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Urinary metabolomic profiling in mice with diet-induced obesity and type 2 diabetes mellitus after treatment with metformin, vildagliptin and their combination

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ABSTRACT

Metformin, vildagliptin and their combination are widely used for the treatment of diabetes, but little is known about the metabolic responses to these treatments. In the present study, NMR-based metabolomics was applied to detect changes in the urinary metabolomic profile of a mouse model of diet-induced obesity in response to these treatments. Additionally, standard biochemical parameters and the expression of enzymes involved in glucose and fat metabolism were monitored. Significant correlations were observed between several metabolites (e.g., *N*-carbamoyl-β-alanine, *N*1-methyl-4-pyridone-3-carboxamide, *N*1-methyl-2-pyridone-5-carboxamide, glucose, 3-indoxyl sulfate, dimethylglycine and several acylglycines) and the area under the curve of glucose concentrations during the oral glucose tolerance test. The present study is the first to present *N*-carbamoyl-β-alanine as a potential marker of type 2 diabetes mellitus and consequently to demonstrate the efficacies of the applied antidiabetic interventions. Moreover, the elevated acetate level observed after vildagliptin administration might reflect increased fatty acid oxidation.

1. Introduction

Obesity represents a major health problem worldwide due to its increasing prevalence and numerous associated comorbidities (Reaven et al., 2004). The combination of obesity-induced insulin resistance and impaired insulin secretion might eventually lead to the development of impaired glucose tolerance or impaired fasting glucose, with further progression to type 2 diabetes mellitus (T2DM) (Conway and Rene, 2004). Considering the large number of complications associated with obesity/T2DM, a comprehensive therapeutic approach to optimally address multiple risk factors is needed (Gaede et al., 2008; Skyler et al., 2009). Moreover, gradual intensification of antidiabetic therapy with frequent necessity for the use of a combination of various antidiabetic drugs with different modes of action is necessary (Inzucchi et al., 2015).

Metformin has been established as a first-line therapy in patients with T2DM, reflecting its efficacy, low cost and several positive metabolic effects in addition to its improvement of glucose control (Inzucchi et al., 2015; NICE, 2015), including a slight reduction in body weight (BW) (Lee and Morley, 1998; Seifarth et al., 2013). When metformin monotherapy does not achieve

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optimal glucose control, a sulfonylurea or dipeptidyl peptidase-4 (DPP-4) inhibitor, such as sitagliptin or vildagliptin, is commonly added as a second drug. The addition of a DPP-4 inhibitor is advantageous compared with the use of sulfonylurea, as it does not induce hypoglycemia or weight gain (Ahren, 2009). However, the optimal second-line drug for patients with T2DM remains a matter of intensive debate (Inzucchi et al., 2015), and innovative approaches are required to identify predictors of the responses to respective drugs or drug classes and their long-term efficacies in various patients with T2DM. This approach will enable individualized selection of therapy for different subtypes of patients with T2DM.

Metabolomics is a potentially useful tool for both assessing the efficacies of different drug compounds and predicting patient responses (Clayton et al., 2006). In particular, NMR-based metabolomics is frequently applied for treatment monitoring (Clayton et al., 2006; Qiu et al., 2008; Samino et al., 2015), with urine as the most investigated biofluid.

The rodent model of high-fat (HF) diet-induced obesity has become one of the most important tools for understanding the etiopathogenesis of human obesity, as it closely mimics the increasing availability of HF foods in modern society (Wang and Liao, 2012); therefore, this model might be appropriate for use in metabolomic studies of therapeutic responses.

The present study examined the urine metabolomic profile and its changes in response to metformin, vildagliptin and their combination in a mouse model of diet-induced obesity (DIO model) and T2DM. The metabolic changes in response to vildagliptin treatment are of particular interest, as DPP-4 is a ubiquitous enzyme that breaks down not only glucagon-like peptide-1 (GLP-1) but also other hormones; thus, this treatment may have some off-target effects that have not yet been identified (Deacon, 2011; Haluzik et al., 2014). Until recently, only a few metabolomic studies have reported on changes in metabolite concentrations following metformin treatment. Some of these studies have utilized a hyphenated LC-MS analytical technique (Zhu et al., 2013), whereas others have employed NMR spectroscopy (Qiu et al., 2008) or even both platforms (Huo et al., 2009), using either urine or blood serum samples. However, to our knowledge, there have been no reports of the metabolic effects of vildagliptin alone or in combination with metformin, although both of these therapeutic approaches have been widely used for the treatment of T2DM (Ahren, 2008; Halimi et al., 2008). Therefore, in the present study, we applied NMR-based metabolomics to delineate the comprehensive therapeutic responses to antidiabetic therapies, specifically vildagliptin monotherapy and vildagliptin/metformin dual therapy, to fill gaps in the current knowledge of their precise mechanisms of action.

2. Materials and methods

2.1. Animals, diets and treatments

All experiments were conducted according to the ethical guidelines for animal experiments and the Czech Republic law No. 246/1992 and were approved by the Committee for Experiments with Laboratory Animals of the Academy of Sciences of the Czech Republic.

Inbred 3-week-old C57BL/6 male mice were obtained from Charles River (Sulzfeld, Germany). The mice were housed under controlled conditions at a constant temperature of 22 ± 2 °C, relative humidity of 45–65% and fixed daylight cycle (6 a.m.–6 p.m.), with 5 mice per cage. The animals were provided free access to water and a standard rodent chow diet ssniff® R/M-H containing 33%, 9% and 58% of calories from proteins, fats and carbohydrates, respectively (ssniff Spezialdiäten GmbH, Soest, Germany).

From 8 weeks of age, the mice were fed a HF diet to induce obesity. The energy content of the HF diet was 5.3 kcal g^{-1}, with 13%, 60% and 27% of the calories derived from proteins, fats and carbohydrates, respectively. The diet was composed of 40% standard chow, 34% powdered cow's milk-based human baby formula, 25% lard, and 1% corn starch w/w (Kopecký et al., 1996). After six weeks of HF diet feeding, oral administration of antidiabetic compounds was initiated, and the mice were kept on the HF diet. The following experimental groups (n = 10) were established: A. water; B. 250 mg/kg/day metformin; C. 20 mg/kg/day vildagliptin; and D. 250 mg/kg/day metformin +20 mg/kg/day vildagliptin. The doses of vildagliptin and metformin used in the combined treatment group were chosen to reflect the clinical situation in patients with type 2 diabetes mellitus (Guarino et al., 2012). The antidiabetic drugs were dissolved in the drinking water, and the doses were continuously adjusted according to the volume of water consumed, which was monitored weekly. In addition to these experimental groups, another 10 mice were fed a standard chow diet (LF – low fat). The untreated group of HF mice provided with water was considered the control group. The BWs were monitored weekly during the dosing period according to the intervals designated in an overview of the study design shown in Fig. 1.

2.2. Biochemical, biometrical and hormonal parameters

2.2.1. Oral glucose tolerance test (OGTT)

The oral glucose tolerance test was performed after overnight fasting on days 50, 51 and 52. At time point 0 (9 a.m.), blood was collected from the tail veins of the mice, and then they were perorally (PO) loaded with a glucose solution (0.2 g/ml) at a dose of 2 g/kg. Blood samples were subsequently collected from the tail veins into heparinized capillaries at 15, 30, 60, 90, 120 and 180 min. The blood glucose concentrations were determined in whole blood using a Glucocard glucometer (Arkray, Kyoto, Japan).

2.2.2. Blood sampling for biochemical analyses and tissue dissection

After 50–52 days, fasted blood samples for biochemical analyses were collected into tubes containing ethylenediaminetetraacetic acid (EDTA) prior to the OGTT, and the blood plasma was subsequently separated and stored at −70 °C. After the OGTT, the mice were sacrificed by decapitation. The visceral adipose tissues (VATs), subcutaneous adipose tissues (SCATs), brown adipose tissues (BATs), perirenal adipose tissues and livers were dissected from all of the mice. The tissue samples were weighed, flash-frozen in liquid nitrogen, and stored at −70 °C for subsequent RNA extraction.

2.2.3. Determination of hormonal and biochemical parameters

The plasma insulin concentrations were measured using an RIA assay (Millipore, St. Charles, MI, USA), and the leptin concentrations were determined by ELISA (Millipore, St. Charles, MI, USA). The blood glucose levels were measured using a Glucocard glucometer (Arkray, Kyoto, Japan). In addition, the plasma triglyceride levels were measured using a quantitative enzymatic reaction (Sigma, St. Louis, MO, USA), and the free fatty acids (FFA) levels were determined using a colorimetric assay (Roche, Mannheim, Germany). All measurements were performed according to the manufacturer's instructions.

2.2.4. Determination of mRNA expression

The adipose tissues (subcutaneous, visceral, and brown) and liver samples were processed as previously described (Maletínská et al., 2011). The mRNA expression of the genes of interest (*Acaca*, *Fasn*, *Lpl*, *Lep*, *Adipoq*, and *Fabp4* in SCAT and VAT; *Acaca*, *Fasn*, *Lpl*, *Pck1*, *Srebf1*, *Cpt1a* and *Cpt1b* in the liver; and *Ucp1* in BAT) was determined using an ABI PRISM 7500 instrument (Applied