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Hepatic S100A9-TLR4-MTORC1 axis normalises diabetic ketogenesis

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Background and aims: Type 1 Diabetes (T1D) afflicts millions of people and is usually diagnosed in children and young adults with an incidence that has been increasing at an alarming annual rate of ~3%. T1D is mainly characterized by hyperglycaemia, however β-cell loss also leads to other serious metabolic derangements such as hypertriglyceridemia, hyperglucagonemia and diabetic ketoacidosis (DKA). DKA is often life threatening (accounting for up to 263 yearly hospitalization events for each 1000 patients) and occurs due to unrestrained ketogenesis, whose incidence is high in diabetic pateints. While insulin therapy reduces ketogenesis this approach is sub-optimal and currently available insulin adjuvants actually increase the risk of developing DKA. To this end, we have identified a ketone normalizing action of S100A9 in T1D. The aims of our study were to uncover where (cell types and tissues) and how (molecular mechanism) S100A9 was exerting this beneficial effect on ketogenesis and to assess its therapeutic potential and clinical relevance.
Materials and methods: We generated insulin deficient mice lacking or re-expressing Toll-Like Receptor 4 (TLR4) only in liver or hepatocytes and mice with hepatic-restricted and hepatocyte restricted loss of Tuberous Sclerosis Complex 1 (TSC1, an mTORC1 inhibitor) in the context of liver overexpression of S100A9. We assessed cellular signaling pathways and circulating metabolic parameters *in-vivo* and *ex-vivo* fatty acid oxidation in these mice to determine the mechanism of action of S100A9. We tested the translational feasibility and safety of an S100A9 based therapeutic in insulin deficient mice through administration of recombinant mouse and human S100A9 (acute and chronic treatment)We analyzed plasma samples of decompensated diabetic patients to determine clinical feasibility of S100A9 treatment.
Results: S100A9 suppresses diabetic ketogenesis via activating mTORC1 signaling downstream of the TLR4-Akt pathway in hepatic non-parenchymal cells. This activation suppresses fatty acid oxidation in the liver. Extracellular S100A9 activates mTORC1 signaling in a cell-autonomous fashion. Administration of recombinant mouse and human S100A9 suppresses DKA in insulin deficient rodents.Chronic treatment of recombinant S100A9 suppresses DKA and displays a promising safety profile in insulin deficient rodents. Plasmatic S100A9 content was only modestly increased in decompensated diabetic patients (possibly indicating a compensatory mechanism to reduce ketogenesis) therefore providing scope for further increasing plasmatic S100A9 as a potential therapeutic clinical avenue in diabetes.
Conclusion: Our results indicate that it acts through the hepatic TLR4-mTORC1 axis in non-parenchymal cells; hence suggesting the existence of an insulin-independent intra-hepatic network able to regulate lipid and ketone metabolism in TID. S100A9 shows promise as a therapeutic,holding potential to reduce insulin needs and consequently diminish unwanted side effects of insulin treatment while improving metabolic control.
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Validation of duodenal targeting by oral pharmacologic duodenal exclusion therapy for treatment of type 2 diabetes

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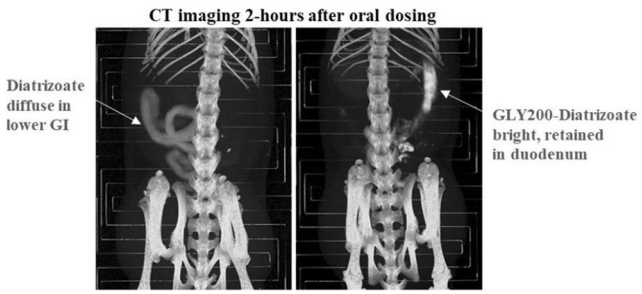
Background and aims: Metabolic surgery is the most successful long-term therapy for T2DM and obesity, reducing macro, microvascular complications, and mortality. The acute and chronic metabolic improvements observed with these surgeries are believed to be, at least in part, a direct consequence of preventing nutrient exposure to the proximal small intestine (duodenum and proximal jejunum exclusion). The use of metabolic surgery and duodenal exclusion devices is limited, however, due to their invasive nature. We developed oral, non-absorbed polymers (GLY100, GLY200) designed to mimic the effects of metabolic surgery non-invasively by augmenting the natural mucus lining of the proximal small intestine to create a temporary barrier. In chronic rodent models of T2DM (GK-Rat and ZDF-Rat), these polymers produced progressive reductions in post-prandial glucose of up to 70% following standardized caloric loads. Improvements in fasting plasma glucose, HOMA-IR, and bodyweight were also observed. The aim of this study was to visualize the retained duodenal barrier in rats using appropriate imaging modalities.
Materials and methods: Imaging of GLY200 after gavage administration to fasted Sprague-Dawley rats was performed using 2 methods: Computed Tomography (CT), and an In Vivo Imaging System (IVIS). For CT imaging, GLY200 was covalently conjugated with diatrizoate via an efficient amide forming reaction and unconjugated diatrizoate was administered as a control. CT images were obtained after transient isoflurane administration 1, 2, and 4 hours after gavage. For IVIS imaging, GLY200 was reacted with FITC to produce a fluorescein-GLY200 conjugate, and FITC-dextran70 was administered as a control. Rats were euthanized 0.5, 1.0, 2, 4, and 8 hours after dosing and the GI-tracts were immediately removed for imaging.
Results: CT imaging (see figure) clearly demonstrated retention of GLY200-diatrizoate within the proximal small intestine 1- and 2-hours after administration, with an intense signal only in the duodenum. In comparison, the diatrizoate control was broadly distributed over the small intestine distal to the duodenum by 1-hour. In IVIS imaging, GLY200-FITC showed intense fluorescence in the proximal small intestine that persisted for 4-hours after administration and was undetectable by 8-hours. In comparison, FITC-dextran70 was distributed broadly throughout the distal intestine and cecum by 30 minutes with no retention in the duodenum.
Conclusion: Our imaging studies confirm that GLY200 conjugate is effectively retained in the duodenum after oral administration. These observations, along with previously reported glycemic improvements in chronic rodent models of T2DM, support the hypothesis that orally administered polymers designed to target the proximal small intestinal gut wall could potentially be used to non-invasively recapitulate the glycemic and weight effects observed with metabolic surgery and duodenal exclusion devices.



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