Cyclosporin stability in Microtainer® EDTA tubes for its dosage over the time

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Introduction

Cyclosporin (CsA), is a first line immunosuppressive agent with a narrow therapeutic range and a large inter-subject pharmacokinetic variability. Therapeutic drug monitoring of CsA, is used to improve patient care.
A previous in vitro (CsA free blood doped) study [1] showed CsA was absorbed into polyethylene or polypropylene blood sample tubes. Edouard Herriot hospital (HEH) conducted this experiment in vivo (in real blood samples) so as to confirm any CsA adsorption.

Experimental method

Samples were taken from pediatric bone marrow transplant patients that had been treated with CsA. Samples (n=43) were collected in Microtainer® EDTA tubes (polyethylene) then analysed with a Dimension® Xpand (Siemens) device.
A Csa antibody conjugated magnetic immunoassay (ACMIA) was performed on the whole blood samples. Within the assay, magnetic particles (CrO_2-CsA) separated freely, then CsA bound to the antibody-enzyme species (Ab-β-gal).
CsA + Ab-β-gal ---> CsA- Ab-β-gal + Ab-β-gal

The "Ab-β-gal" in excess was separated using magnetic particles:
Ab-β-gal + CrO_2-CsA ---> CrO_2-CsA- Ab-β-gal

The "CsA-Ab-β-gal" was then transferred to a spectrophotometric cuvette:
CsA- Ab-β-gal
CPRG ----------> CPR (absorption at 577nm)
(β-galactosidase substrate)

Blood samples were analysed immediately upon arrival to the laboratory (T0). The average time between blood extraction and laboratory arrival was <1h. The blood samples were later analysed again, 3 hours after laboratory arrival (T3).

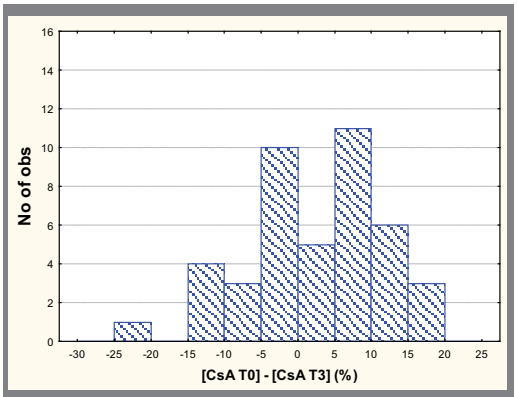
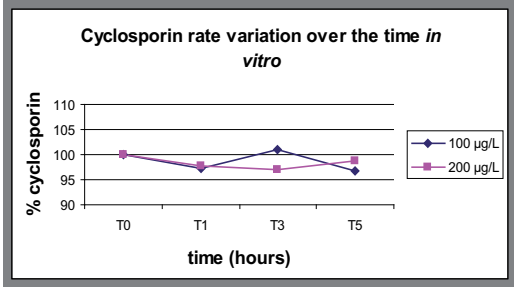


Figure 1



Cyclosporin rate variation over the time in vivo (Figure 1) and in vitro (Figure 2).

Results

Under in vivo conditions, CsA concentrations did not decrease significantly (Fig.1). The CsA concentrations at T0 and T3 were found to be similar, with median CSA concentrations at T0 and T3 of 162.5 µg/L and 160 µg/L, respectively. The CsA immunoassay has an imprecision (CV) around 10%. In an attempt to explain this apparent in vitro vs. in vivo discrepancy, the previous in vitro experiment was reproduced. Once again, CsA adsorption was not observed (Fig.2).

Conclusion and discussion

The previously cited Chollet et al. result could not be confirmed: CsA was not adsorbed in Microtainer® EDTA tubes. Within the previous study, the CsA method required a methanol extraction into a secondary vessel. In this present study, the CsA method used an automated extraction with saponin. The difference between these methods could explain the opposing of results

[1] Chollet F, Evaluation of cyclosporin stability in different tubes for its dosage, September 2006

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