7th International Congress on Drug Therapy in HIV Infection
Poster n: 207

To determine Fos-Amprenavir plasma concentrations in HIV-infected patients: Elisa kit compared to high performance liquid chromatography (HPLC)

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Purpose of the study: To evaluate two different methods (HPLC and TDM ELISA Kit) for fos-amprenavir (fos-APV) plasma determination.

Background

- The protease inhibitor Fos-amprenavir, a prodrug of Amprenavir, is rapidly hydrolyzed to its parent form by cellular phosphatases in the gut epithelium during absorption.
- Routine testing for drug plasma concentration in HIV-infected patients has been hampered by the lack of suitable fast and cost-effective tools; the reference method (High Performance Liquid Chromatography -HPLC) is by far too expensive and requires skilled personnel and continuous maintenance to guarantee consistent performances.
- TDM ELISA kits can offer good routine performances at a fraction of the overall cost per patient.

Methods

- Plasma samples obtained from patients treated with fos-APV were evaluated with HPLC and ELISA.
- Plasma drug concentrations were determined by means of HPLC UV detection using liquid-liquid extraction with methyl-terzbutylether and the internal standard; the lower quantification limit was 50 ng/ml.
- TDM ELISA technology is based on the competition between the drug in the biological sample and an analogue of the same drug chemically conjugated to a revealing enzyme (HRP) for the specific binding site on an antibody of animal origin (range 600-5,000 ng/ml). No extraction is required. Samples were diluted 1:100; samples >5,000 ng/ml were further diluted 1:2 with distilled water. Analysis was performed according to the package insert (for details see: www.biostrands.com) and lasted less than 2 hours.
- Fos-APV plasma levels obtained with the two methods were log transformed and the differences were evaluated by calculating the geometric mean ratios and 95% confidence intervals (CI).
- Data were exponentiated to express the results as geometric means and the ratios of the geometric means on the original scale of measurement.

Results

- Twelve samples obtained from 3 HIV-infected patients at different time points (0, 1, 2, 3 hours post-dosing) were evaluated.
- Median (interquartile IQR) plasma fos-APV was 5,796 ng/ml (3,183-6,755) in HPLC and 6,550 (4,000-7,100) in ELISA.
- Plasma fos-APV resulted 13% increased with ELISA compared to HPLC.
- The geometric mean ratio was 0.79 (confidence interval .IC95%- 0.49-1.27).

Conclusions

- These preliminary data suggest that there are no significant differences between fos-APV plasma levels measured by HPLC and TDM ELISA kits.
- TDM ELISA testing might be useful for routine monitoring of drug plasma level in order: a) to assess minimum target trough concentrations; b) to avoid over-dosage; c) to check for correct compliance.

Fig 1: schematic ELISA test

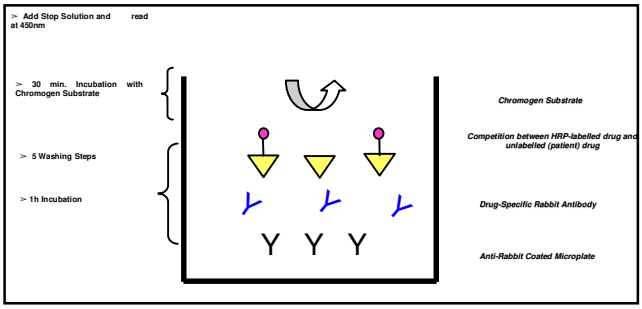


Fig. 2 Curve of calibration

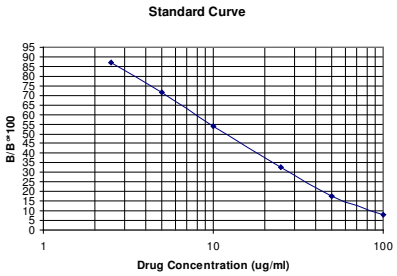


Fig 3: Correlation between HPLC and ELISA

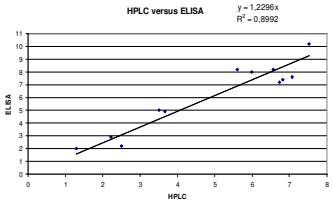
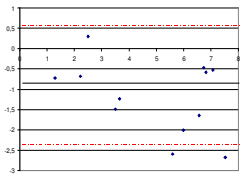


Fig 4: Bland-Altman Test



References

- "Guidelines for the Use of Antiretroviral Agents in HIV-1 Infected Adults and Adolescents" Oct, 29, 2004, DHHS (http://aidsinfo.nih.gov)

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