The effects of metformin on Pdx-1 and insulin gene expression in mouse embryonic and neonatal pancreas

Mahmood Hashemitabar1, Malek Soleimani Mehranjani2, Hamidreza Momeni2, Somayeh Bahramzadeh1*, Fereshteh Negad Dehbashi1, Layasadat Khorsandi1

1 Cellular and Molecular Research Center, Jundishapur University of Medical Sciences, Ahvaz, IR Iran
2 Department of Biology Research Center, Arak University, Arak, IR Iran

ABSTRACT

Background: Metformin is an oral anti-diabetic medication used to treat type 2 diabetes. Metformin treatment increases levels of active GLP-1. Activation of GLP-1 receptor signaling leads to enhanced expression of Pdx-1 mRNA transcripts in β-cell lines. The Pdx-1 gene is critical for β-cell differentiation and expression of the insulin gene.

Objectives: This study investigated the effect of metformin on Pdx-1 and insulin gene expression in mouse embryos and neonates.

Materials and Methods: Pregnant C57BL/6 mice were randomly divided into two groups. The control group received normal saline, and the experimental group received 75, 150, or 250 mg/kg metformin by intraperitoneal injection once daily for 11 days. Half of the pregnant mice were then sacrificed by cervical dislocation on day 19.5 of pregnancy, and the pancreases of the embryos were dissected. The other half of the pregnant mice delivered their pups, and the pancreases of the neonatal mice were removed for assessment of Pdx-1 and insulin gene expression.

Results: The various doses of metformin did not change the expression of Pdx-1 or the insulin gene in the neonatal or embryonic experimental groups compared to the control groups (P > 0.05). Neonates from the metformin-treated groups showed a significant increase in expression of Pdx-1 and insulin compared to embryos from the metformin-treated groups (P < 0.05).

Conclusions: The results indicate that metformin affects the regulatory region of the insulin gene after birth. The insensitivity of embryonic pancreases to metformin is probably due to their lack of functional maturity.

ARTICLE INFO

Article Type: Original Article

Article history:
Received: 2 Nov 2010
Revised: 15 Dec 2010
Accepted: 1 Jan 2011

Keywords:
Pancreas
Pdx-1
Insulin
Mice

1. Background

Diabetes is a chronic metabolic syndrome caused by insulin deficiency. There are approximately 200 million diabetic individuals in the world, only about half of whom are formally diagnosed. These numbers are expected to double by 2030 (1). The disease often results in long-term microvascular, neurological, and macrovascular complications, including retinopathy, nephropathy,
neuropathy, and increased risk of cardiovascular disease (2). Diabetes is the leading cause of blindness, lower limb amputations, and renal failure (3). Type 1 and type 2 diabetes are the two main forms of diabetes (4, 5). Type 1 diabetes accounts for approximately 10% of all cases of diabetes. It is characterized by an absolute insulin deficiency caused by immunological destruction of pancreatic β-cells, the cells that produce and secrete insulin. Type 2 diabetes is more complex in etiology and is characterized by a relative insulin deficiency, reduced insulin action, and resistance to insulin-induced glucose transport in skeletal muscle and adipose tissue. Frank type 2 diabetes is manifest as a continuum of insulin resistance culminating in the failure of increased insulin secretion to compensate for insulin resistance (6, 7). The progression to full diabetes ensues when pancreatic β-cell hypersecretion of insulin fails to compensate for insulin resistance (7).

Metformin is an oral agent commonly prescribed to treat type 2 diabetes (8). The anti-diabetic actions of metformin involve the reduction of hepatic glucose production and/or insulin resistance (9). Metformin treatment increases the levels of active GLP-1 in rats (10). GLP-1 receptor signaling is coupled to the formation of new β-cells through enhanced proliferation of existing β-cells (11) and through induction of islet neogenesis (12). Activation of GLP-1 receptor signaling leads to enhanced expression of mRNA transcripts for glucokinase, GLUT-2, pancreas duodenum homeobox-1 (Pdx-1), and insulin in both normal and diabetic rodents (13). In adults, Pdx-1 regulates genes specific to the β-cell (14).

2. Objectives
This study investigated metformin’s effects on Pdx-1 and insulin gene expression in mouse embryos and neonates.

3. Materials and Methods
3.1. Animals
The study groups consisted of 10 pregnant C57BL/6 mice weighing 25–30 g. The mice were obtained from Ahvaz Jundishapur University of Medical Sciences, Experimental Research Center. This study was approved by the ethics committee of Jundishapur University and was carried out in accordance with its ethical guidelines.

The mice were housed in a temperature- and light-controlled room and were fed with standard laboratory chow and water.

3.2. Experimental design
Pregnant C57BL/6 mice were randomly divided into two groups. The control group received normal saline, and the experimental group received 75, 150, or 250 mg/kg metformin (Sigma, USA) by intraperitoneal injection once daily for 11 days. Half of the pregnant mice were then sacrificed by cervical dislocation on day 19 of pregnancy, and the pancreases of the embryos were dissected. The other half of the pregnant mice delivered their pups, and the pancreases of the neonatal mice were removed for assessment of Pdx-1 and insulin gene expression by semi-quantitative RT-PCR.

3.3. RNA preparation, cDNA synthesis, and RT-PCR
Total pancreatic RNA from pools of six pancreases was extracted using the RNeasy Plus Mini Kit (Qiagen, Canada). RNA (~2 µg) was reverse transcribed using a RevertAid First Strand cDNA Synthesis Kit (Fermentas-EU). PCR reactions were run for insulin and Pdx-1 with primers that allowed for exponential amplification of the transcripts at an equal rate as primers for Gapdh.

All quantifications were performed using mouse Gapdh as an internal standard. PCR was conducted with the following thermal profiles: denaturation at 94 °C for 3 min followed by 35–40 cycles of 30 s at 94 °C, 30 s at the optimal annealing temperatures (listed in Table 1) (15), and 45 s at 72 °C. The program ended with a 10-min extension at 72 °C. The amplified products were subjected to electrophoresis using agarose gels and were stained with ethidium bromide. The ratios of insulin and Pdx-1 to Gapdh were then calculated. Semi-quantitative analysis of PCR products was performed using UVIdoc software.

3.4. Statistical analysis
All values are given as the mean ± SD. Data were analyzed by one-way analysis of variance (ANOVA) using SPSS 17 software. P < 0.05 was interpreted as a statistically significant difference.
4. Results

In the different embryonic experimental groups, there were no significant differences in the expression of Pdx-1 or the insulin gene compared with the control group. In the different neonatal experimental groups, there were no significant differences in the expression of Pdx-1 or the insulin gene compared with the control group (Table 2).

Neonatal mice treated with metformin showed a significant increase in expression of Pdx-1 and the insulin gene compared to the various embryonic treatment groups (P < 0.05). Results from semi-quantitative RT-PCR are reported in Figures 1–3.

5. Discussion

The results from this study demonstrate that metformin at 75, 150, and 250 mg/kg significantly increases expression of Pdx-1 and the insulin gene in neonatal mice compared with embryonic mice.

El-Assaad et al. reported that metformin may counteract the effects of elevated glucose and free fatty acids to decrease β-cell secretory function and viability (16).

Yasuda et al. showed that metformin treatment increases the levels of active glucagon-like peptide-1 (GLP-1) in rats. They concluded that metformin increases GLP-1 secretion (10). The incretin hormone GLP-1 is a gastrointestinal insulin-releasing peptide (17). GLP-1 is known to inhibit glucagon secretion, stimulate insulin biosynthesis, and enlarge pancreatic β-cell mass. GLP-1 causes differentiation of pancreatic AR42J cells into glucagon and insulin-producing cells (18) and promotes a β-cell phenotype through a Pdx-1 dependent pathway (19). Pdx-1 regulates genes that are specific to the β-cell. Specifically, it transactivates the promoter regions of the insulin, glucokinase, GLUT2, and amyloid precursor protein genes (14).

Richardson et al. showed that metformin has marked effects on transcriptional regulation in the pancreatic β-cell and in freshly isolated rat islets of Langerhans. They indicated that metformin stimulates Pdx-1 protein levels and that its effects depend on both exposure time and glucose concentration (20).
The exact mechanism of metformin’s effect on mouse embryonic and neonatal pancreases was not established in this study. The results indicate that metformin affects the regulatory region of the insulin gene after birth and that the insensitivity of the embryonic pancreas to metformin is probably due to its lack of functional maturity.

Financial support
None declared.

Conflict of interest
None declared.

Acknowledgments
This research was supported by a Grant (CMRC-6) from the Research council of the Ahvaz Jundishapur University of Medical Sciences in 2010.

References