Department of Laboratory Medicine

Division of Clinical Chemistry

Studies on the oxysterols

4α- and 4β-hydroxycholesterol

AKADEMISK AVHANDLING
som för avläggande av medicine licentiatexamen vid Karolinska Institutet offentligen försvaras i föreläsningssalen C1-87, Karolinska universitetssjukhuset i Huddinge

Fredagen den 10 juni 2011 kl 10.00

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Stockholm 2011

Abstract

Cytochrome P450 (CYP) enzymes catalyze the metabolism of both foreign and endogenous substances such as drugs and steroids. Enzymes in subfamily CYP3A sum up to 30% of the total CYP content in the liver and metabolize about 50% of all drugs. There is a large inter-individual variability in expression and activity of hepatic CYP3A, mainly due to ethnic, age- and gender-related factors. The CYP3A activity is induced by certain drugs, such as the anti-epileptic drug carbamazepine (CBZ).

Plasma midazolam clearance and the 6β-hydroxycortisol to cortisol ratio in urine have been proposed as clinical markers of CYP3A activity. The suitability of these markers has been discussed as they are difficult to use in for example children and pregnant women due to technical and ethical issues. The plasma level of the endogenous oxysterol 4β-hydroxycholesterol (4b-OHC) has shown to be a marker of CYP3A activity.

In the present project we have studied whether 4b-OHC can be used as a marker of drug induced CYP3A activity in pediatric patients after initiation of treatment with CBZ (Paper 2) and in mothers and neonates at time of birth (Paper 3). In order to increase sample throughput the sample preparation method was optimized (Paper 1). The similar oxysterol 4α-hydroxycholesterol, 4a-OHC, not formed by CYP3A, was determined in parallel (Papers 2-3).

When the sample preparation method was optimized for analysis of 4b-OHC the sample throughput increased about three times by scaling down the sample volume and using solid phase extraction instead of liquid-liquid extraction followed by rotary evaporation. The linear correlation between the two methods was y=1.0x-2.1, r2 = 0.99 (y=new method, n=90).

The plasma level of 4b-OHC was successfully used as a marker of drug induced CYP3A activity in the study of CBZ treatment in children with epilepsy. The CBZ treatment resulted in increased plasma levels of 4b-OHC until at least 8 weeks of treatment. According to the steady plasma levels of CBZ and CBZ-epoxide there was a complete induction of CYP3A within 1-2 weeks and the continued increase of 4b-OHC levels in circulation may be due to slow equilibriums between different compartments.

4b-OHC proved useful also in the study on CYP3A activity in mothers and neonates. Mothers had higher 4b-OHC to cholesterol ratio at delivery as compared to non-pregnant women, indicating increased CYP3A activity during pregnancy. Also the plasma levels of cholesterol and 4b-OHC were higher in the mothers than in the cohort of non-pregnant women. Neonates had lower levels of plasma 4b-OHC and cholesterol at birth as compared to the levels in a cohort of 125 healthy adults. However, the 4b-OHC to cholesterol ratios were similar, indicating similar total CYP3A enzyme activity in neonates as in adults.

In conclusion, 4b-OHC is a non-invasive marker of CYP3A enzyme activity that is easy to use in neonates, children and vulnerable patient groups where probe drugs are difficult or unethical to administer or urine collections are difficult to perform. The blood sample can be taken any time of the day irrespective of food intake which is beneficial in a clinical setting.