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International Immunopharmacology 1 (2001) 1295–1305

International Immunopharmacology

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Long-term intake of Korean red ginseng in HIV-1-infected patients: development of resistance mutation to zidovudine is delayed

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Received 1 November 2000; accepted 26 February 2001

Abstract

We have observed that $CD4^+$ T cell counts in human immunodeficiency virus (HIV)-1-infected patients treated with only Korean red ginseng (KRG) are maintained or even increased for a prolonged period. In the present study, we investigated whether the development of resistance mutations in reverse transcriptase (RT) to zidovudine (ZDV) is delayed by combined therapy with KRG and ZDV. Nested polymerase chain reaction (PCR) and direct sequencing methods were used to define RT codons 41, 67, 70, 210, 215 and 219 of the HIV-1 *pol* gene in DNA from peripheral blood mononuclear cells (PBMC) samples from 18 patients. Nine of these eighteen patients were in the KRG group and had been treated with KRG for 60 ± 15 months (range: 38–82) and ZDV, and nine were in the control group and had been treated with ZDV only. The patients in the KRG group had been treated with ZDV for 75 ± 24 months, and $CD4^+$ T cell counts were maintained from 239 ± 85 to 234 ± 187 μl^{-1} ($P > 0.05$) during the study period, whereas the patients in the control group had been treated with ZDV for 51 ± 31 months, and their $CD4^+$ T cell counts decreased from 272 ± 97 to 146 ± 154 μl^{-1} ($P < 0.01$). In samples within 24 months of ZDV therapy, the overall incidence of 6 resistance mutations to ZDV was 4.2% and 47% in the KRG and control group ($P < 0.01$), respectively. In samples after 24 months of therapy, the incidence was 21.7% and 56.3% in the KRG and control group ($P < 0.01$), respectively. These data suggest that the maintenance of $CD4^+$ T cell counts by ZDV and KRG-intake for a prolonged period might be indirectly associated with delayed development of resistance to ZDV by KRG-intake.

Keywords: Resistance mutation to zidovudine; Reverse transcriptase; Korean red ginseng; $CD4^+$ T cell counts

Abbreviations: HIV-1, human immunodeficiency virus type-1; ZDV, zidovudine; KRG, Korean red ginseng; RT, reverse transcriptase; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; ICD-p24 Ag, immune complex dissociated p24 antigen; CTL, cytotoxic $CD8^+$ T lymphocytes; IL-2, interleukin-2

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1567-5769/01/$ - see front matter
PII: S1567-5769(01)00061-3

1. Introduction

Remarkable advances in the treatment of HIV infection have been made with highly active anti-retroviral drug therapy over the last decade, but HIV infection still remains an incurable disease. Drug resistance is probably the most important factor attributing for the failure of treatment today [1]. Consequently, multidrug therapy has been tried over several years, but it can be poorly tolerated and is compromised by toxicity and noncompliance. Immune system-based therapy combined with antiretroviral therapy is needed as an alternative approach for controlling HIV [2].

In Korea, ZDV monotherapy was introduced in early 1991 to treat HIV-infected patients with $CD4^+$ T cell counts of less than 500 μl^{-1}. Although the effects of low dose ZDV monotherapy (400–600 mg/day) were not maintained up to 12 months [3], it was the only antiretroviral therapy until early 1997. Disease progression in patients with ZDV therapy coincides with the emergence of drug-resistant strains having mutation out of RT codon amino acid positions 41, 67, 70, 210, 215 and 219 [4–7].

Panax ginseng C.A. Meyer is an herbal root, which has been known in China for more than 2000 years [8]. Many scientific investigations have been performed on the active ingredients and their functions since the late 1960s. In regard to antimicrobial effect, Song et al. [9] reported that ginseng treatment reduces bacterial load and lung pathology in chronic *Pseudomonas aeruginosa* pneumonia in rats. Ginseng is now 1 of 12 medicinal herbs commonly used in America [10]. A double-blind study in normal human volunteers revealed a significant increase in neutrophil function, $CD4^+$ T cell counts and NK-function in individuals taking ginseng compared to those taking placebo [11]. It is particularly interesting that the immunostimulating effect of acidic polysaccharide (Ginsan) from ginseng root is blocked in the presence of anti-interleukin (IL)-2 and anti-IFN-γ [12].

We had an opportunity to try KRG for HIV-1-infected individuals for 6 months beginning in late 1991. Surprisingly, we observed various beneficial effects of KRG-intake (5.4 mg daily) in these individuals including increases in the $CD4^+$, $CD8^+$ T cell counts and body weight, and decrease in soluble CD8 antigen (sCD8) in serum [13]. Thereafter, the study was done consecutively for the same target population although the number of patients was increased and there were some interruptions. Moreover, our data showed that intrapatient amino acid variation between clones in the C2/V3 region of HIV-1 from these patients was inversely correlated with the duration of KRG-intake [14]. Among the patients who were involved in the first KRG trial, some have maintained $CD4^+$ T cell counts for 8 years with KRG-intake only. On the other hand, in patients with ZDV monotherapy, the decrease in $CD4^+$ T cell counts usually occurs within 12 months due to the development of resistance to ZDV. This phenomenon was not observed, however, in the patients treated for a prolonged period with ZDV and KRG in combination.

In the present study, we investigated whether the maintenance of $CD4^+$ T cell count in HIV-1-infected patients treated with ZDV and KRG is associated with the delay of the development of resistance to ZDV.

2. Materials and methods

2.1. Patients

This study was approved by the Institutional Review Board of Asan Medical Center of University of Ulsan College of Medicine, and all patients gave their written informed consent before participation. Eighteen patients who were selected from our cohort [13,14] were divided into a KRG group and a control group. In the KRG group, all nine HIV-1-infected patients had taken KRG for more than 36 months (60 ± 15 months; range: 38–82) and ZDV for more than 18 months. All were asymptomatic at enrollment but three patients had a past history for shingles (patients 2, 5 and 8, Table 1). In the control group, nine patients who had taken only ZDV for more than 18 months were selected. KRG-intake was the only significant variable between two groups ($P < 0.01$) (Fig. 1). There was no significant difference in clinical stages and demographics between the KRG group and the control group (Table 1). In detail, mean age and baseline $CD4^+$ T cell counts in the KRG group and the control group were 35 ± 3

Table 1
Baseline characteristics of 18 HIV-1-infected patients

Patient no.	Sex/age	Year of diagnosis	CDC stage[a]	$CD4^+$ T cell count (μl^{-1})	Viral load[b] (pg/ml)
KRG group					
1	M/40	1989	A	209	0
2	M/35	1990	B	131	112
3	M/35	1993	A	135	130
4	M/29	1990	A	198	0
5	M/37	1990	B	227	220
6	M/35	1991	A	246	292
7	M/35	1990	A	280	0
8	F/32	1991	B	350	0
9	M/37	1991	A	374	0
Control group					
10	M/29	1992	B	179	25
11	M/16	1991	A	284	0
12	M/40	1991	B	176	293
13	M/41	1993	B	266	0
14	F/40	1997	A	147	120
15	M/35	1997	A	347	Not tested
16	F/31	1988	A	327	100
17	M/31	1988	A	272	0
18	F/30	1990	A	452	0

[a]A: Asymptomatic; B: Symptomatic.
[b]Immune complex dissociated p24 antigen level in serum.

and 32 ± 8 ($P > 0.05$), and 239 ± 85 and 272 ± 97 μl^{-1} ($P > 0.05$), respectively . The study period since the initiation of ZDV therapy was 75 ± 24 months for the KRG group and 51 ± 31 months for the control group ($P > 0.05$).

2.2. $CD4^+$ T cell counting

$CD4^+$ and $CD8^+$ T cells were measured by FACScan (BD; Becton-Dickinson, CA, USA) flow cytometer after staining of PBMC with phycoerythrin and fluorescein isothiocyanate (FITC)-conjugated antibodies for CD4 and CD8 antigen (Simultest reagent, BD) [13].

2.3. Treatment

Treatment of ZDV monotherapy was begun in early 1991, and triple therapy including protease inhibitors was initiated in late 1997 in South Korea. This study limits the period of ZDV monotherapy prior to triple therapy. The doses of ZDV in the patients were 400–600 mg/day, which were lower doses compared to those in other reports [4–6].

The outpatient-based study on the KRG trial in HIV-1-infected patients was begun at Korean NIH in late 1991, as described previously [13,14]. The KRG used in this study was commercial product which was prepared from six-year-old roots by Korea Ginseng (Chungchungnam-do, South Korea). The quality of KRG used in this trial is guaranteed by the regulation of the South Korean government. Daily dose was 5.4 g and patients were told to take six capsules (300 mg per capsule) orally, three times daily.

2.4. Measurement of ICD-p24 antigen

HIV p24 antigen in serum was detected by the immune complex dissociated (ICD) method (DuPont Medical Products, Boston, USA) [14]. The lower detection limit was 25 pg/ml. Four patients were

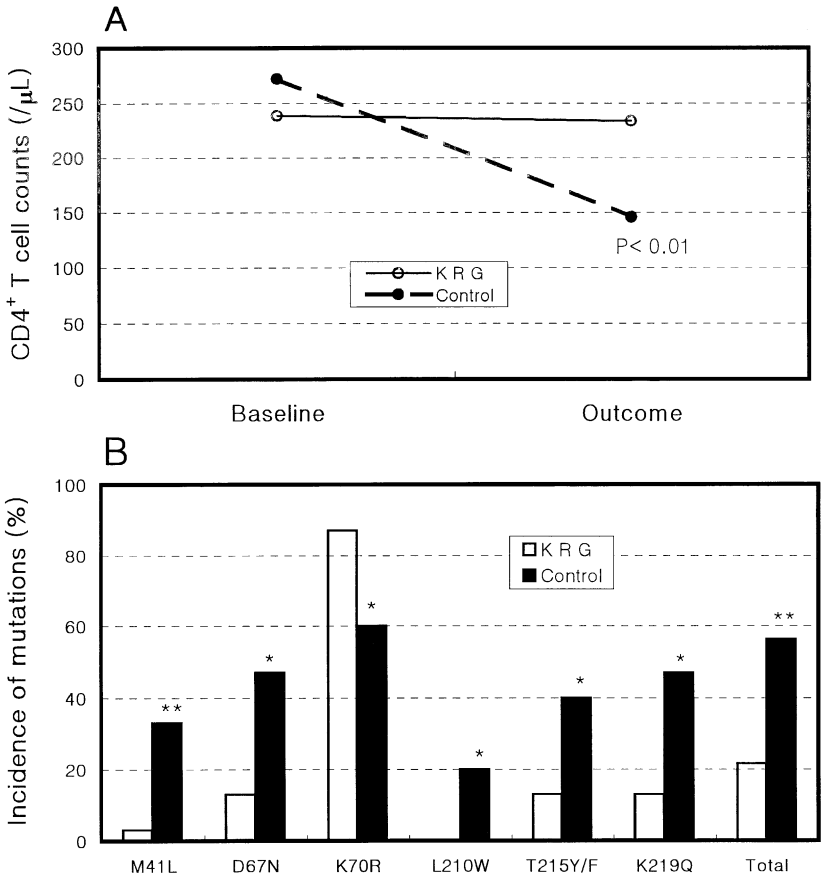

Fig. 1. Comparison of the change of $CD4^+$ T cell counts and incidence of resistance mutations to zidovudine (ZDV) between two groups. The patients in the Korean red ginseng (KRG) group ($n = 9$) had been treated with ZDV for 75 ± 24 months and KRG for 60 ± 15 (range: 38–82). The patients in the control group ($n = 9$) were treated with ZDV only for 51 ± 31 months ($P > 0.05$). (A) $CD4^+$ T cell counts were maintained from 239 ± 85 to 234 ± 187 μl^{-1} ($P > 0.05$) for 75 ± 24 months in the KRG group, whereas it decreased from 272 ± 97 to 146 ± 154 μl^{-1} for 51 ± 31 months in the control group ($P < 0.01$). (B) Comparison of incidence of six resistance mutations to ZDV after 24 months of ZDV therapy. All incidence of mutations except K70R were higher in the control group than in the KRG group, respectively ($P < 0.05$). Total incidence of six mutations to ZDV were 21.7% (39/180) in the KRG group and 56.3% (36/64) in the control group ($P < 0.001$). $^*P < 0.05$, and $^{**}P < 0.01$ by Chi-square test.

positive for p24 antigen in the KRG group and the control group, respectively (Table 1). The tests were performed according to the manufacturer's instructions.

2.5. DNA preparation

DNA for PCR amplification was prepared directly from patients' uncultured PBMC. PBMC were isolated from 10 ml whole blood in sodium heparin tube using Ficoll-Hypaque (Pharmacia, Piscataway, NJ, USA) density gradient centrifugation and divided into two vials and stored at −80°C. One vial of PBMC was thawed and centrifuged. Supernatant was discarded, leaving 200 μl including cell pellets. After homogenization, 10 μl was denatured by heating for 10 min at 95°C.

2.6. PCR and sequencing

Crude cell lysates were used for direct DNA sequencing. Denatured DNA samples (10 μl) were amplified by nested PCR. The outer primer pair were JA99 and RIT 137 [15] and two inner primer pairs

were 523 and 526 for fragment A (257 bp), and 527 and 530 for fragment B (239 bp) [16]. After the first denaturation at 95°C for 3 min, 30 cycles were done under the condition of 94°C for 30 s, 50°C and 72°C for 1 min each, followed by a final extension at 72°C for 10 min. The second PCR was done with 5 μl of the first PCR product. Cycling condition was the same as above, except 58°C for 1 min for primer 523 and 526 and 64°C for 1 min for 527 and 530. The second PCR products were analyzed on 1.5% agarose gels and visualized by ethidium bromide-staining. Sequencing primers used were 523 for fragment A and 527 for fragment B.

2.7. Sequence data

Representative sequences in each patient were registered at GenBank. The GenBank accession numbers of these sequences are in order of duration of therapy as follows: in patient (pt) #1, AF273163, AF282947, and AF282948; in pt #2, AF273143-AF273146; in pt #3, AF273206, AF282956, and AF282957; in pt #4, AF273175 and AF282951; in pt #5, AF273168, and AF282950; in pt #6, AF273184 and AF282952; in pt #7, AF282142 and AF281872; in pt #8, AF282145, AF273177, and AF282146; in pt #9, AF273190, and AF282152; in pt #10, AF281874 and AF273200; in pt #11, AF273189, AF282953, and AF282954; in pt #12, AF273195, and AF282154; in pt #13, AF273207; in pt #14, AF273211, in pt #15, AF282166; in pt #16, AF273158, and AF282134; in pt #17, AF282133, and in pt #18, AF273155.

2.8. Statistical analysis

Data were expressed by mean $\pm$ standard deviation. Paired Student's *t*-test and Chi-square test were used for data analyses between the two groups.

3. Results

3.1. Change in $CD4^+$ T cell counts

In KRG group, nine patients had been treated with both ZDV and KRG for 75 ± 24 months after initiation of ZDV therapy, and $CD4^+$ T cell counts were well maintained (from 239 ± 85 to 234 ± 187 μl^{-1}; $P > 0.05$). The change of $CD4^+$ T cell counts and development of resistance mutations for patients 1 to 3 was described in detail previously [17] and those in patients 6 and 8 are described (Fig. 2). Patient 9, who received combined therapy with ZDV and KRG as early as 6 months after HIV-1 infection, had a steady increase in $CD4^+$ T cell counts with a steady decrease in sCD8 up to 94 months. The total amount of KRG taken by patient 9 during 100 months was 10,002 g. On the other hand, in the control group treated with ZDV only, the $CD4^+$ T cell counts decreased from 272 ± 97 to 146 ± 154 μl^{-1} ($P < 0.01$) during 51 ± 31 months (Fig. 1A). As representative cases among the control group, the change of $CD4^+$ T cell counts and development of ZDV resistance mutations in patients 10 and 11 are described in detail in Fig. 2.

3.2. Comparison of incidence of mutations

In samples obtained within 24 months of the therapy except for baseline data, the incidences of six resistance mutations were 0% (0/8) and 80% (4/5) for M41L ($P < 0.01$), 25% (2/8) and 20% (1/5) for K70R ($P > 0.05$), 0% and 60% (3/5) for L210W ($P < 0.05$), 0% and 100% (5/5) for T215F/Y ($P < 0.01$), and 0% and 20% (1/5) for D67N ($P > 0.05$), in the KRG and control group, respectively. Incidences of all mutations except K70R were higher in the control group than in the KRG group. The total incidence was higher in the control group (47%; 14/30) than the KRG group (4.2%; 2/48) ($P < 0.001$).

In samples after 24 months of therapy, the incidences showed the same characteristics as in the samples obtained within 24 months of therapy; 3% (1/30) and 33% (5/15) for M41L ($P < 0.01$), 87% (26/30) and 60% (9/15) for K70R ($P < 0.05$), 13% (4/30) and 47% (7/15) for D67N/E and K219Q ($P < 0.05$), 0% and 20% (3/15) for L210W, 13% (4/30) and 40% (6/15) for T215F/Y ($P < 0.05$), respectively. Total incidence was also higher in the control group (56.3%; 36/64) than in the KRG group (21.7%; 39/180) ($P < 0.001$) (Fig. 1B). The overall incidence of six mutations associated with ZDV resistance increased gradually depending on the duration of therapy in both groups. However, the

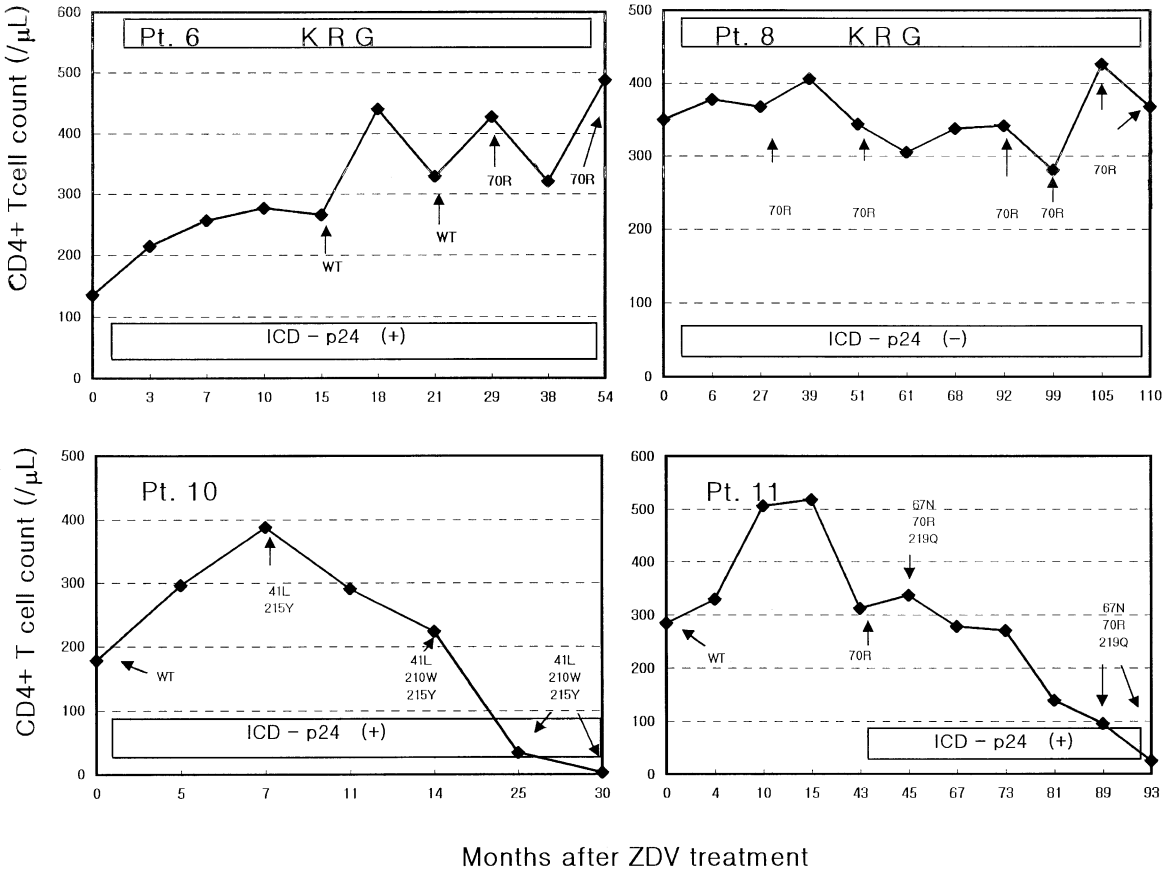

Fig. 2. Comparison of representative cases between the KRG group treated with ZDV and KRG (patients 6 and 8) and the control group treated with ZDV only (patients 10 and 11). Since the initiation of ZDV therapy, the change of $CD4^+$ T cell counts over 57 months in patients 6 and 8 shows a good contrast with that over 30 months in patients 10 and 11. In the change of viral load, immune complex dissociated (ICD)-p24 antigen decreased from 292 to 73 pg/ml in patient 6 and it was continuously negative in patient 8, whereas it increased from negative to 117 and 132 pg/ml in patients 10 and 11, respectively. In addition, patient 11 was infected with HIV-1 from a blood donor in October 1989. The blood donor who has taken KRG only from November 1991 is maintaining $CD4^+$ T cell counts of more than 500 μl^{-1} as of July 2000.

increase was confined to the patients with low baseline $CD4^+$ T cell counts in the KRG group, whereas there was no such findings in the control group.

3.3. The second mutation occurred later in the KRG group than in the control group

The first mutation observed was K70R. Among the available samples, this mutation occurred as early as 10 months after initiation of therapy in patient 1 of the KRG group. It was continuously detected thereafter in the KRG group. In the control group, the first change was at codon 41 and was detected 7 months in patient 10. Because of a lack of samples available from patient 10, we could not know the exact time of the development of the first mutation. The earliest time point the second mutation occurred is 34 months in patient 3 of KRG group, whereas it was also 7 months in control group. The second mutation seemed to occur very slowly in the KRG group. In patient 2, the first T215F was detected 67 months after the initiation of therapy with low doses of ZDV (300 mg daily). And in patient 3, the second mutation, T215F, was first detected 34 months after therapy. These patients (2 and 3) had p24 antigen at baseline and showed T215F at 67 and 34 months in

patients 2 and 3, respectively [17]. In particular, in patient 4, who had taken ZDV of 3495 tablets (100 mg per tablet) intermittently supplied for 73 months, we could not detect any mutation associated with ZDV resistance. This case falls under the "drug holiday". Although the patient had shown high viral titer (220 pg/ml) from baseline, he actually maintained $CD4^+$ T cell counts above the baseline value. It is notable that there was no mutation except K70R in four patients (6, 7, 8 and 9). By reference, it is rare that the development of 215F/Y is detected 60 months after ZDV therapy [18]. However, in patients 8 and 9, T215F was not detected up to 110 and 100 months from initiation of ZDV therapy, respectively (Table 2). Interestingly, mutation at codon 215 in the KRG group was all T215F in three patients, whereas it was T215Y in four out of five patients in the control group ($P < 0.05$).

Table 2
Development of resistance mutations to zidovudine (ZDV) in 32 representative samples among the 58 samples from 18 patients
Hyphens connote identity with wild codon. KRG: Korean red ginseng.

Patient no.	Months with		Wild type codon in RT						$CD4^+$ T cells (μl^{-1})	ICD-p24 Ag (pg/ml)
	ZDV	KRG	41 M[a]	67 D	70 K	210 L	215 T	219 K		
KRG group										
1	10	19	–	–	R	–	–	–	203	NT[b]
	49	38	–	N	R	–	F	Q	14	25
2	62	44	–	–	R	–	–	–	29	NT
	67	49	–	N	R	–	F	Q	10	27
3	19	19	–	–	–	–	–	–	219	60
	29	29	–	–	R	–	–	–	156	106
	34	34	–	N	R	–	F	Q	133	163
4	46	30	–	–	–	–	–	–	397	0
	64	47	–	–	R	–	–	–	189	0
	93	76	L	–	R	–	–	–	228	17
5	6	6	–	–	–	–	–	–	396	70
	73	52	–	–	–	–	–	–	331	329
6	21	17	–	–	–	–	–	–	138	267
	54	50	–	–	R	–	–	–	488	130
7	24	20	–	–	R	–	–	–	274	40
	68	66	–	–	R	–	–	–	222	77
8	27	17	–	–	R	–	–	–	368	0
	110	82	–	–	R	–	–	–	368	0
9	36	16	–	–	R	–	–	–	600	0
	100	76	–	–	R	–	–	–	435	0
Control group										
10	7	0	L	–	–	W	Y	–	388	52
	30	0	L	–	–	W	Y	–	4	117
11	43	0	–	–	R	–	–	–	312	40
	45	0	–	N	R	–	–	Q	337	NT
12	14	0	L	–	–	–	Y	–	174	100
13	27	0	–	N	R	–	–	Q	238	29
14	26	0	–	–	R	–	–	–	138	161
15	19	0	–	N	R	–	F	–	423	14
16	19	0	L	–	–	W	Y	–	281	49
	76	0	L	N	–	W	Y	Q	5	25
17	105	0	–	–	R	–	Y	Q	149	0
18	56	0	–	–	R	–	–	–	370	NT

[a] One letter codon of amino acid for methionine.
[b] Not tested.

3.4. The patients who had low baseline CD4$^+$ T cell counts showed bad prognosis

Patients 1, 2 and 3 with baseline CD4$^+$ T cell counts below 200 μl^{-1} progressed to the late stage of AIDS with increases in p24 antigen compared to baseline value, whereas five patients (5–9) with CD4$^+$ T cell counts of more than 200 μl^{-1} did not show any progression. Concurrently, three out of four patients (1–4) with more than 2 mutations progressed to the late stage of AIDS [17]. Despite very good compliance, patient 2 showed multiple mutations at 67 months of therapy, even though CD4$^+$ T cell counts (154 μl^{-1}) were maintained at greater than baseline value (131 μl^{-1}) for up to 47 months from ZDV initiation and ICD-p24 antigen was suppressed below baseline value. Although the antiretroviral effect of KRG was less strong, its effect was maintained up to 52 and 54 months in patients 5 and 6, respectively. These results show definite contrast compared to those of patients treated with ZDV only.

3.5. Amino acid sequences

Deduced amino acid sequences of RT region obtained at baseline and during ZDV therapy from each

Patient no.	mo.	41 ICTEMEKEGKISKIGPENPYNTPVFAIKKKDSTKWRKLVDFRELNKRTQDF	Consensus
1	0	..........R..	AF273163
	49	..A.....N.NR..	AF282948
2	26	.....R..	AF273143
	67	.....EG.R..	AF273146
3	8	.....	AF273206
	50	.....N.NR..	AF282957
4	0	....I.....R..	AF273175
	93	....L.....NR..	AF282951
5	6	.....	AF273168
	73	.....	AF282950
6	9	.....K....	AF273184
	54	.....R.....K....	AF282952
7	24	.....R..	AF282142
	68	.....R..	AF281872
8	27	.....R..	AF282145
	110	.....SR..	AF282146
9	0	.....R..	AF273190
	100	.....R.....R..	AF282152
10	0	.....	AF281874
	30	....L.N.....	AF273200
11	0	.....	AF273189
	79	.....N.NR..	AF282954
12	14	....L.....	AF273195
	34	....L.....	AF282154
13	47	.....N..R..	AF273207
14	26	.....K....	AF273211
15	19	.....R..	AF282166
16	19	....L.....K....	AF273158
	76	....L.....K....	AF282134
17	105	..A.....N..R..	AF282133
18	56	.....R.....R..	AF273155

Fig. 3. Deduced amino acid sequences in the reverse transcriptase (RT) region that is associated with resistance to ZDV in 18 HIV-1 infected patients. Amino acids in RT codons 41, 67, 70, 210, 215 and 219 mutate as the resistance to ZDV develops. Mo. means the number of the months of ZDV therapy. Dots connote identity with the consensus sequence, whereas mutations are shown by the single amino acid code. Patient 4 shows a premature stop codon (#) at codon 212. There was no significant difference in amino acid sequences between two groups.

```
Patient   169                                                  210  215  219
no.   mo.  EPFRKQNPDIVIYQYMDDLYVGSDLEIGQHRTKIEELRQHLLRWGFTTPDK-Consensus
1      0   D...T...E.....................V....K...K..L..... AF273163
          49 D.......E..T.....................V....E...K..LF...Q AF282948
2     26   ..............................A.............L..... AF273143
          67 ..........................................LF...Q AF273146
3      8   ...........................I.V.............. AF273206
          50 ....R.....................I.V...........F...Q AF282957
4      0   ..............I.............I...........#..... AF273175
          93 ...........................I.................. AF282951
5      6   ..........................................K..... AF273168
          73 ..........................................K..... AF282950
6      9   ..... AF273184
          54 ..........................................K..... AF282952
7     24   ..............................V.............. AF282142
          68 ..... AF281872
8     27   .........L.....................................L..... AF282145
         110 .........L.....................................L..... AF282146
9      0   ...........................I.................. AF273190
         100 K..........................I......E.......... AF282152
10     0   ...........................I.................. AF281874
          30 ..........................................W....Y.... AF273200
11     0   ...........................I.V.............. AF273189
          79 ...........................I.V.............Q AF282954
12    14   .........E.....................I.............Y.... AF273195
          34 .........E.....................I.............Y.... AF282154
13    47   ..........................E......D.............Q AF273207
14    26   ....TK..EM.....................A......H...K..... AF273211
15    19   ..... AF282166
16    19   .....K.....................E..WK...Y.... AF273158
          76 .....KY.....................I.V....E..WK...Y...Q AF282134
17   105   ....TK..EM.................E...A......E...K...Y...Q AF282133
18    56   ..... AF273155
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Fig. 3 (*continued*).

patient are described in Fig. 3. Patient 4 showed a premature stop codon at position 212 at baseline. There was no significant difference in amino acid sequence between the two groups (KRG and control).

4. Discussion

We have observed the maintenance or increase in $CD4^+$ T cell counts in patients treated with KRG since 1991 [13,14]. Mild but consistent decrease in p24 antigen during KRG-intake was also detected. In particular, the decrease in sCD8 level in serum during KRG-intake was most notable. The decrease in sCD8 was 31.3% from 801 to 550 U/ml during 68 months in KRG group. The decrease in sCD8 was maintained as long as KRG was taken. This finding could probably result in the enhancement or long-term maintenance of effector function of cytotoxic $CD8^+$ T lymphocyte (CTL), which is an important characteristic in the long-term nonprogressor [2]. In addition, in cases of combination with ZDV, the beneficial effects associated with KRG-intake mentioned above were particularly notable, and a decrease in $CD4^+$ T cell counts was not detected during at least a few years of therapy. This was in contrast to our previous report on ZDV efficacy [3]. Moreover, our recent report showed that intrapatient amino acid variation between clones in the C2/V3 region of HIV-1 was inversely correlated with the duration of KRG-intake [14]. Based on these obser-

vations, KRG-intake itself is likely to have beneficial effects or selective pressure on HIV-1 in vivo.

We did not obtain information about the level of resistance to ZDV in our patients. However, site-directed mutagenesis experiments have confirmed that six amino acid changes (M41L, D67N, K70R, L210W, T215F/Y, and K219Q) in the HIV-1 RT are responsible for the acquisition of ZDV resistance [4–7]. During ZDV therapy, the mutation at codon 70 tends to be the first to arise as early as 12 weeks after initiation of treatment, followed by the mutation at codon 215 and then other combinations of the mutations listed above. The mutation at codon 215 appears to be the most stable of six mutations and seems to play the leading role in the development of ZDV resistance, as its presence results in a 16-fold increase in resistance relative to the wild type [5].

Regarding the incidence of resistance mutation to ZDV, overall incidence of six mutations was 2.9-fold higher in the control group (53.2%; 50/94) than the KRG group (18%; 41/228) ($P < 0.001$). Surprisingly, we could detect a higher frequency of K70R in the KRG group than in the control group, although single K70R itself did not affect significantly the decrease in drug sensitivity. In fact, $CD4^+$ T cell counts in patients with K70R were maintained in this study. It was well known that the reversal of K70R to wild-type is paralleled by the appearance of mutations at codon 215 [4,5]. However, we could not observe reversal to wild-type in this study. The incidence of six resistance mutations was lower in KRG group than in other reports [18–21]. Even in patients (2, 3, and 4) with baseline $CD4^+$ T cell counts of less than 200 μl^{-1}, the second mutation developed late in the course of treatment. These results are in agreement with the maintenance of $CD4^+$ T cell counts for a prolonged period in those patients. For example, patient 11 (Fig. 2) was infected with HIV-1 from blood donor in October 1989 and his $CD4^+$ T cell counts was less than 50 μl^{-1} in November 1999. The other blood recipient from this donor also shows low $CD4^+$ T cell counts of less than 200 μl^{-1} in July 2000 despite the recent start of antiretroviral therapy without KRG-intake. On the other hand, the HIV-1 donor who has taken KRG only since November 1991 (495 μl^{-1}, 19.7%) is maintaining $CD4^+$ T cell counts (as of July 2000; 609 μl^{-1}, 29.3%). A complete sequence of HIV-1 from the blood donor was determined as a long-term nonprogressor (GenBank accession number; AF224507). These three cases show a good contrast in the change of $CD4^+$ T cell counts although the number of cases is too small to be conclusive.

Several factors associated with the rapid emergence of ZDV resistance are as follows: host factors of the advanced stage of HIV-1 disease and low $CD4^+$ T cell counts; viral factors of high plasma viral load, pre-existing drug-resistant virus, and possibly syncytium-inducing phenotype; and drug-related factors of suboptimal drug levels or poor compliance [21]. The likelihood of developing resistance appears not to be related to drug doses when daily doses of ZDV are > 500 mg [1]. In some patients treated with low doses of ZDV < 500 mg, it is possible that the development of resistance could be delayed by low doses or poor compliance. That possibility is unlikely because of the maintenance of $CD4^+$ T cell counts and continuous suppression of ICD-p24 antigen and sCD8 antigen for more than 8 years.

Recently, IL-2 has been used with other anti-retroviral drugs in HIV-1-infected patients [22] and has given much hope to these patients. NK cell activity, which is depressed by HIV-1 infection, can be restored by the incubation of PBMC with IL-2 [23]. Ginseng polysaccharides stimulate Type 1 cytokines such as IL-2 and IFN-γ in a dose-dependent manner [24]. In regard to the dramatic decrease in sCD8 in patients treated with KRG, the effect of the long-term therapy with KRG, singly or in combination, might be associated with potentiation of CTL function and with the induction of IL-2, which is suppressed in HIV-1-infected patients.

In consideration of the role of KRG as an immunomodulator rather than as a direct antiviral agent, we could strongly recommend KRG-intake from as early stage as possible to get good prognosis over a long term. Early use is recommended because destruction of anatomical structures, such as germinal center and follicular dendritic cells in lymph nodes, which are essential for effective HIV antigen presentation, is less the earlier therapy is begun. Currently, a long-term study of KRG in HIV-1-infected patients is in progress. Although the number of cases in the present study is not sufficient to support a definite conclusion, our data strongly sug-

gest that the combination of antiretroviral drugs with KRG could be a new therapeutic modality to treat HIV-1-infected patients.

Acknowledgements

This work was supported by the grant (2000-062) from Asan Institute for Life Sciences and Korean red ginseng was supported by Korea Ginseng via Korean Society of Ginseng (2000). The authors thank Professor Chun Sik Park, University of Ulsan College of Medicine for the critical reading of this manuscript.

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