

Polymorphisms of orosomucoid in HIV-1 infected patients treated by ritonavir boosted atazanavir (Puzzle 2 - ANRS 107 trial)

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Abstract : L-136

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Abstract

Background : Human orosomucoid (ORM) is a major binding protein for protease inhibitors such as atazanavir (ATV). The polymorphism of ORM could be responsible for interindividual variation in the plasma binding of ATV, which might influence the disposition and virological efficacy of ATV. The objective was to describe the ORM polymorphism in HIV-infected patients (pts) and to determine its possible influence on the ATV pharmacokinetics.

Methods : The study was conducted in 52 highly HIV-1 infected pts (50 males, 41 [29-62] year old) included in the Puzzle 2-ANRS 107 trial. They failed previous HAART therapy and were treated by ritonavir boosted ATV, tenofovir and at least 1 nucleoside reverse transcriptase. The phenotyping of ORM variants was determined in sera after desialylation by isoelectric focusing (Phastsystem, Amersham) on polyacrylamide gels following by immunoblotting. Total ORM plasma concentrations were measured by nephelometry (BN Prospec, Dade Behring) at week 6. ATV pharmacokinetic parameters were determined using a population approach (WinNonMix, Pharsight). Relations between ORM phenotypes, total ORM concentrations and ATV pharmacokinetic parameters were tested using a Kruskal-Wallis test.

Results : Median total ORM concentrations were 1.0 g/L (0.6-1.5). 14 pts had elevated ORM concentration with respect to their sex and their age. The frequencies of the three phenotypes observed ORM1*F1-ORM2, ORM1*F1S-ORM2 and ORM1*S-ORM2 were 34.6%, 55.8% and 9.6% respectively. The allele ORM1*F2 was not observed in this population. All pts expressed a monomorphic allele on ORM2 locus. Total plasma concentrations of ORM were not found to be significantly dependent on ORM phenotypes. Median predicted trough ATV concentrations were 468 ng/mL (89-4978) and were not correlated to ORM phenotype. Median ATV volume of distribution was significantly higher ($p=0.008$) in pts presenting the ORM1*S-ORM2 phenotype than the ORM1*F1S-ORM2 or ORM1*F1-ORM2 (87L vs 77L and 80L). In contrast, ORM phenotype did not significantly impact ATV clearance.

Conclusions : Plasma ORM concentrations were elevated in 27% of these highly pretreated HIV-infected pts. Phenotype frequencies were close to those previously published. The different ORM variants affect ATV volume of distribution. This could be the consequence of different capacities for ATV binding which warrants further studies.

Background

Human orosomucoid (ORM) is a major binding protein for protease inhibitors such as atazanavir (ATV). Its expression is controlled by a cluster of three adjacent genes which code for the proteins ORM1 and ORM2. Moreover there is a polymorphism on the ORM1, which could be responsible for interindividual variation in the plasma binding of ATV influencing the disposition and virological efficacy of ATV. The objective was to describe the ORM polymorphism in HIV-infected patients and to determine its influence on ATV pharmacokinetics.

Material and methods

> ANRS 107 - Puzzle-2 design

- 52 highly HIV-1 infected patients (50 males), who failed previous HAART therapy
- treatment : ritonavir boosted atazanavir (100/300 mg once a day), tenofovir and at least 1 nucleoside reverse transcriptase
- blood samples : at week 6 for the biochemical and pharmacological assays
- patients characteristics at baseline : 41 years (29-62), 65 kg (46-104), 5.0 HIV RNA log 10 copies/mL (3.5-6.1), 196 CD4/μL (2-626)

> Determination of orosomucoid concentrations : nephelometry (BN Prospec, Dade Behring)

> Determination of orosomucoid phenotypes

The technique was validated and the conditions were as follow :

- desialylation by neuraminidase type X to remove the sialic acid moiety of orosomucoid
- separation of the variants : isoelectric focusing (Phastsystem, Amersham) on polyacrylamide gels
- transfer of the variants to a nitrocellulose membrane by passive diffusion
- revelation by immunoblotting

The technique was reproducible within one and between different gels.

> Determination of atazanavir concentrations

- by validated LC/MS/MS assays (LOQ 5 ng/mL, between run CV 5.7%) (Bristol-Myers Squibb, Saint Nazaire, France)
- at week 6 : C trough : prior to drug intake (42 patients) and during a dosing interval (10 patients)

> Determination of atazanavir pharmacokinetic parameters

- population approach (WinNonMix, Pharsight) : one compartment model with first order absorption and elimination

> Statistics

Relations between ORM phenotypes and total ORM concentrations or ATV pharmacokinetic parameters were tested using a Kruskal-Wallis test. Relations between total ORM concentrations and ATV pharmacokinetic parameters were tested using a correlation test. Results are presented as median (range).

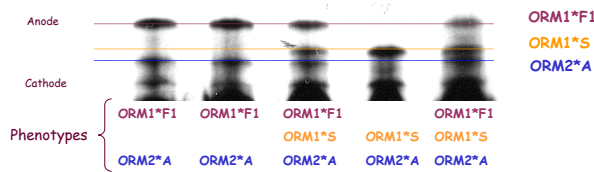
Results

> Total orosomucoid concentrations

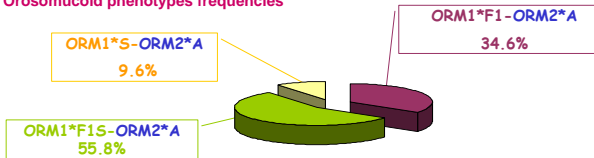
- ORM = 1,0 g/L (0,6-1,5 g/L)
- 14 patients had elevated ORM concentration with respect to their sex and their age.

> Orosomucoid phenotypes identification

- At locus ORM1, three phenotypes controlled by two codominant alleles (ORM1*F1, ORM1*S) were observed including
 - two homozygotes : ORM1*F1 and ORM1*S
 - one heterozygote : ORM1*F1S.
- The allele ORM1*F2 was present in the anodal region of the gel. The allele ORM1*F2 was not observed in this population.
- At locus ORM2, the allele ORM2*A was monomorphic and observed in all sera of patients in the most cathodal region.



> Orosomucoid phenotypes frequencies

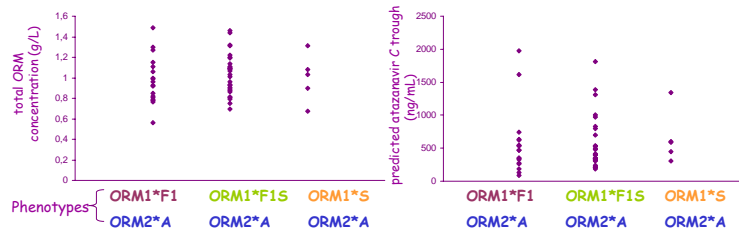


> Atazanavir pharmacokinetics

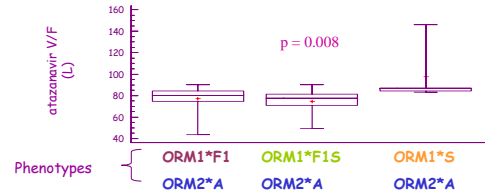
- median trough atazanavir concentration at week 6 : 468 ng/mL (89-4978 ng/mL)
- atazanavir pharmacokinetic parameters (inter-individual variabilities)
 - apparent clearance (Cl/F) : 160 mL/min (34%)
 - apparent volume of distribution (V/F) : 79 L (30%)
 - absorption rate constant (Ka) : 0.74 /h (136%)

> Relations

- total plasma ORM concentrations and trough atazanavir concentrations were independent of ORM phenotypes.



- median ATV volume of distribution was significantly higher ($p=0.008$) in patients presenting the ORM1*S-ORM2 phenotype than the ORM1*F1S-ORM2 or ORM1*F1-ORM2 (87L versus 77L and 80L).



- ATV clearance decreased with increased total ORM concentrations ($p<0.001$). In contrast ATV clearance was independent of ORM phenotype.

Conclusion

Plasma ORM concentrations were elevated in 27% of these highly pretreated HIV-infected patients.

Phenotype frequencies were close to those previously published.

The different ORM variants affect ATV volume of distribution. This could be the consequence of different capacities for ATV binding which warrants further studies. However this is likely without clinical consequences.

Acknowledgements: puzzle 2 study group

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