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Plasminogen deficiency is associated with improved glucose tolerance, and lower DPP-4 activity

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ABSTRACT

Plasminogen (Plg), which is the inactive form of plasmin, deficiency enhanced insulin secretion, and was associated with improved oral glucose tolerance in mice. Additionally, Plg deficiency was associated with lower dipeptidyl peptidase-4 (DPP-4) activity, and enhanced glucagons-like peptide-1 (GLP-1) expression. Plg may regulate the DPP-4 activity and the glucose metabolism.

1. Introduction

Plasminogen (Plg) is converted to plasmin, which is a main component of the fibrinolytic system, through the action of tissue-type plasminogen activator (tPA) or urokinase-type PA (uPA), and the inhibition of the system is achieved mainly by the plasminogen activator inhibitor-1 (PAI-1) or α2-antiplasmin (α2AP). It has been reported that fibrinolytic factors such as tPA, uPA, uPA receptor (uPAR) and PAI-1 are involved in the glucose metabolism [1–3]. However, the physiological role of main fibrinolytic component, Plg in glucose metabolism was not precisely understood.

Dipeptidyl peptidase IV (DPP-4) is ubiquitously expressed in multiple cells, and cleaves numerous substrates including cytokines, neuropeptides, and the incretin hormones [4]. It has been known that the inhibition of DPP-4 elevates the levels of the incretin hormones, which regulates insulin secretion, and DPP-4 inhibitors are novel agents for the treatment of type 2 diabetes. Several studies demonstrate that DPP-4 can interact with Plg, and regulate cell invasion [5]. We herein investigated the role of Plg in the DPP-4 activity and glucose metabolism.

2. Materials and methods

The animal experiments in this study were approved by the Animal Research Committee of Doshisha Women's Collage of Liberal Arts (Approval ID: Y14-021).

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2.1. Animals

The Plg deficient ($Plg^{-/-}$) mice were kindly provided by Prof. D Collen (University of Leuven, Belgium). Wild type, $Plg^{-/-}$ mice littermates were housed in groups of two to five in filter-top cages with a fixed 12 h light, 12 h dark cycle.

2.2. Oral glucose tolerance test (OGTT) in mice

The $Plg^{+/+}$ and $Plg^{-/-}$ mice were fasted for 4 h with free access to water. The $Plg^{+/+}$ and $Plg^{-/-}$ mice were given glucose (1.5 g/kg) by the oral administration, and the blood glucose concentration were measured at the indicated times. In other study, wild-type mice were fasted for 4 h with free access to water. The mice were pre-injected saline or εACA (10 mg/kg) by i.p. injection 60 min before oral glucose tolerance test.

2.3. Measurement of blood glucose concentration

After blood samples were obtained from tail veins, the blood glucose concentration was measured by using a One Touch Ultra™ instrument (Johnson & Johnson Co., Ltd.).

2.4. Measurement of plasma insulin concentration

The collected blood samples were centrifuged to separate plasma, and the plasma insulin concentration was measured by ELISA (Ultra sensitive mouse insulin ELISA kit, Morinaga Institute of Biological Science, Inc.).

2.5. The activity of DPP-4 in the small intestine of mice

The activity of DPP-4 in the small intestine of mice was measured by DPP-4 Assay Kit (Enzo Life Science)

2.6. Immunohistochemical staining of GLP-1

Paraffin sections of the $Plg^{+/+}$ and $Plg^{-/-}$ mice were labeled with anti-GLP-1 primary antibody, then secondarily labeled with FITC–conjugated anti-rabbit IgG (Molecular Probes). The signals were then detected using a laser-scanning microscope. The stained images obtained from separate fields on the specimens ($n = 4$) were analyzed by using ImageJ.

2.7. Statistical analysis

All data are expressed as mean ± SEM. The significance of the effect of each treatment ($P < 0.05$) was determined by analysis of variance (ANOVA) followed by the least significant difference test.

3. Results

3.1. Plg deficiency enhanced insulin secretion, and was associated with improved glucose tolerance in mice

We compared with the plasma insulin concentration in the fed $Plg^{+/+}$ and $Plg^{-/-}$ mice. The plasma insulin concentration in the fed $Plg^{-/-}$ mice was higher than that of fed $Plg^{+/+}$ mice (Fig. 1A). Next, we performed oral glucose tolerance test (OGTT) in $Plg^{+/+}$ and $Plg^{-/-}$ mice. The peak blood glucose concentration after glucose administration in both $Plg^{+/+}$ and $Plg^{-/-}$ mice was similar, but the subsequent blood glucose concentration in the $Plg^{-/-}$ mice was lower than that of $Plg^{+/+}$ mice (Fig. 1B).

3.2. Plg deficiency is associated with lower DPP-4 activity in mice

We examined the activity of DPP-4 in the $Plg^{+/+}$ and $Plg^{-/-}$ mice. The activity of DPP-4 in $Plg^{-/-}$ mice was lower than that in $Plg^{+/+}$ mice (Fig. 2A). Additionally, we confirmed that the expression of the incretin hormones, glucagons-like peptide-1 (GLP-1) in the $Plg^{+/+}$ and $Plg^{-/-}$ mice. The expression of GLP-1 in $Plg^{-/-}$ mice was higher than that in $Plg^{+/+}$ mice (Fig. 2B, C).

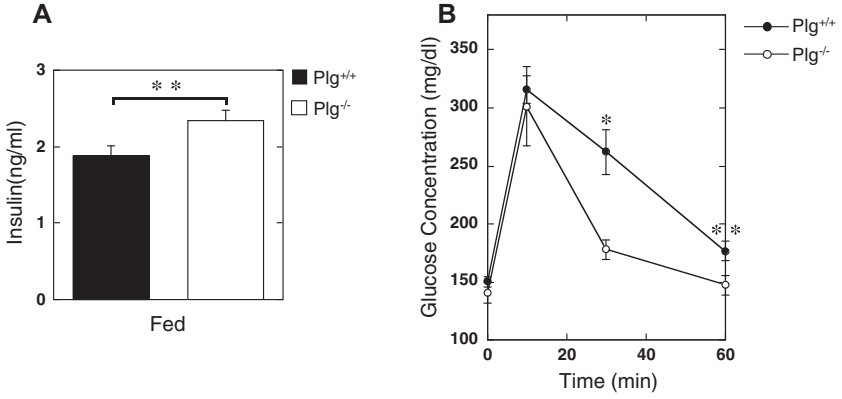

Fig. 1 – Plg deficiency enhanced insulin secretion, and was associated with improved glucose tolerance in mice. (A) The plasma insulin concentration in the fed $Plg^{+/+}$ and $Plg^{-/-}$ mice were measured by ELISA as described in the Materials and Methods ($n = 4$). (B) The blood glucose concentrations were measured at the indicated times after oral administration of glucose to the male $Plg^{+/+}$ and $Plg^{-/-}$ mice as described in the Materials and Methods ($n = 4$). The data represent the mean ± SEM. *, $P < 0.01$; **, $P < 0.05$.