hr/kg) were significantly higher than that of *KR-V* 18-1 (1.1 L/hr/kg). The AUC of *KR-V* 18-1 was greater (8.97 μg · hr/ml) than that of the other compounds, but the Vd (0.58 L/kg) was lower. In the *p.o.* kinetic study (50mg/kg), although the $t_{1/2}$ of *KR-V* 18-1 was shorter than that of the other compounds, the AUC (3.659 μg · hr/ml) and C_{max} (1.891 μg/ml) were markedly higher. In a seperated *in vitro* experiment, only *KR-V* 18-1, of the 5 compounds with bioavailibility, exhibits potent activity against HIV-1 mutant strains. Therefore, *KR-V* 18-1 is expected to become a new potent anti-AIDS drug candidate/lead compound.

Key words : anti-HIV agent, pharmacokinetics, *KR-V*, rat, mutant strain.

Introduction

Zidobudine (3'-azido-2,3-dideoxythymidine, AZT, azidothymidine) is the first FDA-approved anti-HIV drug, nucleoside reverse transcriptase inhibitor (NRTI)[1-3]. This therapy has led to a reduction in the mortality and insidence of opportunistic infections in AIDS patients[4]. A double-blind clinical trial has demonstrated that AZT prolonged the life-expectancy of AIDS patients[5]. However, there is significant dose-related toxicity associated with the administration of AZT manifesting itself as anemia, leucopenia and bone mar-

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row suppression and this remains a limiting factor for its effective use in treatment strategies[6,7]. In addition, pharmacokinetic studies of AZT in phase I trials indicate that the plasma half-life of AZT is approximately 1 hr[8], thus necessitating frequent administration of AZT to maintain therapeutic drug levels.

To overcome the disadvantages assodiated with the toxicity and rapid elimination, non-nucleoside RT inhibitors (NNRTIs) were developed. Starting from the 1-(2-hydroxy) methyl-6-phenylthiothymine (HEPT) derivatives, more than 30 structurally different classes of compounds have been identified as NNRTIs. Two NNRTIs (nevirapine and delavirdine) have been formally licensed for clinical use and several others are (or have been) in preclinical and/or clinical development [tivirapine (TIBO R-86183), loviride (alpha-APA R89439), thiocarboxanilide UC-781, HEPT derivative MKC-442, quinoxaline HBY 097, DMP 266 (efavirenz), PETT derivatives (trovirdine, PETT-4, PETT-5) and the dichlorophenylthio(pyridyl)imidazole derivative S-1153]. The NNRTIs interact with a specific 'pocket' site of HIV-1 RT that is closely associated with, but distinct from, the NRTI binding site. NNRTIs are notorious for rapidly eliciting resistance due to mutations of the amino acids surrounding the NNRTI-binding site[5,9-11]. The NNRTIs inhibit the replication of HIV-1 at concentrations that are three to five orders of magnitude below their cytotoxic threshold[12,13]. However, rapid emergence of drug-resistant mutants caused by point mutations in the RT gene is regarded as an obstacle to the clinical usefulness of NNRTIs[14]. Because of this, many investigators have attempted to find a new potent AIDS drug which is highly effective against mutant HIV strains but has only a low toxicity (Table 1).

In attempts to circumvent previously described disadvantages related to cytotoxicity and drug-resistant variants, *KR-V* series were synthesized by KRICT (Korea Research Institute of Chemical Technology). The *KR-V* series exhibit potent antiviral activity and low cytotoxicity *in vitro*, and involve a very diverse spectrum of structures around the uracil nucleus, like AZT and HEPT (Fig 1). The objective of the present study was to investigate the possibility developing the *KR-V* series a new anti-AIDS agent for oral treatment. Investigating the pharmacokinetics is an indispensable part of such a project. Therefore, this study was carried out to assess the pharmacokinetic properties and oral bioavailability of the *KR-V* series.

Materials and Methods

Chemicals : Nineteen *KR-V* compounds (purity >99%) were investigated in this study. HPLC grade acetonitrile, methanol were purchased from Burdick & Jackson Inc. (USA). All other chemicals were of reagent or analytical grade.

Table 1. Some currently licensed anti-HIV agents

Year	Name	Compound	Abbr.*	T.N.**	Cl.***
1987	Zidovudine	3'-azideo-2,3-dideoxy-thymidine	AZT	Retrovir	RTI[a]
1991	Didanosine	2,3-dideoxyinosine	DDI	Videx	RTI
1992	Zalcitabine	2,3-dideoxycytidine	DDC	Hivid	RTI
1994	Stavudine	2,3-didehydro-3'-deoxythymidine	D4T	Zerit	RTI
1996	Ritonavir		IDV	Norvir	PI[b]
1996	Indinavir	2,3,5-trideoxy-5-[2[](1,1-di-methylethyl) amino]carbony]-4-(3-pyridinylmethyl)-1-piperazinyl]-2-(phenylmethyl)-erythro-pentonamide	RTV	Crixivan	PI

* Abbr. : Abbriviation ; ** T.N. : Trade name ; *** CL. : Classification
[a] RTI : Reverse transcriptase inhibitor.
[b] PI : Protease inhibitor.

Animals : Male specific pathogen-free (SPF) Sprague-Dawley (SD) rats (KRICT, Toxicology Research Center, Breeding Facility, Korea) were obtained at the age of 4 weeks. Animals were housed in polycarbonate cages, five rats/cage at 23±3℃ and 50±10% of relative humidity with a 12 hr light/dark cycle. Animals had free access to rat and mouse pellets (Jeil Feed Co., Korea) and sterilized tap water *ad libitum*.

Pharmacokinetics :

1) Animal treatment : Animals were fasted for approximately 12 hr prior to dosing. After suspension in a 0.1% tween-80 solution, the *KR-V* series were given orally (*p.o.*) or intravenosly (*i.v.*) to rats. The doses were 50 mg/kg b.w. in the p.o. study and 10 mg/kg b.w. in the *i.v.* study. Blood samples (200 μl) were collected from the tail vein using a vacutainer (Becton-Dickinson, USA) into a plastic tube containing heparin as an anticoagulant. Serial samples were obtained at the following times after dosing: (i) In the *i.v.* study, pre-dosing, 5, 10, 20 and 40 min and 1, 1.5, 2, 4, 6, 8 and 24 hr. (ii) In the *p.o.* study, pre-dosing, 0.5, 1, 1.5, 2, 3, 4.5, 6, 10 and 24 hr. Immediately after collection, the blood samples were gently inverted and placed in chipped ice. The samples were centrifuged at 12,000 rpm (Eppendorf 5415C centrifuge, Germany) for 4 min and the plasma was harvested and kept frozen at -20℃ until analysis (maximal storage time was 2 weeks).

2) Extraction procedure : Frozen plasma samples were thawed at room temperature prior to extraction. Each aliquot of serum was mixed with three volumes of MeOH and vortexed (Vortex, Thermolyne, USA). The resulting mixture was centrifuged for 2 min at 12,000 rpm. The supernatant was injected directly onto the HPLC column.

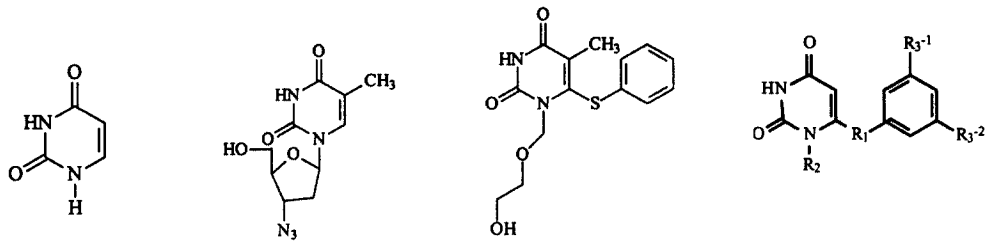
3) Standard calibration curve : Calibration standards for control plasma were prepared using concentrations of 0.1, 0.2, 0.5, 1, 2, 5 and 10 μg/ml. The concentrations of the *KR-V* series were independently calculated using the linear equation $y = ax + b$ of each standard curve, where y represented the peak area of analyte, and x represents the analyte concentration.

4) Pharmacokinetic analysis : Kinetic parameters were obtained using a pharmacokinetic program "MULTI", fitting data to a one-compartment open model. The terminal elimination rate constant (*kel*) was derived from the absolute value of the terminal slope of the log-linear portion of the plasma profile. The elimination half-life ($t_{1/2}$) was calculated by dividing 0.693 by kel. The areas under the plasma concentration *versus* time curve (AUC) were obtained by the

Table 2. Antiviral activity of *KR-V* series

Compound No.	CC_{50}[a] (μM)	EC_{50}[b] (μM)	SI[c]	Compound No.	CC_{50}[a] (μM)	EC_{50}[b] (μM)	SI[c]
1	735	0.0032	233,000	11	227	0.0015	151,000
2	643	0.0049	132,000	12	353	0.00087	417,000
3	67	0.00094	72,000	13	415	0.0039	106,000
4	398	0.0024	169,000	14	360	0.0038	95,000
5	158	0.0012	132,000	15	217	0.0024	92,000
6	99	0.00097	103,000	16	206	0.0067	30,000
7	121	0.001	121,000	17	9.5	0.0014	7,000
8	189	0.0014	135,000	18	22.6	0.0026	9,000
9	163	0.0021	78,000	18-1	22.6	0.0026	9.000
10	68	0.00074	92,000				

[a] 50% Cytotoxicity concentration based on the reduction of number of viable mock-infected MT-4 cells.
[b] 50% Effective concentration based on the inhibition of HIV-1-induced CPE in MT-4 cells.
[c] Selectivity index: CC_{50}/EC_{50}.



Uracil AZT HEPT *KR-V*

Fig 1. Chemical structure of anti-HIV drugs having an uracil nucleus.

trapezoidal rule. The peak plasma concentration (C_{max}) and the time to reach peak concentration (t_{max}) were obtained from the predicted value of the fitted curve. The bioavailibilities of the *KR-V* series after *p.o.* administration were calculated from the ratio of the AUC after *p.o.* dosing to that after *i.v.* dosing, normalized for the difference in dose levels.

Results

Pharmacokinetics : Of the 19 *KR-V* series, only 5, *KR-V* 3, 10, 14, 16, and 18-1, showed adequate oral bioavailability in the rats. The plasma concentration profiles for the

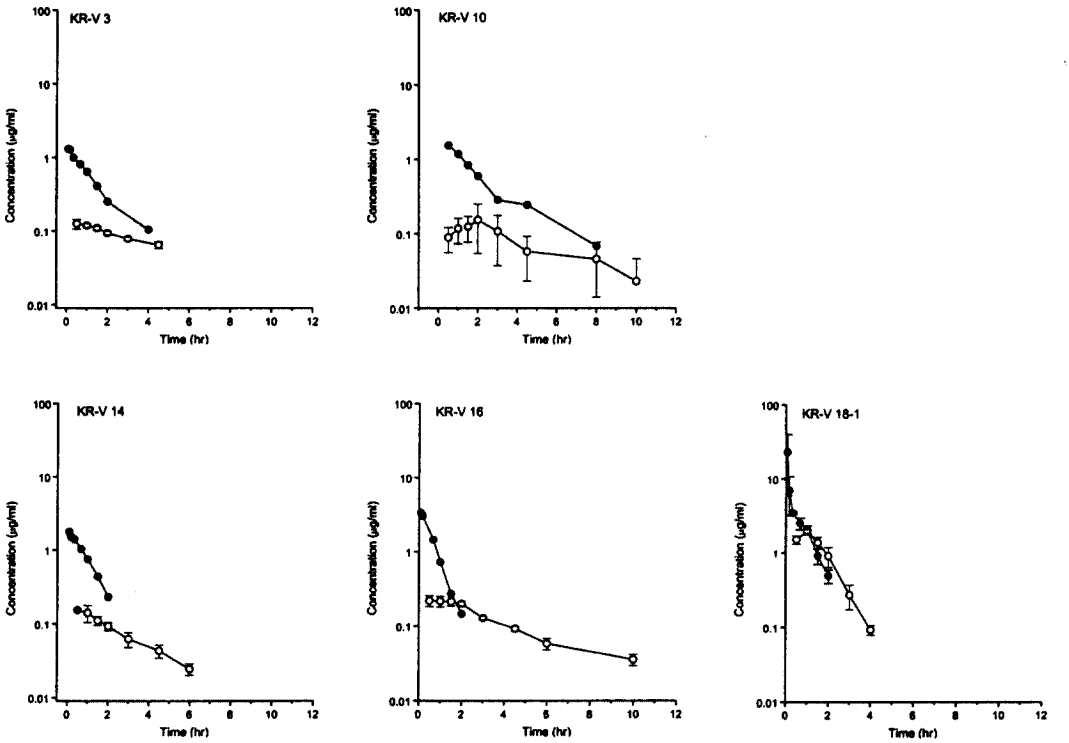


Fig 2. Plasma concentration-time profiles of *KR-V* 3, 10, 14, 16 and 18-1 following intravenous administration at dose of 10 mg/kg (n = 1 rat) and oral administration at 50 mg/kg (n = 3 rats). The values are mean ± SEM(-○- p.o., -●- i.v.).

Table 3. Pharmacokinetic parameters of *KR-V* 3, 10, 14, 16 and 18-1 after *i.v.* administration of 10 mg/kg to rats

KR-V No.	Pharmacokinetic parameter value[a]			
	$t_{1/2}$ (hr)	$AUC_{0-\infty}$ (μg.hr/ml)	CL_{total} (L/hr/kg)	Vd (L/kg)
3	0.85±0.047	1.72±0.074	5.83±0.253	7.11±0.173
10	0.69±0.075	1.56±0.133	6.40±0.545	6.41±0.331
14	0.73±0.042	1.98±0.088	5.05±0.226	5.29±0.138
16	0.43±0.021	2.44±0.091	4.09±0.153	2.55±0.059
18-1	0.36±0.052	8.97±1.867	1.12±0.233	0.58±0.173

[a] Valeus are the estimated±clculated SEM(n=1).
$t_{1/2}$, a half-life of elimination; CL_{total}, total body clearance; Vd, volume of distribution.

Table 4. Pharmacokinetic parameters of *KR-V* 3, 10, 14, 16 and 18-1 after oral administration of 50 mg/kg to rats

KR-V No.	n	Pharmacokinetic parameter value[a]				
		C_{max} (μg/ml)	t_{max} (hr)	$AUC_{0-\infty}$ (μg.hr/ml)	$t_{1/2}$ (hr)	F (%)
3	3	0.13±0.011	0.55±0.319	0.73±0.066	3.49±0.363	8.53±0.764
10	2	0.13±0.048	1.33±0.281	0.82±0.422	2.91±0.538	10.5±5.403
14	3	0.15±0.015	0.53±0.119	0.51±0.074	1.95±0.235	5.14±0.743
16	2	0.23±0.029	0.87±0.061	1.06±0.046	2.55±0.502	8.65±0.377
18-1	3	1.89±0.110	0.70±0.092	3.66±0.483	0.56±0.076	8.16±1.077

[a] Values are mean±SEM (n = 3).
C_{max}, maximum concentration; t_{max}, time to reach maximum concentrations; $t_{1/2}$, a half-time of elimination; F, bioavailability.

5 compounds are shown in Fig 2 and the pharmacokinetic parameters are summarized in Table 3 and 4. After *i.v.* administration (10 mg/kg), the CL_t of *KR-V* 3, 10, 14, 16 and 18-1 was 5.8, 6.4, 5.1, 4.1 and 1.1 L/min/kg, respectively, and the Vd was 7.1, 6.4, 5.3, 2.5 and 0.6 L/kg, respectively. After the 50 mg/kg *p.o.* dose, the $t_{1/2}$ was 3.5, 2.9, 2.0, 2.6 and 0.6 hr, respectively, and the AUC was 0.7, 0.8, 0.5, 1.1 and 3.7 μg hr/ml, respectively. The bioavailibility of *KR-V* 3, 10, 14, 16 and 18-1 was calculated to be 8.5, 10.5, 5.1, 8.6 and 8.2%, respectively.

Anti-HIV activity against HIV-1 mutant strains : Of the 19 *KR-V* compounds, *KR-V* 5, 6, 12 and 18-1 exhibited positive anti-HIV activity against HIV-1 mutant strains including 100-Ile, 103-Asn, 138-Lys and 181-Cys (Pharmaceutical Screening Center, KRICT).

Discussion

AZT is the first FDA-approved anti-HIV drug. However, a significant toxicity and short half-life are factors which continue to limit its effective use. NNRTIs such as HEPT inhibit the replication of HIV-1 at concentrations that are three to five orders of magnitude below their cytotoxic threshold[12]. However, the rapid emergence of drug-resistant mutants is regarded as a significant obstacle to their clinical usefulness. Because of this, there is a need to develop a new anti-HIV agent with potent activity against these mutants but also with lesser cytotoxicity.

The *i.v.* and *p.o.* pharmacokinetics were studied to assess the bioavailability of the *KR-V* series. Of the 19 *KR-V* compounds, only *KR-V* 3, 10, 14, 16 and 18-1 showed adequate oral bioavailability in rats. An one-compartment open model with first order elimination adeuately described the plasma concentration-time data. In the *i.v.* kinetic study, at a dose of 10 mg/kg, the $t_{1/2}$ of the 5 compounds was similar, but the CL_t of *KR-V* 3, 10, 14 and 16 (>4.0 L/hr/kg) were significantly greater than that of *KR-V* 18-1 (1.1 L/hr/kg). The AUC of *KR-V* 18-1 was greater (8.97 μg · hr/ml) and the Vd was smaller (0.58 L/kg) than those of other compounds. In the *p.o.* kinetic study, at a dose of 50 mg/kg, although the $t_{1/2}$ of *KR-V* 18-1 was shorter than that of the other compounds, the AUC (3.66 μg · hr/ml) and C_{max} (1.89 μg/ml) were markedly greater. The lower value of CL_t and higher value of AUC for *KR-V* 18-1 indicates that this compound is less subject to hepatic and/or renal elimination than the other compounds[15]. The $t_{1/2}$ of *KR-V* 3, 10, 14 and 16 were longer following *p.o.* administration than after *i.v.* administration. These features, coupled with the increased $t_{1/2}$ after oral dosing, suggest a possibility of prolonging absorption, the so-called flip-flop mechanism[16].

In the *in vitro* experiment, only the *KR-V* 18-1, among the 5 bioavailable compounds, exhibitd potent activity against HIV-1 mutant strains including 100-Ile, 103-Asn, 138-Lys and 181-Cys. This point is very critical in selecting new anti-AIDS drug candidates. As shown in Table 2, the cytotoxicity of *KR-V* 18-1 (CC_{50} 22.6 μg/ml) is lower than that of AZT (CC_{50} 4.6 μg/ml). The antiviral activity of *KR-V* 18-1 (EC_{50} 0.0026 μg/ml) is more potent than that of HEPT (EC_{50} 7 μg/ml) and is comparable with that of AZT (EC_{50} 0.003 μg/ml). Moreover, the oral bioavailibility of *KR-V* 18-1 (14%) is similar to that of HEPT (11%)[5,17]. From these overall data, including the antiviral activity, mutant activity and oral bioavailibility, the most positive findings from the investigation of new *KR-V* lead compounds were obtained with *KR-V* 18-1.

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새로운 항HIV-1제, *KR-V* series의 개발을 위한 약물동태연구

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국문초록 : 새로운 항HIV 후보물질인 19개의 *KR-V* 경구제제의 생체이용률을 평가하기 위해 랫드에서 정맥 및 경구투여후 약물동태를 연구하였다. 혈장내 *KR-V* 화합물들의 검출은 HPLC-UVD 법을 이용하여 분석하였다. 19개 *KR-V* series중 *KR-V* 3, 10, 14, 16 및 18-1만이 랫드에서 경구로 흡수되어 생체이용성을 나타내었다. 10mg/kg의 정맥투여후 약물동태 연구에 있어서는 5개 물질, *KR-V* 3, 10, 14, 16 및 18-1의 소실 반감기는 서로 비슷하였으나 *KR-V* 3, 10, 14 및 16의 총청소율(CL_{total}, >4L/hr/kg)은 *KR-V* 18-1(1.1L/hr/kg) 보다 유의성 있게 높았다. *KR-V* 3, 10, 14 및 16에 비해 *KR-V* 18-1이 혈중곡선하면적(AUC, 8.97μg · hr/ml)은 크고 겉보기분포용적(Vd, 0.58L/kg)은 적었다. 50mg/kg의 경구투여후 약물동태 연구에 있어서는 *KR-V* 18-1의 반감기가 다른 4개의 물질에 비해 비록 짧았지만 경구 AUC (3.659μg · hr/ml), 최고혈중농도(C_{max}, 1.891μg/ml)는 현저히 높았다. 또한 별도의 *in vitro* 실험결과, 생체이용률을 나타낸 이들 5개의 물질들 중 *KR-V* 18-1만이 HIV-1 돌연변이종(mutants)에 대한 억제효과를 나타내었다. 따라서 *KR-V* 18-1이 항에이즈(AIDS)제의 새로운 후보물질 혹은 선도물질로서 가능성이 기대되었다.

Key words : anti-HIV agent, pharmacokinetics, *KR-V*, rat, mutant strain.