

Natural Polymorphism of NS3 Protease Domain Strains HCV-1 in HCV and HIV-HCV Coinfected Patients: Virological and Clinical Implication for Drug Resistant Viruses

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Introduction

HCV NS3 protease is a chymotrypsin-like serine-protease responsible for cleavage of the non-structural proteins of HCV and plays a pivotal role in viral life cycle. Selection of drug-resistant mutants was evidenced by in vitro and clinical studies with HCV NS3-4A protease inhibitors (PIs). It appeared that mutations V36A/M, T54A, I71T, T72I P88L, R155Q A156T, and D168V were selected in each studies conferring resistance level to each PIs.

Aim

The aim of this study was first to describe the natural polymorphism of NS3 sequence in different HCV-1 strains, and second to compare the diversity of the protease in HCV monoinfected patients and in HIV/HCV coinfecting patients receiving an HIV protease inhibitor.

Patients & Methods

24 mono-infected genotype1 patients (11 Genotype 1A and 13 Genotype 1B) and 24 HCV-HIV coinfecting patients receiving anti-HIV PI therapy, were selected in this study. NS3 protease domain was amplified by RT-PCR. PCR products were purified and directly sequenced (54-197). Multiple alignments of nucleotides and deduced amino acid sequences were inferred by Clustal_X version 1.64b. Fisher's exact test was used to compare proportions of mutation at position 36, 54, 71, 72, 88, 155, 156 and 168. Wilcoxon rank-sum test was used to estimate clinical and virological differences between HCV monoinfected patients and HIV-HCV co-infected patients.

Figure 1: HCV Genotype 1A and 1B Amplicons

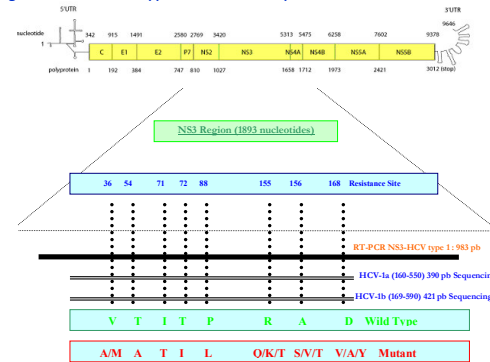


Figure 2: NS3 Protease Sequence

APFTAYSQQT	RQLGCLITS	LTGRDKNQVDG	EVQVLSATQ	SPLATCVNSV	CWTVYH*GAG
	71-72	88			
SKTLAKGKP	ETQMYINVDQ	D*LVGWPAFPG	ARSMTPCTGG	SSDLVLYTRH	ADVVPVRRRG
			155-156	168	
DSRGELLSPR	PISYLKGS*YG	GPLLCPGSHVV	VGIFRAVCT	RGVAKAVDFV	PVESMELTWR

*Catalytic Triad

Table: Resistance Mutations Associated with Viral Resistance Class: In Vitro and In Vivo Study

	BSLH 2001	BCH-6	BCH-002004	VX-950	ITMH-101
T54A	ND	ND	L ¹	ND	ND
R109K	L ¹	L ¹	L ¹	L ¹	ND
I159V	L ¹	L ¹	L ¹	L ¹	ND
R155Q	L ¹	ND	ND	ND	ND
A156S	L ¹	ND	ND	ND	ND
A158T	ND	ND	ND	ND	ND
A159V	ND	ND	ND	ND	ND
D168A	ND	ND	ND	ND	ND
D168V	ND	ND	ND	ND	ND
V170A	ND	ND	ND	ND	ND

1 Tong, X. et al. Antiviral Research (2006), 70, 28-38.
 2 Yu, M. et al. J. Biol. Chem. (2005), 281, 8205-8215.
 3 Lu, L. et al. A. A. C. (2004), 48, 2259-2266.
 4 Lin, C. et al. J. Biol. Chem. (2004), 279, 17508-17514.
 5 Lin, C. et al. J. Biol. Chem. (2005), 280, 36784-36791 and Mo, H. et al. A. A. C. (2005), 48, 4305-4314.
 6 Sewert, S. et al. DDW Digestive Disease Week, Los Angeles May 21-24 2006.
 N.D.: Not Described, S: susceptible, L: Low level of resistance, M: Medium level of resistance, H: High level of resistance. B-L, B-M-30, H-30.

Results

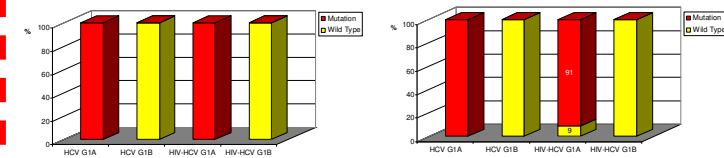
The mutation rates observed in the different positions were not different for HCV infected and HIV-HCV coinfecting patients (respectively 19% and 18%). No difference in amino acid sequences were found between Genotype 1A and Genotype 1B patients.

Mutations were observed only in Genotype 1A patients whatever the type of infection (HCV or HIV-HCV)(cf. Figure 2). Diversity on the protease was more frequently observed in positions 71 and 72, whereas positions 36, 54, 155, 156, and 168 were well conserved whatever the subtype 1 nor the HIV coinfection status.

Figure 3

Mutation in position 71(I → V or L) regarding the type of infection and Genotype (1A or 1B)

Mutation in position 72(T → I or V) regarding the type of infection and Genotype (1A or 1B)



Conclusions

- In this cohort of HCV infected patients and HIV-HCV coinfecting patients, the natural strains of the NS3 protease domain related to resistance to HCV-PI were well conserved among 1B subtype.
- There is a natural polymorphism for 1A subtype at position 71 (I → V or L) and 72 (T → I or V), whatever the status (HCV or HIV-HCV).
- There is no influence of the anti-HIV PI therapy on the mutation rate in the NS3 protease.
- This finding could have implication for monitoring of patients receiving anti-HCV PIs.

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