Pharmacologic monitoring of NTBC treatment by using dried blood spot and plasma samples in Chilean hereditary tyrosinemic type-1 patients under tyrosine restricted diet

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Background

Tyrosinemia type 1 (Tyr-1) is an inborn error of metabolism caused by defects in tyrosine metabolism and characterized by accumulation of tyrosine and toxic degradation products, such as succinylacetone (SA). Treatment of Tyr-1 patients is based on nitisinone (NTBC) administration and tyrosine and phenylalanine-restricted diet. Monitoring levels of NTBC in plasma samples has been recommended, which is crucial for dosage adjustment, current clinical guidelines recommend keep NTBC in plasma between 40-60 µM in order to hinder the SA formation. Only a few reports have validated the optimal therapeutical range of NTBC in dried blood spot (DBS) samples that ranges 20-40 µM.

The aim of the present study was to evaluate a correlation between NTBC level in DBS and plasma samples, to established the optimal therapeutic range of NTBC in both type of samples by analyzing the level of SA excreted in the urine and the hepatocarcinogenic biomarker alpha-Fetoprotein in our group of our active Tyr-1 patients

Methodology

Fifteen Tyrosinemia Type-1 Patients

- One-year follow-up in our Center
- Quarterly biochemical and clinical control (48 control in total)
  - NTBC analysis in DBS and plasma
  - SCA levels in urine
  - alpha-Fetoprotein serum levels
  - Plasma Aminoacids
- Parameter Correlations with NTBC levels and Range of NTBC.
Results

1.- NTBC levels in plasma and DBS are well-correlated

In a paired study of 43 plasma and DBS samples, we identified the correlation coefficient for NTBC concentrations between the two types of samples. We found significant correlation between NTBC levels measured in DBS and plasma (Spearman r: 0.8046, p<0.0001) with a conversion factor of 2.57 (Figure 1), which we can use to transform NTBC values from DBS into plasma values. Given our determination of equivalency between the two types of samples and considering the most accepted range for NTBC levels in plasma samples (40-60 μmol/L), the calculated range for NTBC in DBS would be 15-24.5 μmol/L (38.6-64 172 μmol/L in plasma).

Figure 1. Correlation of NTBC concentration for forty-three plasma (μmol/L) and DBS (μmol/L) samples (r = 0.8046; p<0.001; 95%CI 0.74 - 0.92). The complement table on right shows the conversion values for each range with a factor of 2.57. DBS: Dried blood spot.
Results

2.- Optimal therapeutic range of NTBC based on succinylacetone excretion and alpha-fetoprotein.

To explore the optimal therapeutic range of NTBC in our HT-1 cohort, paired samples were sorted by NTBC concentration (in DBS): 0-14.9 μmol/L; 15-24.9 μmol/L; 25-34.9 μmol/L and >35 μmol/L. For each range, we determined the mean concentration of SA in urine and the hepatocarcinogenic biomarker, αFP (Figure 2). Recommended optimal management values for SA and αFP were identified for the NTBC range in DBS of 15-24.9 μmol/L (SA below 0.5 mmol/mol creatinine and αFP below 10 μg/L). The mean for SA was 0.2 mmol/mol creatinine (95% CI range: 0–0.4) and 8.3 μg/L for αFP (95% CI range: 4.8–10.6). No significant correlation was found between NTBC and SA or αFP in paired-sample comparison.

Figure 2. NTBC concentration ranges associated with SA excretion in urine and αFP in plasma. Graph A: SA; Graph B: αFP. Dotted lines represent maximum allowable concentration, SA: 0.5mmol/mol creatinine in urine and αFP: 10 μg/L in plasma. The complement table shows the mean plus standard deviation per variable. The values observed in the clinical guide’s suggested range for NTBC are in blue. SA: Succinylacetone; αFP: alpha-fetoprotein
**Results**

By segregating NTBC concentration as we previously did, we evaluated whether higher NTBC levels significantly influence amino acid levels. Figure 3 shows that the median Tyr levels (Fig.3A) are within the recommended range (400-600 μmol/L) when NTBC concentrations in DBS are up to 34.9 μmol/L. However, in samples with higher NTBC levels (>35 μmol/L), elevated Tyr median outside the acceptable reference range were observed. (No statistical difference was found for lower NTBC ranges). By contrast, Phe concentration remained within optimal values for all NTBC ranges (Fig. 3B). Methionine levels showed an upward trend dependent on NTBC concentration but remained within good metabolic control limits (Fig. 3C).

**Conclusion**

Our results revealed in our cohort of HT-1 patients that lower NTBC concentration in blood than the indicated in consensus guidelines might be sufficient to control levels of SA and αFP, the main factors that induce severe hepatic complications, such as hepatocarcinogenic. The full impact of NTBC on the entire physiological system is not yet fully understood. Adjusting the NTBC dose to an effective minimum, patient-by-patient, might improve their neurocognitive, biochemical, physical outcomes, and ultimately the prognosis for the disease.

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