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Therapeutic modulation of cerebral L-lysine oxidation in glutaric aciduria type I: The role of the L-pipecolate pathway

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Glutaric aciduria type I is a rare inborn error of cerebral L-lysine metabolism belonging to the group of "cerebral" organic acidurias. It is caused by an autosomal recessive deficiency of the mitochondrial flavoprotein glutaryl-CoA dehydrogenase (GCDH; EC 1.3.99.7). Biochemically, glutaric aciduria type I is characterized by an accumulation of glutaric acid, 3-hydroxyglutaric acid, glutaconic acid and glutarylcarnitine in tissues and body fluids. In the majority of untreated patients, striatal injury and, subsequently, secondary dystonia develop acutely within encephalopathic crises precipitated by catabolic stress or insidiously between age 3 to 36 months. These children often lose previously acquired motor skills and develop dystonia, dyskinesia, dysarthria and feeding problems. The severity of symptoms correlates with the extent of striatal injury. Glutaric aciduria type I is regarded as treatable neurometabolic disorder, if patients are diagnosed neonatally (preferably by newborn screening) and metabolic treatment is started immediately. Evidence-based guideline recommendations for metabolic treatment include (1) low L-lysine diet, (2) carnitine supplementation and (3) emergency treatment during episodes that are likely to induce catabolic stress.
The current understanding of L-lysine breakdown postulates that cerebral and *extra*cerebral tissues metabolize L-lysine along two different pathways; pipecolate pathway being the major cerebral and saccharopine pathway being the predominant *extra*cerebral route. Understanding cerebral L-lysine metabolism and its therapeutic modulation, however, is the prerequisite for an optimization of current treatment strategies. A major shortcoming of our current understanding about cerebral L-lysine metabolism is the function and localization of L-pipecolate oxidase (EC 1.5.3.7), the key enzyme of cerebral pipecolate pathway. Using *Gcdh*$^{-/-}$ mice, a useful model for glutaric aciduria type I, the major aim of our study was to investigate cerebral L-lysine oxidation in subcellular compartments. Thus, a substantial focus was put on the establishment of an isolation protocol for brain peroxisomes from adult mice, specifically targeting the most unexplored and controversially discussed step of cerebral L-lysine breakdown, which is L-pipecolate oxidation. Specific peculiarities of adult mouse brains (myelin-rich tissue, different density ranges of brain peroxisomal subpopulations, similar density ranges of brain peroxisomes and other subcellular fractions in Nycodenz™) required a careful adaptation of various isolation steps. Eventually, we present an isolation procedure for brain peroxisomes, consisting of crude separation of organelles via differential centrifugation (1,000×g_{max}, 3,500×g_{max} and 25,000×g_{max}), removal of myelin by means of a sucrose gradient and enrichment of peroxisomes using a continuous 15 to 30% Nycodenz™ gradient. Moreover, by optimizing recently published methods for the isolation of murine liver peroxisomes, we were able to investigate and compare L-pipecolate oxidation, to our knowledge for the first time, in brain and liver peroxisomal subpopulations of adult *Gcdh*$^{-/-}$ mice and thus, to analyze the importance of L-lysine degradation along the pipecolate pathway in brain and liver. Furthermore, since a decrease of glutaric acid concentrations could be observed in both the brain and liver of *Gcdh*$^{-/-}$ mice after clofibrate exposure, presenting a promising novel therapeutic option for glutaric aciduria type I, we investigated alternative peroxisomal degradation routes that might be responsible for the drop of glutaric acid in those tissues.

Our enzymatic findings highlighted that L-pipecolate oxidation was detectable in brain peroxisomal subpopulations of *Gcdh*$^{-/-}$ mice. Moreover, since cerebral L-pipecolate oxidase activity was quite low (6.6 ± 2.03 µU/mg protein) and only occurred in the purest brain peroxisomal subpopulation, we conclude that very likely L-pipecolate is metabolized peroxisomally in the murine brain. However, we could not detect a significant change of cerebral L-pipecolate oxidase activity between untreated and clofibrate-exposed *Gcdh*$^{-/-}$ mice. Furthermore, since we provide data, that an alternative hepatic degradation pathway for glutaryl-CoA oxidation is irrelevant in untreated *Gcdh*$^{-/-}$ mice and exposure to clofibrate accounts for a strong induction of hepatic glutaryl-CoA oxidation, we conclude that clofibrate-mediated induction of peroxisomal fatty acid oxidation is the most likely explanation for the reduction of glutaric acid concentration in the liver of *Gcdh*$^{-/-}$ mice, but not in the brain. Findings also suggested that palmitoyl-CoA and glutaryl-CoA are most probably metabolized by the same enzyme in murine liver, namely palmitoyl-CoA oxidase. This conclusion derives from localization, nearly identical activity patterns and similar inducibility by clofibrate for both palmitoyl-CoA and glutaryl-CoA oxidation.

Additionally, by means of isolation protocols for brain and liver peroxisomes we provide evidence that different peroxisomal subpopulations account for various metabolic pathways. Whereas cerebral low-density subpopulations are predominantly involved in D-amino acid oxidation, intermediate-density subpopulations account for L-pipecolate oxidation. This however, differs from the liver, where intermediate-density subpopulations mainly metabolize D-amino acids, and high-density subpopulations predominantly account for palmitoyl-CoA and glutaryl-CoA oxidation (fatty acid oxidation).

In summary, we could demonstrate that it is indeed highly likely that L-pipecolate oxidation takes place in brain peroxisomes of *Gcdh*$^{-/-}$ mice. Based on the current findings we conclude that brain peroxisomes appear to be integral organelles of cerebral L-lysine degradation with regard to L-pipecolate oxidation and liver peroxisomes are involved in the alternative breakdown of accumulating glutaryl-CoA in *Gcdh*$^{-/-}$ mice. Moreover, peroxisomes in both tissues might be promising therapeutic targets, since we could show that clofibrate exposure reduces cerebral and hepatic glutaric acid concentrations. We assume that peroxisomal or mitochondrial targets are responsible for the clofibrate-mediated reduction of glutaric acid in the brain, while it is inducible peroxisomal glutaryl-CoA oxidation in the liver of *Gcdh*$^{-/-}$ mice. Thus, by applying clofibrate, we present an interesting novel treatment possibility for the reduction of glutaric acid levels in the brain and liver of *Gcdh*$^{-/-}$ mice, however, further studies will be needed to investigate the neurological outcome of our biochemical observations.