Determination of dolutegravir serum concentrations in daily clinical practice using a new HPLC method

Bestimmung von Dolutegravir Serumkonzentrationen in der klinischen Praxis der HIV Therapie mittels einer neuen HPLC-Methode

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Background

Dolutegravir (DTG, Fig. 1) is a new and promising antiretroviral agent for the therapy of HIV. DTG has shown dose dependent serum concentrations above but not below a daily dose of 50mg. In serum a protein adjusted 90 % inhibitory concentration (IC90) of 63.75 ng/ml (152 nmol/L) has been determined [1]. The compound is mainly metabolized by UDP-glucuronosyltransferase (UGT) (Cytochrome P450 metabolism only 8%). In general, this reduces the potency of drug-interactions However, a reduction of DTG exposition of more than 50% has been shown for the antiretrovirals etravirine, efavirenz and also for e.g. Al/Mg-antacids and rifampicin; other concomitant drugs can result in a significant increase of DTG level by inhibiting metabolism [2]. We established a high performance liquid chromatography (HPLC) assay for determination of DTG concentration and evaluated DTG serum concentrations in daily clinical practice of HIV patients.

Method

A sensitive and selective method for the determination of DTG with HPLC and UV detector was established. Chinoxalin was used as internal standard; samples were acidified with equal volume of sodium acetate buffer (pH 4.6) followed by liquid-liquid extraction with diethylether. 20µl of resolved residue average patients concentration was allocated into two groups with equal samples were drawn between 0 and 19 hours after last intake of DTG (p < 0.001) (Fig.4). The average concentration per patient (range 1-4, mean 1.99 samples/patient) was 2437 (median 1988 ng/ml, range 75 - 3 10013 ng/ml).

Table 1: Intraassay and interassay precision and accuracy of the HPLC assay for determining DTG concentration in serum.

Results

From 102 patients (84 male/18 female, mean age 47.4 years) DTG levels have been determined in 203 samples.

The mean concentration of all samples was 2272 ng/mL (median 1757 ng/mL, standard deviation (SD) 1712 ng/mL, range 35 – 10013 ng/mL (Fig. 3). Trough concentrations (20-28h after last application) were significantly lower than concentrations determined at 1456 ng/mL (median 1230 ng/mL, SD 1008 ng/mL, range 35 – 5572 ng/mL) compared to concentrations (mean 3518 ng/ml, median 2707ng/ml, SD 2321 bg/ml, range 75 – 9027 ng/ml) in blood samples drawn between 0 and 19 hours after last intake of DTG (p < 0.001) (Fig.4).

The average concentration per patient (range 1-4, mean 1.99 samples/patient) was 2437 (median 1988 ng/ml, range 75 - 3 10013 ng/ml).

Table 1: Intraassay and interassay precision and accuracy of the HPLC assay for determining DTG concentration in serum.

Figure 1: Dolutegravir

Figure 2: Representative HPLC chromatogram of DTG, and IS (internal standard) in a patients serum sample with a DTG concentration of 2654ng/ml.

Figure 3: DTG concentration in all samples; (median - black bar; 50% interquartile range - blue box)

Figure 4: DTG concentration in relation to the last intake of DTG medication

Figure 5: DTG concentration in relation to gender (a) and age (b); (median - black bar; 50% interquartile range - blue box)

Age and gender had no significant impact on DTG levels. Mean concentrations for female patients was 3212 ng/ml (median 2143) and 2271 ng/ml (1977 ng/ml) for the male patients (p 0.0624) (Fig. 6).

Figure 6: interindividual variation in 8patients with 2 or more trough concentrations

Conclusion

HPLC can be useful for the determination of DTG concentrations. Despite several potential drug interactions and a broad range of serum levels shown here, all except one DTG concentrations (99,5%) evaluated in this real-life setting have been above the previously published IC90. Similar to previous results age and gender had no influence on DTG serum concentration.

References: