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Natural product vindoline stimulates insulin secretion and efficiently ameliorates glucose homeostasis in diabetic murine models

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ABSTRACT

Ethnopharmacological relevance: *Catharanthus roseus* (L). Don (*Catharanthus roseus*) is a traditional anti-diabetic herb widely used in many countries, and the alkaloids of *Catharanthus roseus* are considered to possess hypoglycemic ability.
Aim of the study: To systematically investigate the potential anti-diabetic effects and the underlying anti-diabetic mechanisms of vindoline, one of the alkaloids in *Catharanthus roseus*.
Materials and methods: The regulation of vindoline against the glucose-stimulated insulin secretion (GSIS) was examined in insulinoma MIN6 cells and primary pancreatic islets. Insulin concentration was detected by Elisa assay. Diabetic models of *db*/*db* mice and type 2 diabetic rats induced by high-fat diet combining with streptozotocin (STZ/HFD-induced type 2 diabetic rats) were used to evaluate the anti-diabetic effect of vindoline *in vivo*. Daily oral treatment with vindoline (20 mg/kg) to diabetic mice/rats for 4 weeks, body weight and blood glucose were determined every week, oral glucose tolerance test (OGTT) was performed after 4 weeks.
Results: Vindoline enhanced GSIS in both glucose- and dose-dependent manners (EC_{50} = 50 μM). It was determined that vindoline acted as a Kv2.1 inhibitor able to reduce the voltage-dependent outward potassium currents finally enhancing insulin secretion. It protected β-cells from the cytokines-induced apoptosis following its inhibitory role in Kv2.1. Moreover, vindoline (20 mg/kg) treatment significantly improved glucose homeostasis in *db*/*db* mice and STZ/HFD-induced type 2 diabetic rats, as reflected by its functions in increasing plasma insulin concentration, protecting the pancreatic β-cells from damage, decreasing fasting blood glucose and glycated hemoglobin (HbA_{1c}), improving OGTT and reducing plasma triglyceride (TG).
Conclusion: Our findings suggested that vindoline might contribute to the anti-diabetic effects of *Catharanthus roseus*, and this natural product may find its more applications in the improvement of β-cell dysfunction and further the potential treatment of type 2 diabetes.

1. Introduction

Type 2 diabetes is a severe worldwide disease, which mainly characterizes insulin resistance and the progressive failure of pancreatic β-cells (Leahy, 1990). Under insulin resistant conditions, pancreatic β-cells increase insulin release to overcome the reduced ability of insulin with its deficiency, thereby maintaining normal glucose tolerance. Once β-cells fail to secrete enough insulin to compensate for the decreased insulin sensitivity, type 2 diabetes occurs (Kasuga, 2006). Decreased insulin concentration can lead to hyperglycemia, which promotes the progressive deterioration of β-cells leading to more severe and chronic health problems including high blood pressure (Qi et al., 2012), vision loss, renal failure (Mazzucco et al., 2005), stroke and death (Snell-Bergeon and Wadwa, 2012). Although the pathophysiological mechanisms of type 2 diabetes are complex, the consensus has been reached that β-cell dysfunction is the key contributor to this disease (Prentki and Nolan, 2006; Thomas et al., 2009). To date, several anti-diabetic drugs have been clinically used to improve β-cell dysfunction, including sulfonylureas, dipeptidyl

Abbreviations: *Catharanthus roseus*, *Catharanthus roseus* (L). Don; GSIS, glucose-stimulated insulin secretion; HFD, high-fat diet; STZ, streptozotocin; OGTT, oral glucose tolerance test; HbA_{1c}, glycated hemoglobin; TG, plasma triglyceride; TEA, tetraethylammonium chloride; Gly, glybenclamide; FSK, forskolin; LAME, linoleic acid methyl ester.

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peptidase-4 inhibitors and incretin hormone GLP-1 analogs. Unfortunately, none of them has been successful in controlling long-term microvascular and macrovascular complications (Zarich, 2009). Recently, to improve curative effects, many medicinal herbs and their extracts are being widely used in the treatment of diabetes in many countries (Malviya et al., 2010).

Catharanthus roseus is originated from the island of Madagascar, and is now growing naturally in many tropical countries and treated as ornamental (Magnotta et al., 2006). In some countries, hot water decoction of the leaves and/or the whole plant are used for diabetic treatment (Nammi et al., 2003a). Previous reports revealed that the alcoholic extract of the leaves of *Catharanthus roseus* exhibited the blood glucose lowering activity (Chattopadhyay, 1999) and the prophylactic activity against the alloxan monohydrate-induced necrotic actions of β-cells (Chattopadhyay et al., 1991). It was later shown that the ethyl acetate fraction of the ethanolic extract of *Catharanthus roseus* was the most effective in glucose lowering against the STZ-induced hyperglycemia rats (Ahmed et al., 2009).

Vindoline (Fig. 1a) as one of the alkaloids is highly present in young leaves and twigs of *Catharanthus roseus* (Srivastava et al., 2004), and oral administration of vindoline (100 mg/kg) had an acute hypoglycemic effect on rats (Svoboda and Root, 1964). Additionally, leaf powder of *Catharanthus roseus* was reported to increase plasma insulin in STZ-induced diabetic rats (Nammi et al., 2003a; Rasineni et al., 2010). All these results thereby suggested that the blood glucose lowering effect of vindoline might be possibly associated with insulin secretion.

In the current work, we systematically investigated the effect of vindoline on insulin secretion in insulinoma MIN6 cells and primary pancreatic islets. It was found that vindoline obviously

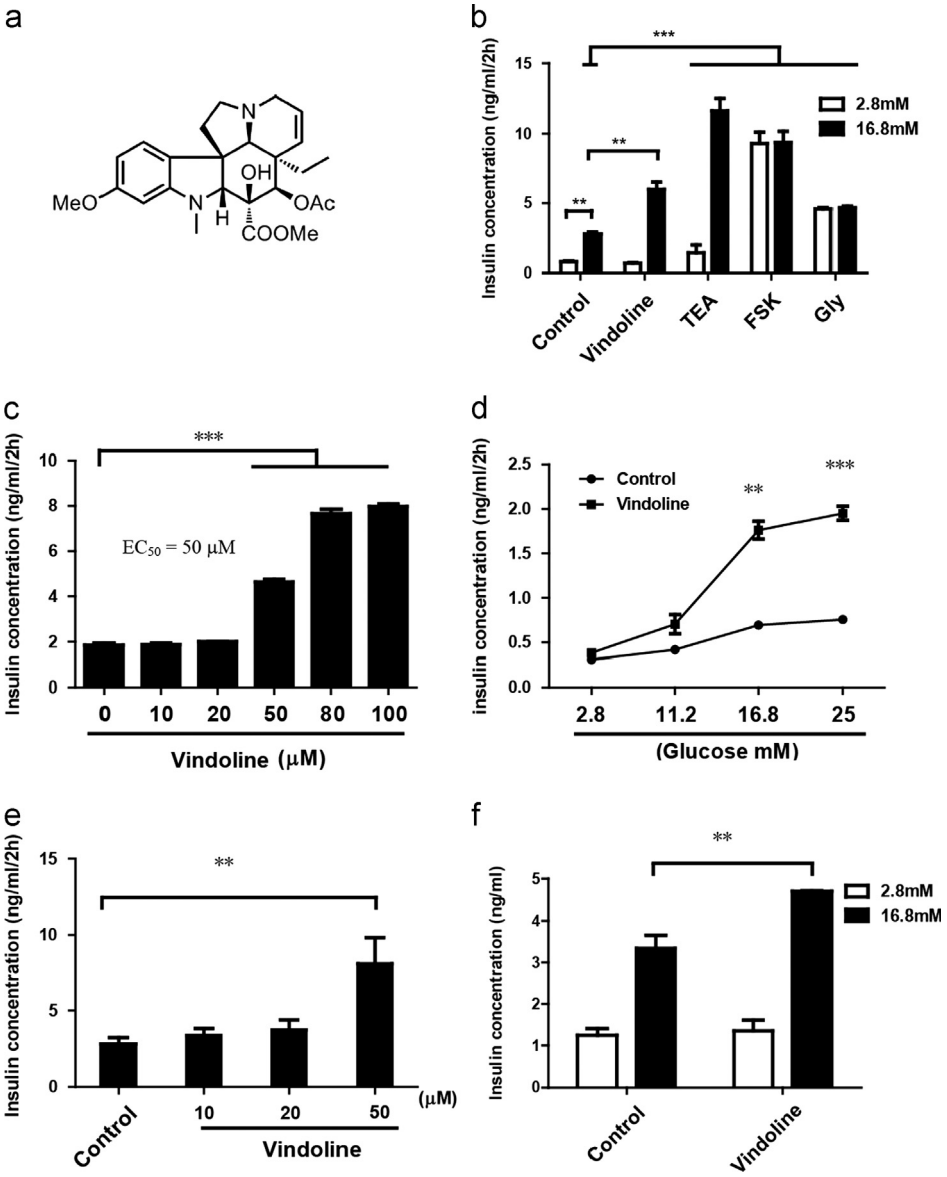

Fig. 1. Vindoline enhanced GSIS in both MIN6 cells and primary islets. (a) Chemical structure of vindoline. (b) After 2 h incubation with glucose-free KRB buffer, MIN6 cells were stimulated with the indicated agents (50 μM vindoline, 15 mM TEA, 10 μM FSK, 0.5 μM Gly) in 2.8 mM (white) or 16.8 mM (black) of glucose, and insulin secretion was determined. (c) After 2 h incubation with glucose-free KRB buffer, MIN6 cells were stimulated with 16.8 mM glucose in the presence of vindoline (10, 20, 50, 80, and 100 μM) for 2 h, and insulin secretion was thus determined. (d) After 2 h incubation with glucose-free KRB buffer, MIN6 cells were stimulated with 2.8, 5.6, 11.2, 16.8, and 25 mM glucose in the presence or absence of vindoline (50 μM) for 2 h, and insulin secretion was thus determined. (e) Primary mice islets were isolated and incubated with glucose-free KRB buffer for 2 h, then these islets were stimulated with vindoline (10, 20, and 50 μM) for 2 h, and insulin secretion was determined. (f) After 2 h incubation with glucose-free KRB buffer, primary islets were stimulated with 2.8 and 16.8 mM glucose in the presence or absence of vindoline (50 μM) for 2 h, and insulin secretion was determined. All data were presented as means ± S.E.M ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$).