Glycomics and Lipidopics

Extended Abstract

Mining for Oxysterols in Cyp7b1−/− Mouse Brain and Plasma: Relevance to Spastic Paraplegia Type 5

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Abstract:

Deficiency in cytochrome P450 (CYP) 7B1, also known as oxysterol 7α-hydroxylase, in humans leads to hereditary spastic paraplegia type 5 (SPG5) and in some cases in infants to liver disease. SPG5 is medically characterized by loss of motor neurons in the corticospinal tract. In an effort to gain a better understanding of the fundamental biochemistry of this disorder, we have extended our previous profiling of the oxysterol content of brain and plasma of Cyp7b1 knockout (-/-) mice to include, amongst other sterols, 25-hydroxylated cholesterol metabolites. Although brain cholesterol levels do not differ between wild-type (wt) and knockout mice, we find, using a charge-tagging methodology in combination with liquid chromatography–mass spectrometry (LC–MS) and multistage fragmentation (MSn), that there is a build-up of the CYP7B1 substrate 25-hydroxycholesterol (25-HC) in Cyp7b1-/- mouse brain and plasma. As reported earlier, levels of (25R)26-hydroxycholesterol (26-HC), 3β-hydroxycholest-5-en-(25R)26-oic acid and 24S,25-epoxycholesterol (24S,25-EC) are similarly elevated in brain and plasma. Side-chain oxysterols including 25-HC, 26-HC and 24S,25-EC are known to bind to INSIG (insulin-induced gene) and inhibit the processing of SREBP-2 (sterol regulatory element-binding protein-2) to its active form as a master regulator of cholesterol biosynthesis. We suggest the concentration of cholesterol in brain of the Cyp7b1-/- mouse is maintained by balancing reduced metabolism, as a consequence of a loss in CYP7B1, with reduced biosynthesis. The Cyp7b1-/- mouse does not show a motor defect; whether the defect in humans is a consequence of less efficient homeostasis of cholesterol in brain has yet to be uncovered. Cytochrome P450 (CYP) 7B1 (cytochrome P450 family 7 subfamily B member 1) was first identified in 1995 and found to be primarily expressed in brain in rodents. CYP7B1 is an oxysterol- and steroid- 7α-hydroxylase, accepting many oxysterols and cholestenoic acids as substrates as well as steroids including dehydroepiandrosterone (DHEA). In humans, deficiency in the enzyme was first revealed in a ten-week-old boy presenting with severe liver disease. In more recent studies, treatment of another infant with this enzyme deficiency with chenodeoxycholic acid has proved successful in resolving liver disease [6]. CYP7B1 is expressed in human hippocampus and interestingly CYP7B1 mRNA is significantly reduced in dentate neurons from Alzheimer's disease subjects. In mice, deletion of Cyp7b1 results in a mild phenotype despite elevation of tissue and plasma levels of its oxysterol substrates 25-hydroxycholesterol (25-HC) and 26-hydroxycholesterol, presumably the 25R-epimer, (26-HC, also known as 27-hydroxycholesterol, see Supplementary Materials, Table S1 for abbreviations, common and systematic names) [8]. In light of this mouse data, it was surprising when Tsaousidou et al. found in 2008 that sequence alterations in CYP7B1 were associated with hereditary spastic paraplegia type 5 (SPG5) in humans [9]. Subsequent studies have confirmed patients suffering from SPG5 have a metabolic phenotype characteristic of inactive CYP7B1. In an effort to understand the differences between mouse and human with respect to defective CYP7B1, we embarked on a sterolomic investigation of mouse brain and plasma exploiting enzyme-assisted derivatization for sterol analysis (EADSA) and liquid chromatography–mass spectrometry (LC–MS) with multistage fragmentation (MSn). We previously found that in Cyp7b1 knockout (Cyp7b1-/-) mouse brain the concentration of cholesterol was similar to that of the wild-type (wt, Cyp7b1+/+), as were the levels of 24S-hydroxycholesterol (24S-HC) [14]. On the other hand, concentrations of (25R)26-hydroxycholesterol (26-HC), 3β-hydroxycholest-5-en-(25R)26-oic acid (3β-HCA) and 24S,25-epoxycholesterol (24S,25-EC) were elevated, presumably being CYP7B1 substrates. Now, delving deeper into the sterolome, we reveal that 25-HC and 26-hydroxydesmosterol (26-HD) are also elevated in Cyp7b1-/- mouse brain, whereas 7α,25-dihydroxysterols are reduced in abundance, but other oxysterols 24R-hydroxycholesterol (24R-HC), 7α- and 7β-hydroxycholesterol (7α-HC, 7β-HC) do not change in concentration between the two genotypes. We suggest that elevated levels of 25-HC, 26-HC and 24S,25-

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EC in brain reduce the expression of cholesterol biosynthetic genes by inhibiting the processing of SREBP-2 (sterol regulatory element-binding protein 2) to its active form as a master transcription for the mevalonate pathway, thereby reducing cholesterol synthesis and compensating for its reduced metabolism (via the CYP7B1 pathway), thus maintaining cholesterol levels in Cyp7b1-/- mouse brain at wild-type levels. 25-HC, 24S,25-HC, 3β-HCA, and in some studies 26-HC, have all been found to be ligands to the liver X receptors (LXRα, NR1H3; LXRβ, NR1H2), activation of which increases the expression of ATP-binding cassette transporter A1 (ABCA1) and apolipoprotein E (APOE), transporter and carrier proteins important for maintaining correct sterol levels in neurons [19] and avoiding overload by potentially toxic oxysterols. Brain and plasma samples were from male mice of 13 and 23 months of age. Cyp7b1-/- and Cyp7b1+/+ mice were littermates generated from Cyp7b1+/- crosses at the University of Edinburgh animal facilities. All mice were housed under standard conditions (7:00 am to 7:00 pm light/dark cycle, 21 °C) with food and water available ad libitum. Tissue sampling was performed under the aegis of the UK Scientific Procedures (Animals) Act, 1986, amended in 2012 to comply with the European Directive 2010/63/EU. The study was conducted under PPL No.70/7870 with prior approval from the University of Edinburgh Animal Welfare and Ethical Review Body. All mice were sacrificed in the morning by cervical dislocation, trunk blood collected and brains removed, frozen on powdered dry ice and stored at −80 °C. Sterols including oxysterols were extracted from brain as described in. In brief, mouse brain was homogenised in ethanol containing isotope-labelled internal standards and oxysterol- and cholesterol-rich fractions separated by solid phase extraction (SPE) on a reversed phase C18 column. The oxysterol-rich fraction was then divided into two aliquots (a and b) and the first treated with cholesterol oxidase enzyme from Streptomyces sp. to oxidise 3β-hydroxy-5-ene groups to 3-oxo-4-ene equivalents, suitable for subsequent derivatisation with the Girard P (GP) reagent (i.e., fraction a). The second aliquot of the oxysterol fraction was treated with GP reagent directly, in the absence of cholesterol oxidase (i.e., fraction b). This allowed the differentiation of oxysterols with a native 3-oxo-4-ene structure (fraction b) from those with a 3β-hydroxy-5-ene structure (i.e., (fraction a)–(fraction b)). The cholesterol-rich fraction was treated separately, but in the same way as the oxysterol-rich fraction. Plasma samples were prepared as described in Autio et al. and Crick et al. by extraction into ethanol followed by SPE to separate cholesterol- and oxysterol-rich fractions . The oxysterols were then derivatised with GP reagent with (fraction a), or without (fraction b), prior oxidation by cholesterol oxidase. For plasma analysis, two GP reagents were used [2H0]GP and [2H5]GP with either fraction a or with fraction b, respectively. This allowed the duplex LC-MS analysis of oxysterol fractions prepared with (fraction a) or without (fraction b) treatment with cholesterol oxidase The oxidised/derivatised oxysterol-rich fractions were analysed by LC-MS (MSn) using an Ultimate 3000 LC system (Thermo Fisher Scientific, Loughborough, UK) and LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, Loughborough, UK) as described in Meljon et al. and Crick et al. . In brief, GP-derivatised oxysterols were separated on a reversed phase Hypersil Gold C18 column (Thermo Fisher Scientific) using a methanol/acetonitrile/0.1% formic acid gradient. The eluent was directed to an electrospray ionisation source (ESI) and analysed by high-resolution (60,000 at m/z 400) MS and MS3 ([M]+→[M-Py]+→, where "-Py" corresponds to the loss of the pyridine group from the molecular ion M+) scans performed in parallel in the Orbitrap and LTQ linear ion-trap, respectively. Quantification was performed using the isotope dilution method.

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