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Importance of Alternative Enzymatic Pathways Involved in the Detoxification of Methylglyoxal in Diabetes

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The glyoxalase system is a highly specific enzyme system existing in all mammalian cells. It is responsible for the detoxification of dicarbonyl species, primarily methylglyoxal (MG). It has been implicated to play an essential role in preventing the increased formation of advanced glycation end products under certain pathological conditions such as diabetes. This study has shown, that the decrease of GLO1 under hyperglycemic conditions is only partly leading to an increase in MG or MG-derived AGEs. This finding contradicts previous findings but it could be a result of tissue specific susceptibility and therefore possible compensation of GLO1 through alternative detoxification pathways. To define those alternative detoxification pathways in a specific manner, a total loss of GLO1 is mandatory, which was claimed to be lethal. In this study, the creation of the first glyoxalase 1 knock-out model ($GLO1^{-/-}$) in mammalian Schwann cells using the CRISPR/Cas9 technique was achieved. Neither elevated concentrations of MG nor associated protein modifications were observed in $GLO1^{-/-}$ cells. Alternative detoxification of MG in $GLO1^{-/-}$ was achieved by increased catalytic efficiency of aldose reductase (AKR1b3) towards hemithioacetal (product of GSH and MG), which is most likely caused by *S*-nitrosylation of aldose reductase. The hemithioacetal was mainly converted into lactaldehyde, which was paralleled by a loss of reduced glutathione. Inhibition of aldose reductase in $GLO1^{-/-}$ cells was thereby associated with an increased sensitivity against MG, elevated intracellular MG levels, associated modifications, as well as increased oxidative stress. These *in vitro* data suggest that aldose reductase can compensate for the partial loss of GLO1. This might be of clinical importance within the context of neuronal diseases caused by an impaired glyoxalase system. The transfer of this finding from a single cell culture model to a complex organism is obligatory to define the contribution of alternative detoxification pathways in humans. The deficit of GLO1 and the subsequent increase in MG has been reported to be one the five mechanisms by which hyperglycemia causes diabetic late complications. $GLO1^{-/-}$ mice appear healthy and showed no elevated MG or MG-H1 levels under hyperglycemic conditions, which confirmed the *in vitro* findings presented in this work. The enzymatic efficiency of various oxidoreductases in the liver and kidney towards MG were increased in the $GLO1^{-/-}$ mice. Both subclasses, ALDHs and AKRs, were upregulated in especially during hyperglycemic conditions. The functional relevance of this was confirmed by the altered distribution of alternative detoxification products. Therefore the kidney, where AKRs have naturally high activity, accumulated hydroxyacetone and lactaldehyde, whereas the liver accumulated high amounts of pyruvate as a result of increased ALDH activity towards MG. Furthermore, it was shown that MG-dependent AKR activity is a clinical relevant pathway in human patients suffering from diabetes. Overall, these data suggest that in the absence of GLO1, AKR can effectively compensate to prevent the accumulation of MG. The combination of metabolic, enzymatic and genetic factors may therefore provide a better means of identifying patients which are at risk for the development of late complications caused by impaired detoxification systems.