Farnesoid-X-receptor (FXR) agonist INT-747 regulates the mRNA expression of hepatic cationic amino acid transporters CAT-2A and CAT-2B

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Background-Aims: The asymmetric dimethylarginine (ADMA), a potent inhibitor of constitutive and inducible nitric oxide synthase (NOS), is metabolized by dimethylarginine dimethylaminohydrolase (DDAH) to citrullina and dimethylamine (Teerlink et al., 2009). In addition, ADMA is able to interfere with NO synthesis by competing with arginine and symmetric dimethylarginine (SDMA) for cellular transport across cationic amino-acid transporters (CATs) (Closs et al, 1997). Interestingly, liver abundantly express CATs, especially CAT-2A and CAT-2B, and the extensive hepatic expression of CAT-2A mRNA suggests a higher uptake of ADMA in this organ as compared with heart, lung and kidney (Hattori et al., 1999). Previous results have shown that activation of Farnesoid X receptor (FXR) by GW4064 led to up-regulation of DDAH-1 and CAT-1 in mouse liver and kidney (Hu et al., 2006). The goal of our study was to evaluate the effect of INT-747, an FXR agonist, in the control of the hepatic mRNA expression of CAT-2A, CAT-2B and DDAH1. In addition the serum and hepatic levels of ADMA were also evaluated. **Methods:** To investigate the effect of FXR activation, male WISTAR rats (n=16) were orally administered 10 mg/kg/day of the INT-747 (Intercept Pharmaceuticals Inc.), an FXR agonist, in vehicle (methylcellulose 1%) for 5 days or with vehicle alone. Serum levels of AST, ALT, Alkaline Phosphatase (AP), total and direct bilirubin were determined. Hepatic biopsies were used for mRNA expression of CAT2A, CAT2B and DDAH by RT-PCR. DDAH activity was also quantified. The determination of hepatic and serum levels of ADMA was performed by Elisa Kit. **Results:** No changes in serum AST, ALT, AP, total and direct bilirubin levels were observed after FXR agonist treatment. A significant reduction in mRNA expression of CAT2A and CAT2B was detected after 5 days treatment: a decreased CAT-2A expression was found in INT-747 group as compared with vehicle-treated rats, 1.64±0.2 vs 2.71±0.4, respectively ($p \leq 0,04$). The same trend was detected for CAT-2B expression: 0.47±0.08 vs 1.02±0.1, respectively, $p \leq 0,006$. A marked increase in serum ADMA levels after INT-747 administration was found as compared with vehicle-treated rats: 1,27±0,03 and 0,79±0,07 micromol/l, respectively, $p \leq 0,001$. No changes in hepatic ADMA content was detected in vehicle and INT-747-treated group (pmol/mg prot: 12,6±1,8 and 13,3±1.3, respectively, $p \geq 0,05$). The evaluation of mRNA expression and activity of DDAH showed that no changes were detectable after 5 day of INT-747 administration. **Conclusions:** The CAT-2B are low capacity transporters that have a high affinity for cationic amino acids and in particular present high affinity for ADMA (Closs et al, 1997). In contrast, CAT-2A, an alternate splice variant of CAT-2B, possesses low affinity but high transport capacity. In this study we found, for the first time documented, that the INT-747 administration results in a reduction of hepatic expression of CAT-2A and CAT2B mRNA. Because, at the dose considered in this study, no changes in DDAH1 expression and activity were detected, the increased serum levels of ADMA is probably due to a decreased uptake in the liver. These data confirm and support the crucial role of the liver in the metabolism of ADMA by taking up large amounts of ADMA from the systemic circulation and suggest the possibility to control this function by the FXR receptor modulation.

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