Research Article

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mPGES-2 deletion remarkably enhances liver injury in streptozotocin-treated mice via induction of GLUT2

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Background & Aims: Microsomal prostaglandin E synthase-2 (mPGES-2) deletion does not influence *in vivo* PGE_2 production and the function of this enzyme remains elusive. The present study was undertaken to investigate the role of mPGES-2 in streptozotocin (STZ)-induced type-1 diabetes and organ injuries.
Methods: mPGES-2 wild type (WT) and knockout (KO) mice were treated by a single intraperitoneal injection of STZ at the dose of 120 mg/kg to induce type-1 diabetes. Subsequently, glycemic status and organ injuries were evaluated.
Results: Following 4 days of STZ administration, mPGES-2 KO mice exhibited severe lethality in contrast to the normal phenotype observed in WT control mice. In a separate experiment, the analysis was performed at day 3 of the STZ treatment in order to avoid lethality. Blood glucose levels were similar between STZ-treated KO and WT mice. However, the livers of KO mice were yellowish with severe global hepatic steatosis, in parallel with markedly elevated liver enzymes and remarkable stomach expansion. However, the morphology of the other organs was largely normal. The STZ-treated KO mice displayed extensive hepatocyte apoptosis compared with WT mice in parallel with markedly enhanced inflammation and oxidative stress. More interestingly, a liver-specific 50% upregulation of GLUT2 was found in the KO mice accompanied with a markedly enhanced STZ accumulation and this induction of GLUT2 was likely to be associated with the insulin/SREBP-1c pathway. Primary cultured hepatocytes of KO mice exhibited an increased sensitivity to STZ-induced injury and higher cellular STZ content, which was markedly blunted by the selective GLUT2 inhibitor phloretin.
Conclusions: mPGES-2 deletion enhanced STZ-induced liver toxicity possibly via GLUT2-mediated STZ uptake, independently of diabetes mellitus.

Keywords: mPGES-2; Glucose transporter 2; Streptozotocin; Diabetes; Prostaglandin E_2.
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Abbreviations: mPGES-2, microsomal prostaglandin E synthase 2; STZ, streptozotocin; WT, wild type; KO, knockout; GLUT2, glucose transporter 2; GSH, glutathione; COX, cyclooxygenase; HO-1, haem oxygenase 1; 15-PGDH, 15-hydroxyprostaglandin dehydrogenase; SREBP-1c, sterol regulatory element-binding protein 1c; Hct, haematocrit; PAS, Periodic acid–Schiff; EM, electron microscopy; PPAR, peroxisome proliferator-activated receptors; FAS, fatty acid synthase; HTGL, hepatic triglyceride lipase; TG, triglyceride; DGAT, diglyceride acyltransferase; TBARS, thiobarbituric acid reactive substances; ROS, reactive oxygen species; NADPH, nicotinamide adenine dinucleotide phosphate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; AKT, protein kinase B; IR, insulin receptor.

Introduction

Microsomal prostaglandin E synthase 2 (mPGES-2) was initially isolated from the microsomal fraction of bovine heart [1], and the cDNAs of the human and monkey homologues were subsequently cloned [2]. This enzyme is synthesized as a Golgi membrane-associate protein, and the formation of the mature cytosolic enzyme needs proteolytic removal of the N-terminal hydrophobic domain [2,3]. mPGES-2 is constitutively expressed and most abundant in brain, heart, kidney, and small intestine [4]. Although mPGES-2 was originally found to be glutathione (GSH)-independent [2], a recent study more convincingly demonstrated the GSH-dependent property of mPGES-2 [5]. *In vivo* mPGES-2 forms a complex with GSH and haem and only haem-free mPGES-2 exhibited PGE_2 synthetic activity under *in vitro* conditions [5]. In agreement with this study, the *in vivo* evidence from mPGES-2 KO mice did not show that this protein is responsible for the PGE_2 production under basal or pathophysiological conditions [6]. Therefore, the functional role of mPGES-2 remains elusive.

The gene map of mPGES-2 is close to chromosome region 9q34.13, which is closely associated with obesity or body weight [7]. More interestingly, several recent reports strongly indicated the association of mPGES-2 arg298His polymorphism with type-2 diabetes or metabolic syndrome [8–11], which highly suggests a potential role of mPGES-2 in the regulation of energy metabolism, especially glucose metabolism.

STZ, a nitrosourea analogue, is not only a widely used reagent to reproduce the animal model of type-1 diabetes by destroying pancreatic β-cells, but is also a FDA-approved drug for the treatment of metastatic cancer of pancreatic islet cells. STZ is similar to glucose transported into cells via the glucose transporter 2 (GLUT2) rather than via other GLUTs and is particularly toxic to pancreatic β-cells due to the high expression of GLUT2, which is a well-documented fact in STZ-induced pancreatic β-cell damage in mouse and rat [12–16]. STZ induces DNA fragmentation via DNA alkylation and subsequent activation of poly ADP ribose polymerase (PARP-1) leading to the depletion of NAD (+) and ATP [17–19], which finally results in cell necrosis. Moreover, pancreatic β-cells are not the only target of STZ cytotoxicity, as DNA damage by STZ has also been found in liver and kidney cells [20].

To define the role of mPGES-2 in diabetes, we treated mPGES-2 KO mice with streptozotocin (STZ) to induce type-1 diabetes. To our surprise, mPGES-2 KO mice exhibited severe lethality and liver toxicity few days after STZ treatment, despite similar glucose levels. In the present study, we extensively characterized the hepatic phenotype of mPGES-2 KO mice and also provide the underlying mechanism, involving the change of STZ-transport by GLUT2 in the liver.

Materials and methods

Animals

mPGES-2 mutant mice were generated in our lab. This mouse colony was propagated at the University of Utah and maintained on a mixed C57/BL6x129/Sv background under a 12:12-h light-dark cycle (lights on at 6:00 a.m. and lights off at 6:00 p.m.). In all studies, 3- to 4-month-old male mice were used. All procedures were conducted according to the principles and guidance of the University of Utah Institutional Animal Care and Use Committee.

Specific methods

The methods for the generation of the STZ diabetic model, the CCl_4 liver injury model, primary hepatocyte culture, cell viability, STZ measurement, biochemical assays, DNA fragmentation, quantitative RT-PCR (qRT-PCR), Western blotting, immunohistochemistry, and statistical analysis are shown in the Supplementary data section. Primers for qRT-PCR are listed in Table 1.

Table 1. Sequences of primers for real-time PCR.

Gene	Primer sequence	Accession number
GAPDH	5'-gtcttcactaccatggagaagg-3' 5'-tcatggatgaccttggccag-3	M32599
mPGES-1	5'-agcacactgctggtcatcaa-3' 5'-ctccacatctgggtcactcc-3'	BC024960
mPGES-2	5'-gctggggctgtaccacac-3' 5'-gattcacctccaccacctga-3'	NM_133783
COX-2	5'-aggactctgctcacgaagga-3' 5'-tgacatggattggaacagca-3'	NM_011198
COX-1	5'-cattgcacatccatccactc-3' 5'-ccaaagcggacacagacac-3'	BC023322
TNF-α	5'-tccccaaagggatgagaag-3' 5'-cacttggtggtttgctacga-3'	NM_013693
MCP-1	5'-gctctctcttcctccaccac-3' 5'-acagcttctttgggacacct-3'	NM_011333
HO-1	5'-ggtgatggcttccttgtacc-3' 5'-agtgaggcccataccagaag-3'	NM_010442
p47phox	5'-gtcgtggagaagagcgagag-3' 5'-cgctttgatggttacatacgg-3'	NM_010876
p67phox	5'-ggccaagtgaaaaactactg-3' 5'-gcctcataactgaagattgc-3'	NM_010877
BAK	5′-cgctacgacacagagttcca-3′ 5′-tccatctggcgatgtaatga-3′	NM_007523
BAX	5′-tgcagaggatgattgctgac-3′ 5′-gatcagctcgggcactttag-3′	NM_007527
Caspase-3	5′-atgggagcaagtcagtgga-3′ 5′-ggcttagaatcacacacacaaag-3′	NM_009810
Glut1	5'-tcaacacggccttcactg-3' 5'-cacgatgctcagataggacatc-3'	NM_011400
Glut2	5'-tgtgctgctggataaattcgcctg-3' 5'-aaccatgaaccaagggattggacc-3'	NM_031197
Glut4	5'-gtaacttcattgtcggcatgg-3' 5'-agctgagatctggtcaaacg-3'	AB008453
DGAT1	5'-ttccgcctctgggcatt-3' 5'-agaatcggcccacaatcca-3'	NM_010046
DGAT2	5'-agtggcaatgctatcatcatcgt-3' 5'-tcttctggacccatcggccccagga-3'	NM_026384
HTGL	5'-ctgagcaccaagaagcactc-3' 5'-tggaagagcaggaatctgg-3'	X58426
FAS	5'-gctcctcgcttgtcgtct-3' 5'-gccttccatctcctgtcatc-3'	NM_007988
PPARα	5'-cgggtcatactcgcgggaaag-3' 5'-tggcagcagtggaagaatcg-3'	NM_011144

Results

The lethal phenotype of mPGES-2 KO mice following STZ treatment

To investigate the role mPGES-2 in type-1 diabetes, a single i.p. injection of STZ at 120 mg/kg was administered to WT and KO mice. Unexpectedly, after four days of STZ application, six of nine mPGES-2 KO mice died, and the remaining three were close to death. In contrast, WT mice did not show any noticeable abnormality after STZ treatment (Supplementary Fig. 1A). The six dead and three close-to death KO mice consistently exhibited a yellowish liver and an enlarged stomach, contrasting the nearly normal appearance of these organs in WT mice. The STZ-treated KO mice animals also showed lower body temperature (WT/STZ 34.2 ± 0.54 °C *vs.* KO/STZ 27.8 ± 1.61 °C, p <0.05), higher haematocrit (Hct) (WT/STZ 52.03 ± 0.84 *vs.* KO/STZ 64.13 ± 2.6, p <0.05) and lower blood glucose level (WT/STZ 436.75 ± 46.7 mg/dl *vs.* KO/STZ 272 ± 18.02 mg/dl, p <0.01), as compared with control animals or STZ-treated WT mice (Supplementary Fig. 1B–D). Liver HE staining showed mild hepatocyte oedema in WT mice but a severe global steatosis of hepatocytes in KO mice (Supplementary Fig. 1F).

To avoid lethality, a separate experimental analysis was performed at day 3 of the STZ treatment. At day 3, all STZ-treated KO mice were still alive showing a yellowish liver and enlarged stomach (Fig. 1A), as well as increased Hct (WT/STZ 51 ± 0.4% *vs.* KO/STZ 56.9 ± 1.8%, p <0.05) and slightly reduced body temperature (WT STZ/35.7 ± 0.26 °C *vs.* KO/STZ 31.86 ± 1.35 °C, p <0.05) compared to the control animals or STZ-treated WT mice, with the exception that the blood glucose increment was similar in STZ-treated WT and KO mice (WT/STZ 291.75 ± 49.53 mg/dl *vs.* KO/STZ 278.0 ± 40.77 mg/dl, p >0.05) (Supplementary Fig. 1B–D). Interestingly, mPGES-2 KO mice exhibited a significant elevation of plasma insulin levels in parallel with a