P476

Antiretrovirals Concentrations in the Olfactory Mucosa of HIV-positive Patients

Andrea Calcagno[1], Amedeo De Nicolò[1], Mattia Trunfio[1], Tiziano Allice[2], Imperiale Daniele[3], Claudia Bartoli[4], Valeria Ghisetti[2], Enrica Amasio[4], Antonio D'Avolio[1], Giovanni Di Perri[1], Stefano Bonora[1].

1 Unit of Infectious Diseases, Amedeo di Savoia hospital, Department of Medical Sciences, University of Torino, Torino; 2 Laboratory of Microbiology and Molecular Biology, Ospedale Amedeo di Savoia, ASL "Città di Torino", 3. Unit of Neurology and 4 Unit of Eye-Nose-Throat Medicine, Ospedale Maria Vittoria, ASL "Città di Torino", Torino, Italy

Correspondence to

Andrea Calcagno
Clinica Universitaria di Malattie Infettive
Ospedale Amedeo di Savoia
C.so Svizzera 164
10149, Torino, Italy
+390114393884
andrea.calcagno@unito.it

Background

- Antiretrovirals (ARVs) long-term efficacy and HIV-functional cure require the abolition of viral replication in every body compartment as well as the effect of the immune system.
- The olfactory mucosa (OM) is a unique tissue at the intersection of the central nervous (CNS) and lymphatic system; preliminary data suggest that HIV may persist in the OM despite antiretroviral treatment.[1]
- Differential ARVs exposures have been described in cerebrospinal fluid (CSF), brain tissue and lymph nodes.[2-4]

Patients and Methods

Inclusion Criteria

- HIV-positive patients on combination antiretroviral treatment (cART) undergoing lumbar punctures (LPs) for clinical reasons.

Procedure

- Patients underwent nasal brushing[1] and LP less than 72 hours apart;
- Swabs were tested for Olfactory Mucosa Protein (if positive sampling was deemed adequate) and HIV RNA (modified CAP/CTM v.2.0 assay);
- One swab was inserted in 0.9% saline solution containing vial, then centrifugated (800 rpm, 5 min at 4°C); the surnatant was transferred to three 1mL vials and the swab to a dry vial and all were frozen at -80°C.

Analysis

- Frozen swabs were weighted upon extraction and then inserted in PTFE tubes along with 40µl of internal standard (marked with stable isotopes) working solution plus 500 µl of water:methanol solution (30:70 v:v);
- These tubes were then vortex-mixed 10 sec and sonicated for 10 minutes at 40°C;
- The dry extracts were dissolved in 110 µL of water:acetonitrile:acetic acid (94.9:5:0.1 v:v:v) solution and 10 µl of acid phosphatase (0.5 X) and incubated for 1 hour at 37°C, in order to convert phosphate metabolites of NRTIs to the free form;
- The resulting extracts were analyzed by using HPLC/MS-MS, obtaining absolute amounts (ng): these results were then normalized for the estimated weight of the extracted material (the difference between the initial weight and after the extraction process);
- The lower limit of quantification was 0.3 ng per sample, corresponding to a mean concentration of 3 pg/mg of OM swab.

Aim of the study

Comparing ARV concentrations in the OM, CSF and plasma

Results (n=31)

	n (%) or median (IQR)
Gender (male)	25 (80.6%)
European ancestry	30 (96.8%)
Age (years)	51.4 (46.2-58.2)
Body Mass Index (Kg/m^2)	23.5 (19.7-27.7)
CD4 (cell/uL)	649 (360-965)
plasma HIV RNA >50 copies/mL	2 (6.5%)
if detectable plasma HIV RNA (cps/mL)	103/433
CSF HIV RNA >50 copies/mL	4 (12.9%)
if detectable CSF HIV RNA (cps/mL)	120/137/157/301218
OM HIV RNA >50 copies/mL	1 (3.2%)
if detectable OM HIV RNA (cps/mL)	116
CSF cells (n/mm^3, median/range)	0 (0-2)
CSF serum albumin ratio	6.2 (4.3-7.4)
CSF neopterin (ng/mL)	1.12 (0.73-1.87)
Diagnosis:	
Neurocognitive impairment	15 (48.4%)
Syphilis	5 (16.1%)
Asymptomatic (previous CSF escape)	3 (9.7%)
PML	2 (6.5%)
White matter hyperintensities	2 (6.5%)
Bickerstaff brainstem encephalitis	1 (3.2%)
Hepatic encephalitis	1 (3.2%)
Drug-associated dizziness	1 (3.2%)
Vertigo	1 (3.2%)

After processing, a median of **101 mg (89-123) of OM cells** were retrieved. All samples were OMP+ at histological analysis.

OM concentrations were detectable in 78 out of 99 measurements

TFV (10/12) and ABV (3/6) were often below the lower limit of quantification (3 pg/mg)

OM concentrations as well as OM to plasma and OM to CSF ratios are shown in the Table.

A marginal correlation between OM and plasma (rho=0.30, p=0.007) and CSF concentrations (rho=-0.22, p=0.072) was observed.

	Samples	OM concentrations (pg/mg)	OM to plasma ratio (‰)	OM to CSF ratio (‰)
Lamivudine	7	**15** (10-38)	**10** (7-51)	**175** (62-550)
Emtricitabine	14	**4** (1-12)	**10.4** (0-20)	**42** (0-54)
Abacavir	6	**1** (0-9.7)	**0** (0-72)	**0** (0-14)
Tenofovir (TDF)	10	**0**	**0**	**0**
Tenofovir (TAF)	2	**0**	**0**	**0**
Nevirapine	2	**10-12.5**	**2.3-2.6**	**5.7-33.2**
Rilpivirine	4	**5.5** (1.7-15.2)	**76** (25-76)	**6***
Etravirine	3	**9** (5-9)	**34** (6-34)	**1000** (136-1000)
Atazanavir	1	**160**	**356**	**26666**
Darunavir	11	**106** (22-286)	**58** (17-83)	**11047** (2650-16426)
Ritonavir	4	**133** (45-360)	**1190** (458-1190)	**0***
Cobicistat	11	**288** (119-662)	**1052** (731-7721)	**0***
Raltegravir	6	**12** (4.5-45.7)	**18** (6-93)	**70** (38-220)
Elvitegravir	3	**3** (0-3)	**4.4***	**0***
Dolutegravir	9	**8** (4.5-16)	**7** (2-20)	**1636** (775-2602)
Maraviroc	4	**57** (34-73)	**193** (47-193)	**5333***

* Only one available sample

Conclusions and Further Developments

- We developed a novel method for quantifying ARVs in mucosal tissues obtained through nasal brushing swabs.
- In this small pilot study antiretroviral concentrations in the OM were highly variable with protease inhibitors showing the highest exposure.
  - Olfactory mucosal exposure of some ARVs exceeds CSF concentrations and confirms the recent studies challenging the CSF to CNS pharmacological correlation[3,5]
- Further studies are needed to compare ARVs' penetration (measured through a minimally invasive technique) in comparison to residual viral replication.

1. Calcagno A, et al. AIDS 2018
2. Hammond ER, et al. Am J Epidemiology 2014
3. Srnivas N, et al. Xenobiotica 2018
4. Fletcher CV, et al. PNAS 2014
5. Curley P, et al. AAC 2016