Symposium

[S-16] S-16: TDM of 5-FU

Chairs: Edward Chu, USA / Yasutsuna Sasaki, Japan
Wed. Sep 27, 2017 10:30 AM - 12:00 PM Room D (1F)

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[S-16-4] Determination of endogenous concentrations of uracil and dihydrouracil em dried saliva spots by LC-MS/MS: a new tool to access dihydropyrimidine dehydrogenase deficiency

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Background

Dihydropyrimidine dehydrogenase (DPD) deficiency is responsible for approximately 80% of severe toxicities related to fluoropyrimidines. DPD also converts endogenous uracil (U) to dihydrouracil (UH2), and the ratio between their concentrations has been used as an indicative of DPD deficiency. Considering the ease of collection of saliva samples and the intrinsic logistical advantages of dried samples, the availability of a dried saliva spots (DSS) assay for measuring [U]/[UH2] ratios has the potential to allow identification of patients prone to fluoropyrimidines severe toxicity. The aim of this study was to develop and validate a LC-MS/MS assay for the measurement of U and UH2 concentrations in DSS samples.

Methods

Nine 18 mm DSS discs (~450 μL) were cut in four pieces, transferred to a screw cap tube and added of 5 mL of extraction solvent (ethyl acetate:2-propanol 85:15, v/v) and 100 μL of IS (5-FU 2 g/mL). After homogenization for 30 min at 800 rpm the organic layer was dried at 60 ˚C. The extract was reconstituted with 100 L of water and 25 μL was injected onto the LC-MS/MS. Separation was performed in a C18 column (150×2.1 mm, 1.7 μm) at 10˚C, mobile phase acetic acid 0.5% and acetonitrile 0.1% formic acid (gradient 98:2 to 50:50, v/v). MS conditions were: electrospray ionization, capillary voltage 4.5 kV; vaporizer 202 ˚C; capillary 300 ˚C. Transitions for quantitation were (m/z) 113/70 for U; 115/55 for UH2; 131/114 for IS. The method was applied to 40 healthy volunteers paired DSS and fresh saliva samples.

Results

The assay was linear from 10 to 1,000 ng/mL (r>0.99), accurate 89-112% and precise RSD 5.7-13.0%. Mean extraction yield was 58% U and 49% UH2. The [UH2]/[U] ratio was stable in DSS for 9 days at 45 ˚C (90.7 to 102.6%). Concentrations of U, UH2 and the metabolic ratio were highly concordant between matrices (rs=0.83; 0.70 and 0.83, respectively). Using a [UH2]/[U] cut-off of 1.16 for identification of slow DPD metabolizers, 97.5% concordance was achieved between matrices.

Conclusions

DSS samples could be a useful alternative for DPD activity screening, particularly in locations with limited access to highly equipped laboratories.