DRUG MONITORING AND VIRAL RESPONSE TO LOPINAVIR/RITONAVIR OR SAQUINAVIR/RITONAVIR CONTAINING REGIMENS IN INDIVIDUALS INFECTED WITH THE HUMAN IMMUNODEFICIENCY VIRUS TYPE1

B. FALKENSAMMER, W. FASSER[1], K. SCHERER[1], A. ZEMANN[1], W. PARSON[2], H. ULMER[3], M.P. DIERICH and H. STOIBER

Department of Hygiene, Microbiology and Social Medicine and Ludwig-Bolzmann-Institute for AIDS research, Innsbruck Medical University;
[1]Institute for Analytical Chemistry and Radiochemistry, Innsbruck Medical University;
[2]Department of Legal Medicine, Innsbruck Medical University;
[3]Institute for Biostatistics, Innsbruck Medical University, Austria

Received April 5, 2004 - Accepted September 29, 2004

The aim of this study was to correlate results of therapeutic drug monitoring, genotypic resistance and viral response to lopinavir/ritonavir (LPV/r) or saquinavir/ritonavir (SQV/r) containing antiretroviral regimens. The retrospective short-term study included 20 patients with LPV/r and 20 patients with SQV/r containing highly active antiretroviral therapy (HAART). At baseline 7 LPV/r patients and 10 SQV/r patients had CD4$^+$T cell counts above 410 cells/μl. After 6 months CD4$^+$T cells had doubled in 5 LPV/r and 2 SQV/r patients. In LPV/r patients the mean serum concentration of lopinavir (LPV) was 2.6 ppm and 67% of all LPV/r samples had 50 or fewer viral copies/ml. In SQV/r patients the mean serum concentration of saquinavir (SQV) was 2.1 ppm. 79% of all SQV/r samples had 50 or fewer viruses/ml. Pharmacoenhanced regimens efficiently suppress human immunodeficiency virus type 1 (HIV-1) and the risk of developing resistance mutations is therefore reduced. The implementation of drug monitoring is an additional tool to determine optimal treatment conditions.

Protease Inhibitors (PIs) have significantly changed antiretroviral therapy. With their introduction impressive long term effects regarding viral suppression have been achieved. Treatment guidelines recommend the use of PIs for initial therapies in HIV-1 infected individuals (1).

The HIV protease targets amino acid sequences in the gag and gag-pol polyproteins, which need to be cleaved before nascent viral particles can mature (2). All PIs are metabolised by cytochrome p450 (CYP 450) isoenzyme 3A4 present in the liver and small intestine. CYP 450 is responsible for numerous interactions between PIs and other drugs. Serum concentrations are decreased by coadministration of CYP3A4 inducers or increased by CYP3A4 inhibitors (3). Certain combinations of PIs can exploit these pharmacokinetic interactions in order to improve the plasma pharmacokinetic profile of the boosted drug (4). Ritonavir (RTV) at low doses is used as a booster agent (pharmacoenhancer) when co-administered with another PI, for example with SQV or LPV.

Pharmacoenhanced treatment regimens have several advantages: higher drug exposures provide increased antiretroviral effects, the genetic barrier can be raised (4), and an increased concentration in the central nervous system as well as other body compartments can be achieved. Furthermore, these PI combination therapies reduce the pill burden. Additionally they widen the range of "salvage" therapy settings.

Key words: HIV-1, therapeutic drug monitoring, pharmacoenhanced antiretroviral regimens, genotypic resistance, virologic response

Mailing address: Dr. Barbara Falkensammer
Department of Hygiene,
Microbiology and Social Medicine
Fritz-Pregl-Str. 3 - 6010 Innsbruck, Austria
Phone: +43 512 507 3438 - Fax: +43 512 507 2870
Barbara.Falkensammer@uibk.ac.at

0394-6320 (2005)

Therapeutic drug monitoring (TDM) is a helpful tool to avoid supratherapeutic antiretroviral serum levels, which might cause side effects (5-7). There are significant interindividual differences concerning pharmacokinetics, such as intestinal activity, inflammation, liver and renal function, body mass indices or sex.

In the case of drug levels being too low, escape mutants are likely to occur. The major amino-acid mutations associated with clinical resistance have been mapped to the protease and reverse transcriptase enzymes (8). In general, initial single amino acid mutations yield only a slight chance, by less than a factor of 5, of reduced drug sensitivity (9-10). However, secondary mutations that may accumulate in viral subsets of HIV-infected individuals may cause high-level drug resistance. In addition, cross resistance mutations among PIs frequently occur in these patients (11-12). Having acquired resistance to PIs, viral load levels and CD4+T cell counts may return to pretreatment values.

Pharmacological studies are required in order to evaluate several factors influencing virologic responses to LPV/r- and SQV/r-containing antiretroviral regimens. The aim of this study was to evaluate the importance of serum drug levels in response to combination therapies containing LPV/r or SQV/r regimens.

MATERIALS AND METHODS

Patients

A non-randomised, single-centre, retrospective, short-term study was performed. Forty patients, older than 18 years, at different clinical stages of HIV-1 infection were included. Their viral load levels were below 6.1 $\log_{10}$ copies/ml. Exclusion criteria were active cytomegalovirus, toxoplasmosis or tuberculosis infection and pregnancies

All 40 patients were treated with 2 nucleoside reverse transcriptase inhibitors (NRTIs). Half of the patients were additionally administered LPV and RTV (400mg/100mg) twice a day, while the second half was treated with SQV and RTV (1000mg/100mg) twice a day. Determination of PI serum levels and virologic response parameters were performed regularly every 3 months – at baseline and then at least twice more. In order to measure drug levels, blood samples were taken from the patients two hours after drug administration (C_{max} [ppm]). Baseline data, including age, sex, history of antiretroviral treatment and progression to acquired immunodeficiency syndrome (AIDS) according to CDC (Centre of Disease Control) guidelines were determined. Four patients were randomly chosen from each group and their serum level determined prior (C_{min} [ppm]) and two hours post (C_{max} [ppm]) drug administration. The local ethic commission at the University Innsbuck approved the study.

Virologic and immunologic parameters

HIV-1-RNA quantification was performed using the Cobas Amplicor HIV-1 Monitor Test (Roche Molecular Systems, Brachburg, NJ, USA) according to the manufacturer's instructions. Lymphocyte subsets were determined as described by Zangerle et al. (13), who defined the lowest non-pathologic limit with 410 CD4+T cells/µl. Urinary neopterin was determined as previously described (14) and 161 µmol/mol was recommended as the highest normal limit.

Genotypic mutation analysis

In order to detect mutations viral protease genotypes were performed using primer [5´-CTC AGG TCA CTC TTT GGC AAC-3´] and primer [5´-GTA TGG TAA ATG CAG TAT ACT TCC-3´] as described previously (15-16).

Sequencing reactions were performed using the Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA) (17). DNA was loaded on a CE 310 Genetic Analyser (PE Biosystems, Foster city, CA) and electrophoresis was conducted according to the manufacturer's recommendations. Analysis was performed using ABD Prism Sequencing Analysis Software, Version 3.0, and Sequence Navigator Software, Version 1.0.1 (Perkin Elmer, Norwalk, CT).

The PI resistance-associated-mutations complied by Shafer (8) were identified manually comparing nucleotide viral sequence derived from these patients with the wild-type-strain HIV_{NL4-3} (accession number: M 19921). Genotypic results were confirmed by another independent laboratory, where viral isolation, amplification and sequencing procedures were separately performed.

Serum level measurements

Prior to high performance liquid chromatography (HPLC) analysis, serum samples were filtered to remove debris and preconcentrated by solid-phase extraction

(SPE) with C2 cartridges (Varian, Harbor Cityt, CA, USA). HPLC analyses were performed as published previously (18) using a Zorbax SB-C18 protected by a guard column (Zorbax SB-C18 guard carts, Agilent Technologies, Vienna, Austria). Absorbance detection of SQV and RTV was performed at 210 nm, detection of LPV was carried out at 239 nm.

Statistical analysis

Correlations of viral load level, CD4+T cell counts and neopterin levels of the two different therapeutic groups were calculated with either Chi-Quadrat (Pearson) or with the Fishers exact test. P values were calculated and a value <0.05 was considered statistically significant.

RESULTS

In this study 40 HIV-positive individuals were investigated. The majority of these patients were sexually infected (Table I). Treatment histories prior to initiation of pharmacoenhanced regimens were comparable in both therapy groups (Table I). Due to virologic failure or side effects their treatment regimens were switched to either LPV/r or SQV/r combined with two NRTIs.

Patients in the LPV/r group had baseline mean RNA levels of 2.4 $\log_{10}$ copies/ml (Fig. 1a) and in the SQV/r group of 2.3 $\log_{10}$ copies/ml (Fig. 1b). After 3 months of treatment with these two different pharmaco-enhanced regimens LPV/r and SQV/r patients exhibited mean viral load levels of 2.1 $\log_{10}$ copies/ml and 1.9 $\log_{10}$ copies/ml, respectively. After six months the mean viral load levels were 1.9 $\log_{10}$ copies/ml and 1.7 $\log_{10}$ copies/ml for the LPV/r and SQV/r group patients, respectively. It has to be emphasized, that data points representing viral loads below 1.69 $\log_{10}$ copies/ml (detection limit of 50 copies/ml) are drawn at an arbitrary level of 1 $\log_{10}$ copies/ml in the graphs. However, the calculated geometric mean values were obtained by setting these values to 1.69 $\log_{10}$ copies/ml to avoid a false positive trend.

At the time of inclusion, 7 LPV/r-patients and 10 SQV/r-patients (Tab. II) had CD4+T cell levels of more than 410 cells/µl (range, LPV/r: 4-589 CD4+T cells/µl; SQV/r: 72-1500 CD4+T cells/µl).

Table I. *Baseline characteristics of the 20 patients of the LPV/r combination therapy and the 20 patients of the SQV/r combination therapy.*

		LPV/r	SQV/r
Age (years) [mean (range)]		38.5 (25-52)	40.4 (28-59)
Female sex (%)	5	0	25
HIV transmission rate (%)			
• haemophile		5	0
• homosexual contact		30	35
• heterosexual contact		30	30
• injecting drug use		35	35
Duration of prior antiretroviral therapy (months) [mean (range)]			
• PIs		23.2 (0-53)	19.2 (0-52)
• NRTIs		38.6 (0-110)	40.1 (2-86)
• NNRTIs		1.3 (0-8)	2.7 (0-17)
Stage of disease - AIDS (%)	8	5	65

PIs, protease inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors.

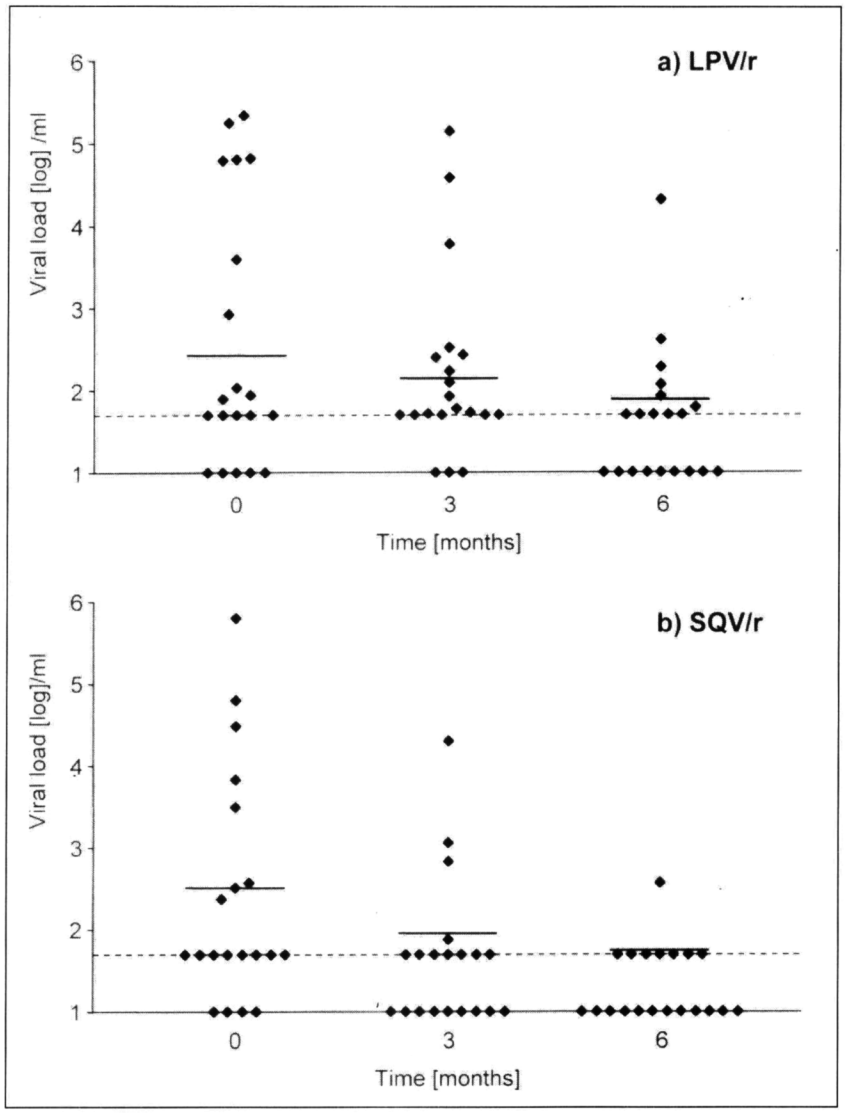

Fig. 1. *Development of viral load levels [log]/ml in the LPV/r and in the SQV/r group.*
Vertical bars present viral load levels. In the LPV/r and in the SQV/r group mean viral load levels were decreasing from 2.4 log and 2.3 log at the beginning, to 2.1 log and 1.9 log after 3 months of pharmacoenhanced therapy and to 1.9 log and 1.7 log after 6 months.

After 6 months of pharmacoenhanced treatment regimens 9 patients in the LPV/r group and 14 patients in the SQV/r group displayed CD4+T cell levels above 410 cells/µl.

As indicated in Table III, 5 LPV/r and 2 SQV/r patients had doubled their CD4+T cell counts within this time period. Viral load reductions of more than 2 $\log_{10}$ copies/ml were observed in 3 patients in the LPV/r group and 5 patients in the SQV/r group. Neopterin levels developed very similarly in both groups – at baseline one patient in each group (Tab. II) had less than 161 µmol/mol (range, LPV/r: 113-956 µmol/mol; SQV/r: 125-717 µmol/mol). Having received pharmacoenhanced antiretroviral therapy for 6 months, 5 patients in the LPV/r and 6 patients in the SQV/r group had neopterin levels below 161 µmol/mol. Reductions of urine neopterin levels of more than 200 µmol/mol (Table III) were observed in 4 LPV/r patients and 3 SQV/r patients during the course of treatment.

Genotypic mutations: it was possible to perform genotypic resistance analysis in 4 LPV/r-patients with 10 serum samples taken at different time points. In one patient a mutation was identified at position 33. The nonpolymorphic mutation L33F can enhance resistance to compounds such as indinavir (IDV), amprenavir (APV) and LPV (8). In some patients mutation M46L and I47V could be detected as well. They may be associated with reduced drug susceptibility towards IDV, RTV, APV and LPV, when other additional mutations are present. There was no patient in this group

Table II. *CD4+T cells and Neopterin levels.*

	$CD4^+$T cells: > 410/µl (n=20)			Neopterin: < 161 µmol/mol (n=20)		
	LPV/r	SQV/r	p-values	LPV/r	SQV/r	p-values
Baseline	7	10	0.5	1	1	1
3 months	10	14	0.3	3	4	1
6 months	9	14	0.2	5	6	1

A higher but not statistically significant number of patients with SQV/r containing antiretroviral treatment had CD4+T cells counts above 410/µl compared to patients with LPV/r containing regiments. Considering neopterin levels no differences between the two study groups could be determined.

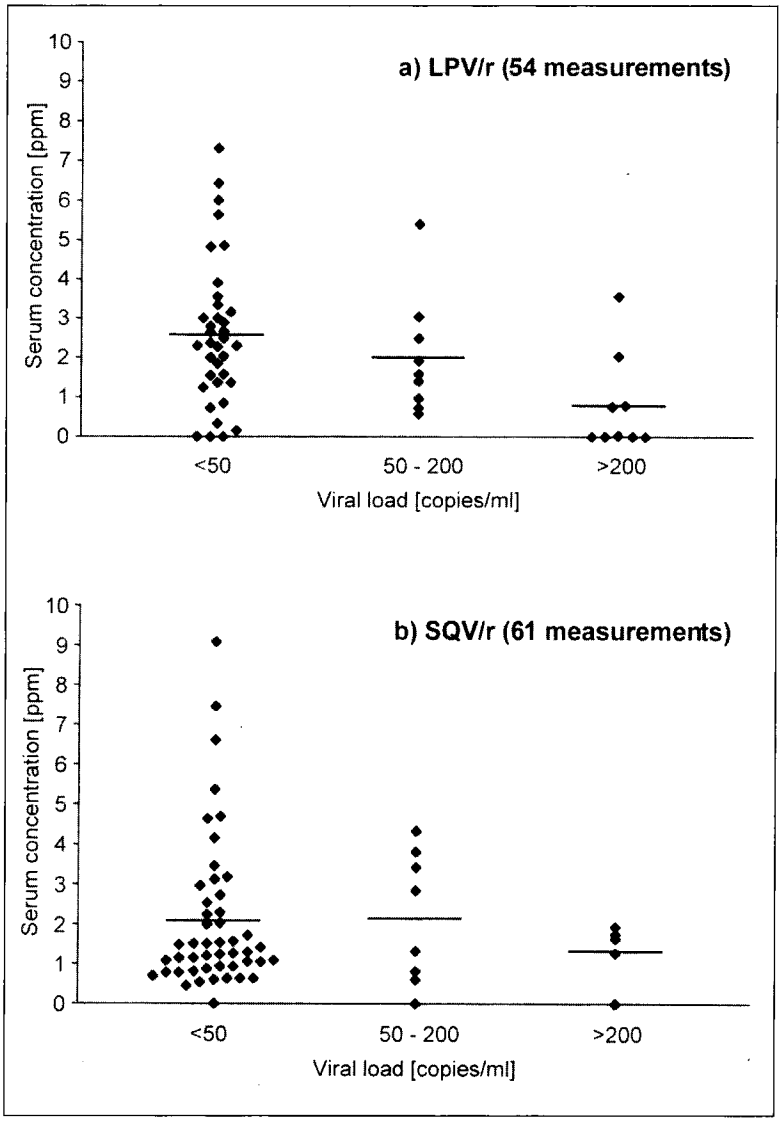

Fig. 2. *Correlation of serum level measurements [ppm] with viral load levels [copies/ml].*
Vertical bars present mean serum concentrations at all points of time with the exception of baseline parameters. The majority of patients had no detectable viral load levels with 2.6 ppm in the LPV/r group and with 2.1 ppm in the SQV/r group. Mean drug concentrations were measured in the LPV/r patients with 2.0 ppm and in SQV/r patients with 2.2 when viral load levels were between 50 and 200 copies/ml. Few patients had more than 200 viral copies/ml. In this case, mean LPV drug concentrations were 0.8 ppm and mean SQV drug concentrations were 1.3 ppm.

Table III. *Summary of developments of CD4⁺T cell counts, viral load levels and neopterin levels within the study period.*

	LPV/r	SQVr
$CD4^+$T cell increase: <50%	12	12
$CD4^+$T cell increase: 50-100%	3	6
$CD4^+$T cell increase: >100%	5	2
Viral load reduction: <1 log_{10} copies/ml	11	9
Viral load reduction: 1-2 log_{10} copies/ml	6	6
Viral load reduction: >2 log_{10} copies/ml	3	5
Neopterin reduction: <100 µmol/mol	10	10
Neopterin reduction: 100-200 µmol/mol	6	7
Neopterin reduction: >200 µmol/mol	4	3

$CD4^+$T cell increased more than 100% in 5 patients of the LPV/r compared to 2 patients in the SQV/r group. Viral load reductions of more than 2 log_{10} copies/ml were observed in 3 LPV/r patients and in 5 SQV/r patients. 4 LPV/r and 3 SQV/r patients had significant neopterin reductions of more than 200 µmol/mol during the observation period.

with more than 2 changes in the PI genome which were related to decreased protease sensitivity.

Due to low viral load levels in the SQV/r group, genotypic resistance analysis could be performed in only 3 patients with 6 serum samples. Aminoacid changes were detected in positions 33 and 46. Surprisingly, there was no resistance mutation directly associated with SQV. For genotypic resistance analysis lowest viral amplification limit was 2.6 log_{10} RNA copies/ml.

Therapeutic drug monitoring: in all 40 patients therapeutic drug monitoring was performed by HPLC. Serum samples were taken from patients at baseline, after 3, 6, and in several cases also 9 and 12 months later. LPV and SQV levels were determined and their concentrations [ppm] were correlated with viral load levels. In the LPV/r group the mean serum concentration of LPV was 2.6 ppm and 67% of all LPV/r samples had 50 or fewer viral copies/ml (Fig. 2a). In the SQV/r group the mean serum concentration of SQV was 2.09 ppm and 79% of all SQV/r samples also had 50 or fewer copies/ml (Fig. 2b). Complete suppressive viral response had not been achieved when the mean serum concentration of LPV was 2.0 ppm in the LPV/r group as viral load levels

were between 50 and 200 copies. When viral load levels were higher than 200 copies/ml (9 measurements), median lopinavir concentrations were 0.8 ppm.

Low dose RTV was measured in 65% of all LPV serum samples with a mean concentration of 0.3 ppm whereas it was detectable in 90% of all SQV serum samples with a mean concentration of 0.8 ppm.

Additionally, four patients were randomly chosen from each group and their serum level determined prior and two hours post drug administration. These results were correlated with current viral load levels (Table IV). In one patient of each group drug serum levels increased less than 0.5 ppm within two hours. Nevertheless, none of these 8 patients had more than 200 viral copies/ml.

Patients with high detectable serum levels suffered adverse effects such as tiredness (7 LPV/r patients, 6 SQV/r patients; data not shown), diarrhoea (6 LPV/r patients, 0 SQV/r patients) and nausea (3 LPV/r patients, 1 SQV/r patient). It is to note that the patient who had highest lopinavir levels (7.3 ppm) suffered particularly from these effects. Statistical analyses showed that diarrhoea occurred significantly more often after treatment with lopinavir than with saquinavir ($p=0.02$, Fishers exact test, data not shown). However, taking into consideration other side effects, resistance developments, virologic and immunologic parameters both lopinavir and saquinavir in combination with ritonavir containing antiretroviral regimens achieved similar results.

DISCUSSION

In patients treated with either LPV or SQV containing antiretroviral regimens, drug serum levels, genotypic resistance mutations, virologic and immunologic parameters were determined.

Table IV. *Serum levels of LPV and SQV were determined prior (C_{min} [ppm]) and 2 hours post (C_{max} [ppm]) drug administration. In one patient in each group serum levels rose less than 0.5 ppm.*

Patients	C_{min} [ppm]	C_{max} [ppm]	DC	Viral Load [log]
LPV/r-01	2.3	2.8	0.5	0.6
LPV/r-05	n.d.*	1.3	1.3	1.3
LPV/r-06	0.6	1.3	0.7	1.6
LPV/r-20	1.7	3.2	1.5	1.5
SQV/r-03	0.5	0.6	0.1	2.0
SQV/r-05	1.6	2.7	1.1	1.6
SQV/r-09	0.3	0.8	0.5	n.d.*
SQV/r-11	1.0	7.3	6.3	1.6

**n.d. (not detected)*

LPV levels increased in the patients between 0.5 and 1.5 ppm, the SQV drug concentrations varied between 0.1 and 6.3 ppm. The levels of the antiretrovirals were determined two hours after administration by HPLC as described in the Materials and Methods section.

The duration of exposure to different classes of antiretrovirals was comparable between LPV/r and SQV/r patients (Table I). There was a slight, although not significant difference between the two study groups.

The virologic response parameters obtained in this study indicated that there are very few differences when comparing treatment success. After 6 months of pharmacoenhanced antiretroviral regimens 9 patients in the LPV/r group and 14 patients in the SQV/r group had CD4+T cell levels of more than 410 cells/µl (Table II). During these 6 months the CD4+T cell level doubled in 5 LPV/r and 2 SQV/r patients (Table III). Both LPV/r and SQV/r containing antiretroviral regimens seemed to be very efficient. Due to pharmacoenhanced antiretroviral therapies mean viral load levels in both study groups had decreased by more than 0.5 $\log_{10}$ copies/ml (Fig. 1) within 6 months. A reduction of viral load levels of more than 2 $\log_{10}$ copies/ml could be found in 3 LPV/r and 5 SQV/r patients (Tab. III) within this time period.

As indicated in Fig. 2a, 67% of all viral load measurements in the LPV/r group were below 50 copies/ml and 79% were under the limit of detection in the SQV/r group (Fig. 2b). Furthermore patients with undetectable viral load levels had mean drug concentrations of 2.6 ppm for lopinavir and 2.1 ppm for saquinavir in pharmacoenhanced treatment regimens.

Some patients in the LPV/r group had very low or even no detectable drug serum levels. 5 out of 9 LPV/r patients had viral load levels higher than 200 copies/ml and undetectable serum levels (Fig. 2a). Thus in patients who received LPV/r, drug levels seem to correlate with viral load. In the SQV/r group (Fig. 2b) patients with more than 200 viral copies/ml had mean SQV levels of 1.3 ppm indicating that despite detectable amounts of SQV in the serum viral replication was not completely suppressed. Since no SQV mutations were detectable in the protease genome of patients´ isolates, other reasons such as mutations in the reverse transcriptase genome may be responsible for this incomplete suppression.

Serum levels determined in this study were in accordance with HPLC data published by Langmann et al. (19) and Boffito et al. (20). In their study responders had 5.9 ± 1.8 µg/ml of detectable lopinavir. When analysing results of serum level concentrations prior (C_{min} [ppm]) and two hours post drug administration (C_{max} [ppm]) (Tab. IV), in one patient of each group minor rising drug levels were detected (<0.5 ppm). Surprisingly, these two patients had fewer than 200 viral copies/ml. The reasons for this phenomenon are unknown and may be due to delayed resorption of the compound.

TDM provides valuable information to clinicians as there are a number of factors that influence a successful therapy. Serum levels may be affected by high interpatient variabilities which are due to differences in resorption, metabolism and elimination of the antiretrovirals. Additional important factors are food intake, diarrhoea and renal as well as liver activity, which may be decreased by coinfections such as hepatitis C, liver dysfunctions or cirrhosis (21). Furthermore, sex, body mass indices, and drug-drug interactions may also influence drug serum levels (22). Nowadays pharmacoenhanced treatment regimens take advantage of pharmacokinetic boostering effects by inhibiting CYP 450. Enhanced drug levels may cause unnecessary side effects. Nevertheless, suboptimal drug levels cause reduced antiretroviral efficacy and may promote the development of resistance mutations.

Genotypic resistance analyses in this study showed, that in patients with viral load levels higher than 2.6 $\log_{10}$ copies/ml only 3 patients had acquired mutations in position 46 or 47. Amplifying low viral load levels (2.6 $\log_{10}$ copies/ml), the risk of multiplying non representative quasispecies is increased. However, in 16 serum samples the only mutations directly associated with PI resistance were detectable in positions 46 and 47.

The 46 or 47 mutation alone does not induce resistance towards LPV, however, in combination with mutations in positions 50, 82, 84, and 90 LPV will have reduced suppressive effects (8, 23). Surprisingly, in the SQV/r study group no SQV mutation was detectable at all. These genotypic results may indicate that RTV-boostered regimens raise the genetic barrier (4) and reduce the possibility of mutation developments. Some RTV mutations [46, 47, 32] could be detected in both study groups. As RTV is the booster substance in these combination regimens rather than the suppressive drug, these mutations seem to have no negative effect. Problems may occur in cases where RTV is

subsequently applied as the suppressive substance.

Due to different mutation patterns of LPV and SQV, each of these PIs may be an alternative drug, for substituting each other.

In order to follow clinical progression parameters, viral load levels, $CD4^+T$ cell counts, and neopterin levels were determined at baseline and then every 3 months. Human monocytes/ macrophages appear to constitute the most relevant source of neopterin (24) when activated with interferon-γ (INF-γ). Therefore the production of neopterin closely correlates with INF-γ concentrations (25) and the activation of cell mediated immunity. Thus neopterin provides useful diagnostic and prognostic information as well as insight into disease progression. Although neopterin levels correlate with HIV infection (26-27), this soluble marker is not HIV specific and is also increased during other viral infections or infections with intracellular bacteria such as mycobacteria tuberculosis.

There were no significant differences between neopterin level developments of patients in the LPV/r and in the SQV/r group (p=1, Fishers exact test). In both groups neopterin levels decrease indicating that both regimens are successful. A reduction of neopterin levels of more than 200 mg/dl was observed for 4 patients in the LPV/r group and 3 patients in the SQV/r 3 during the study (Table III).

The use of RTV as a boostering agent is increasingly used in clinical practice nowadays as this approach has several advantages. Interpatient variabilities can be diminished (4), twice- or possibly once-daily applications are enabled and these treatment regimens are not necessarily food dependent. In addition, pill burden can be reduced and, as a consequence, the costs are lowered as well.

Pharmacoenhanced regimens represent a good strategy in active antiretroviral treatment, which appears to be very efficient. Further investigations will be required in order to determine serum doses to prevent on the one hand mutation developments of viral particles and on the other hand unnecessary side effects.

As the present study is limited in scale a more detailed evaluation will be necessary in order to prove assumptions concerning therapeutic drug monitoring. However, the data obtained from the investigated patients give good indications of correlations of drug serum levels, viral progression parameters and genotypic resistance.

ACKNOWLEDGEMENT

The authors wish to thank Prof. R. Zangerle and Dr. M. Sacletti for their clinical advice and the HIV cohort in Innsbruck for providing serum samples. This study was further supported by ABBOTT, the OENB (P 8504), the L. Bolzmann Society for AIDS Research and the Federal State of Tyrol. Saquinavir was kindly provided by Roche (Basel, Switzerland).

REFERENCES

1. **Carpenter C., M. Fischl, S. Hammer, M. Hirsch, D. Jacobsen, D. Katzenstein, J. Montaner, D. Richman, M. Saag, R. Schooley, M. Thompson, S. Vella, P. Yeni and P. Volberding**. 1997. Antiretroviral therapy for HIV infection in 1997: updated recommendations of the International AIDS Society – US Panel. *JAMA 277:1962.*
2. **Kramer R., M. Schaber, A. Skalka, K. Ganguly, F. Wong-Staal and E. Reddy**. 1986. HTLV-III gag protein is processed in yeast cells by the virus pol-protease. *Science 231:580.*
3. **Leibenguth P., C. Le Guellec, J. Besnier, F. Bastides, M. Mace, M. Gaudet, E. Autret-Leca and G. Paintaud**. 2001. Therapeutic drug monitoring of HIV protease inhibitors using high-performance liquid chromatography with ultraviolet or photodiode array detection. *Ther. Drug Monit. 23:679.*
4. **Moyle G. and D. Back.** 2001. Principles and practice of HIV-protease inhibitor pharmacoenhancement. *HIV Med. 2:105.*
5. **Langmann P., M. Zilly, B. Weissbrich, S. Desch, T. Vath and H. Klinker**. 2002. Therapeutic drug monitoring of indinavir in HIV-infected patients undergoing HAART. *Infection 30:13.*
6. **Moyer T., Z. Temesgen, R. Enger, L. Estes, J. Charlson, L. Oliver and A. Wright**. 1999. Drug monitoring of antiretroviral therapy for HIV-1 infection: method validation and results of a pilot study. *Clin. Chem. 45:1465.*
7. **Back D., S. Khoo, S. Gibbons, M. Barry, and C. Merry**. 2000. Therapeutic drug monitoring of antiretrovirals in human immunodeficiency virus infection. *Ther. Drug Monit. 22:122.*
8. **Rhee S., M. Gonzales, R. Kantor, B. Betts, J. Ravela**

and R. Shafer. 2003. Human immunodeficiency virus reverse transcriptase and protease sequence database. *Nucleic Acids Res. 31:298.*

9. **el-Farrash M., M. Kuroda, T. Kitazaki, T. Masuda, K. Kato and M. Hatanaka**. 1994. Generation and characterization of a human immunodeficiency virus type 1 (HIV-1) mutant resistant to an HIV-1 protease inhibitor. *J. Virol. 68:233.*
10. **Otto M., S. Garber, D. Winslow, C. Reid, P. Aldrich and P. Jadhav.** 1993. In vitro isolation and identification of human immunodeficiency virus (HIV) variants with reduced sensitivity to C-2 symmetrical inhibitors of HIV type 1 protease. *Proc. Natl. Acad. Sci. USA 90:7543.*
11. **Condra J., W. Schleif, O. Blahy, L. Gabryelski, D. Graham, J. Quintero, A. Rhodes, H. Robbins, E. Roth and M. Shivaprakash.** 1995. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. *Nature 374:569.*
12. **Molla A., M. Korneyeva, Q. Gao, S. Vasavanonda, P. Schipper, H. Mo, M. Markowitz, T. Chernyavskiy, P. Niu, N. Lyons, A. Hsu, G. Granneman, D. Ho, C. Boucher, J. Leonard, D. Norbeck and D. Kempf.** 1996. Ordered accumulation of mutations in HIV protease confers resistance to ritonavir. *Nat. Med. 2:760.*
13. **Zangerle R., S. Steinhuber, M. Sarcletti, M.P. Dierich, H. Wachter, D. Fuchs and J. Most**. 1998. Serum HIV-1 RNA Levels Compared to Soluble Markers of Immune Activation to Predict Disease Progression in HIV-1 Infected Individuals. *Int. Arch. Allergy Immunol. 116:228.*
14. **Wachter H., D. Fuchs, A. Hausen, G. Reibnegger and E. Werner**. 1989. Neopterin as marker for activation of cellular immunity: immunologic basis and clinical application. *Adv. Clin. Chem. 27:81.*
15. **Falkensammer B., R. El Attal, B. Mullauer, M. Sarcletti, W. Parson, M.P. Dierich and H. Stoiber.** 2002. Loss of zidovudine related mutations in the reverse transcriptase gene of HIV after switching therapy. *Eur. J. Med. Res. 7:379.*
16. **Walter H., B. Schmidt, K. Korn, A. Vandamme, T. Harrer and K. Uberla**. 1999. Rapid, phenotypic HIV-1 drug sensitivity assay for protease and reverse transcriptase inhibitors. *J. Clin. Virol. 13:71.*
17. **Parson W., T. Parsons, R. Scheithauer and M. Holland.** 1998. Population data for 101 Austrian Caucasian mitochondrial DNA d-loop sequences: Application of mtDNA sequence analysis to a legal case. *Int. J. Legal Med. 111:124.*
18. **van Heeswijk R., R. Hoetelmans, R. Harms, P. Meenhorst, J. Mulder, J. Lange and J. Beijnen.** 1998. Simultanous quantitative determination of the HIV protease inhibitors amprenavir, indinavir, nelfinavir, ritonavir and saquinavir in human plasma by ion-pair high-performance liquid chromatography with ultraviolet detection. *J. Chromatogr. B. Biomed. Sci. Appl. 719:159.*
19. **Langmann P., M. Zilly, B. Weissbrich, C. Schlor, T. Vath, E. Richter and H. Klinker**. 2000. Therapeutic drug monitoring of saquinavir in patients during protease inhibitor therapy with saquinavir alone or in combination with ritonavir or nelfinavir. *Eur. J. Med. Res. 5:59.*
20. **Boffito M., I. Arnaudo, R. Raiteri, S. Bonora, A. Sinicco, A. Di Garbo, H. Reynolds, P. Hoggard, D. Back and G. Di Perri.** 2002. Clinical use of lopinavir/ritonavir in a salvage therapy setting: pharmacokinetics and pharmacodynamics. *AIDS 16:2081.*
21. **Back D., G. Gatti, C. Fletcher, R. Garaffo, R. Haubrich, R. Hoetelmans, M. Kurowski, A. Luber, C. Merry and C. Perno**. 2002. Therapeutic drug monitoring in HIV infection: current status and future directions. *AIDS 16(S1):5.*
22. **Dasgupta A. and P. Okhuysen**. 2001. Pharmacokinetic and other drug interactions in patients with *AIDS. Ther. Drug Monit. 23:591.*
23. **Carrillo A., K. Stewart, H. Sham, D. Norbeck, W. Kohlbrenner, J. Leonard, D. Kempf and A. Molla**. 1998 In vitro selection and characterisation of human immunodeficiency virus type 1 variants with increased resistance to ABT-378, a novel protease inhibitor. *J. Virol. 72:7532.*
24. **Baier-Bitterlich G., H. Wachter and D. Fuchs**. 1996. Role of neopterin and 7,8-dihydroneopterin in human immunodeficiency virus infection: marker for disease progression and pathogenic link. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 13:184.*
25. **Fuchs D., M. Malkovsky, G. Reibnegger, E. Werner, G. Forni and H. Wachter**. 1989. Endgenous release of interferon-gamma and diminished response of peripheral blood mononuclear cells to antigenic stimulation. *Immunol. Lett. 23:103.*
26. **Amirayan-Chevillard N., H. Tissot-Dupont, Y. Obadia, H. Gallais, J. Mege and C. Capo.** 2000. Highly active antiretroviral therapy (HAART) and circulating markers of immune activation : specific effect of HAART on neopterin. *Clin. Diagn. Lab. Immunol. 7:832.*
27. **N. Gianotti and A. Lazzarin.** 2003. Managing failure to antiretroviral drugs in HIV-1-infected patients. *Int. J. Immunopathol. Pharmacol. 16:9.*