Myricetin: a potent approach for the treatment of type 2 diabetes as a natural class B GPCR agonist

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ABSTRACT: The physiologic properties of glucagon-like peptide 1 (GLP-1) make it a potent candidate drug target in the treatment of type 2 diabetes mellitus (T2DM). GLP-1 is capable of regulating the blood glucose level by insulin secretion after administration of oral glucose. The advantages of GLP-1 for the avoidance of hypoglycemia and the control of body weight are attractive despite its poor stability. The clinical efficacies of long-acting GLP-1 derivatives strongly support discovery pursuits aimed at identifying and developing orally active, small-molecule GLP-1 receptor (GLP-1R) agonists. The purpose of this study was to identify and characterize a novel oral agonist of GLP-1R (i.e., myricetin). The insulinotropic characterization of myricetin was performed in isolated islets and in Wistar rats. Long-term oral administration of myricetin demonstrated glucoregulatory activity. The data in this study suggest that myricetin might be a potential drug candidate for the treatment of T2DM as a GLP-1R agonist. Further structural modifications on myricetin might improve its pharmacology and pharmacokinetics.—Li, Y., Zheng, X., Yi, X., Liu, C., Kong, D., Zhang, J., Gong, M. Myricetin: a potent approach for the treatment of type 2 diabetes as a natural class B GPCR agonist. FASEB J. 31, 2603–2611 (2017). www.fasebj.org

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Type 2 diabetes mellitus (T2DM) remains a serious global health threat, and its prevalence is increasing at an epidemic rate (1). The evidence suggests that the incidence of T2DM is increasing in children and adolescents (2–5). T2DM can lead to metabolic syndrome, which is a cluster of metabolic abnormalities that includes glucose intolerance, hypertension, hyperlipidemia, and a noninfective inflammatory state (6–8). Additionally, the metabolic syndrome induced by T2DM can increase the risk of cardiovascular disease (CVD), which is the primary cause of premature death in patients with type 2 diabetes (9–11). The development of novel antidiabetic medicines has not ceased in the decades since the discovery of the utility of insulin. Developments in molecular biology and cell biology have contributed to novel drugs that act on updated targets or mechanisms of T2DM, such as glucagon-like peptide 1 (GLP-1).

GLP-1 was discovered in 1990 and is gut hormone that is released from intestinal L cells after oral glucose administration (12). The function of GLP-1 is to stimulate the secretion of insulin, which balances abnormal blood glucose levels (13–17). Compared with the direct administration of insulin, GLP-1 is an intelligent approach to achieving blood glucose control in a blood glucose level–dependent manner because GLP-1 stimulates the secretion of insulin based on increased glucose levels. In healthy conditions, the function of GLP-1 is halted to prevent the risk of hypoglycemia, which is a main side effect of the use of insulin by patients with type 2 diabetes (18, 19). GLP-1 has been demonstrated to regulate blood glucose concentrations by mechanisms that include enhancing insulin synthesis/secretion, suppressing glucagon secretion, slowing gastric emptying, and enhancing satiety (20). GLP-1 is also capable of inhibiting the apoptosis of β-cells, which suggests that GLP-1 might recover the β-cell functions that are impaired in patients with T2DM (21). The distinct clinical utility of GLP-1 makes it a potent therapeutic strategy for T2DM. However,
the poor stability of GLP-1 (3–5 min) has significantly limited its clinical utility because of the rapid degradation, which is catalyzed by the enzyme dipeptidyl peptidase IV (DPP-IV) (22). The extremely poor stability renders the therapeutic administration of GLP-1 impractical; therefore, many efforts have focused on altering the pharmacokinetic properties of GLP-1 and identifying a synthetic GLP-1 receptor (GLP-1R) agonist.

Myricetin is a flavonoid extracted from the leaves of Myrica rubra (Lour.) Sieb. et Zucc. (23). Accumulating evidence suggests that myricetin possesses antidiabetic properties that are mediated via regulation of the transport of glucose through the function of glucose transporter-2 in Xenopus laevis oocytes (24). It has also been hypothesized that the glucose-regulating effects of myricetin are partially attributable to associations with glucose transporter-4 (25, 26). In addition to the associations with glucose transporters, myricetin might exhibit effects on other glucose regulation mechanisms (27). Myricetin has been observed to increase the activity of glycogen synthase 1 in the hepatocytes of rats with diabetes (28, 29). Recent evidence suggests that myricetin administration might be beneficial for increasing insulin sensitivity and inhibiting islet β-cell apoptosis (30). However, the effects of myricetin on glucose regulation in animal models of type 2 diabetes are not fully understood.

Myricetin has been hypothesized to be a natural GLP-1R agonist in this study because the physiologic characteristics of myricetin described in the literature are similar to those of GLP-1, including the inhibition of β-cell apoptosis, glucoregulation, and the prevention of hyperglycemia. In the present study, the effects of myricetin on GLP-1R and the role of myricetin in glucose clearance were investigated in vitro and in vivo.

MATERIALS AND METHODS

Materials

The cAMP kits were purchased from CisBio Bioassays (Bedford, MA, USA). The rat INS-1 cell line was obtained from Ying Li (Tianjin Medical University General Hospital). The rat insulin and leptin detection kits were purchased from Phoenix Technology, Inc. (Beaverton, OR, USA). A 1-touch blood glucose meter and filters were purchased from Abbott (Shanghai, China). Myricetin (HPLC purity ≥98.0%) was obtained from Sigma-Aldrich (6760; St. Louis, MO, USA). The other chemicals were purchased from Sigma-Aldrich unless otherwise specified.

Animals

Kuming mice, male ZDF (fa/ fa) rats, lean male ZDF rats, and male Wistar rats were purchased from Shanghai Laboratory Animal Co. (China Academy of Sciences, Shanghai, China). Male GLP-1R-knockout (GLP-1RKO) mice were derived from heterozygous mating pairs (AppTech.12630.TJIPR; WuXi, Shanghai, China). These GLP-1R-deficient mice are a knockout model in which the transgenic construct contains a PGK-neo cassette replacing 2 coding exons of the GLP1R gene in the same transcription orientation, along with 4.8 and 3.5 kb of the GLP1R sequence 5’ and 3’ to the PGK-neo sequence. The loss of these exons equates to absence of the first and third transmembrane domains and the intervening sequence of the GLP1R.

Ethics statement

All animal studies were performed in accordance with the approved guidelines of the Animal Experiments Inspectorate of China. All experimental protocols were approved by the Tianjin Institute of Pharmaceutical Research committee (TJJPR0267-003-02).

Receptor binding assay

Binding affinity measurement was carried out in 96-well microtiter plates in buffer containing 25 mM Hepes/0.1% bovine serum albumin (pH 7.4). Myricetin was dissolved in buffer. 125I-GLP-1(7–37) (80 kBq/pmol) (APP TEC; WuXi) was dissolved in buffer and added at 50,000 cpm per well. Nonspecific binding was determined with 1 M GLP-1 solution. Buffer (165 μl) with or without GLP-1 was added to each well, followed by 10 μl myricetin/25 μl plasma membrane/25 μl tracer. The plates were then incubated at 37°C for 1 h. The bound tracer and the unbound tracer were separated by vacuum filtration (Millipore vacuum manifold; Millipore, Billerica, MA, USA). Prism software (GraphPad, La Jolla, CA, USA) was used for all curve fittings. The binding curves were fitted as 1-site competition, and the saturation data were fitted as 2-site binding.

cAMP stimulation assay

For the cAMP assay, INS-1 cells (1.0 × 10^5 cells; passage number, 2) were seeded and plated in 96-well opaque white plates. After 24 h, the medium was replaced with RPMI 1640 medium containing 500 μM 3-isobutyl-1-methylxanthine (an inhibitor of cAMP phosphodiesterase). Subsequently, myricetin was titrated into the incubations to a final concentration of 3 μM. The assay plate was titrated with 2.5 μl/well cAMP and an equal volume of anti-cAMP conjugate after 1 h of incubation. The homogeneous time-resolved fluorescence signal was read on a SpectraMax M5 (Molecular Devices, Sunnyvale, CA, USA) microplate reader after 60 min. The ratio of the absorbances at 665 and 620 nm (×10,000) was calculated and plotted.

Glucose-dependent insulin secretion assay in islets

Male Wistar rats (8–9 wk old; ~300 g) were killed by CO2 asphyxiation, and the islets were isolated. The common bile duct was cannulated with a 27-gauge needle, and the pancreas was dissected with 10 ml of HBBS (Sigma-Aldrich) containing 2% bovine serum albumin (Sigma-Aldrich) and 1 mg/ml collagenase (Sigma-Aldrich). Subsequently, the pancreas was removed and digested in HBBS at 37°C. The islets were purified on a Histopaque (Histopaque-1077:Histopaque-11991 mixture; Sigma-Aldrich) gradient for 18 min at 750 g. The islets were cultured overnight in RPMI-1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (Thermo Fisher Scientific, Waltham, MA, USA).

The insulin secretion was examined by performing a static incubation assay in EBBS culture (Thermo Fisher Scientific). The islets were incubated in Earle’s balanced salt solution containing 3.3 mM glucose at 37°C for 30 min. Three size-matched islets were then selected and incubated in 0.3 ml of Earle’s balanced salt solution containing the indicated amount of glucose (normal blood glucose condition of 3.3 mM or high blood glucose conditions of 30, 60, or 120 mM).
condition of 16.7 mM) in the presence of 3 μM myricetin at 37°C for 90 min. The supernatants were collected and assayed for secreted insulin using a rat insulin RIA kit (Meso Scale Diagnostics, Rockville, MD, USA).

**Insulin secretion assay in Wistar rats**

The insulin secretion assays were performed by injecting these derivatives into male Wistar rats (n = 6 per group; ~300 g). GLP-1 (100 μg/kg body weight) was subcutaneously injected, and myricetin (250 μg/kg body weight) was administered orally. Glucose (2 g/kg body weight, which is a standard dose for glucose administration) was administered intraperitoneally 30 min after the peptide injection. Blood samples were collected via tail vein incisions at the indicated times after glucose administration. The blood samples were assayed for the insulin levels using a rat insulin RIA kit.

**Glucose tolerance test**

To clarify whether myricetin possessed glucose-regulating activity, single-dose glucoregulatory assays were performed. In these assays, myricetin was administered once, and the blood glucose levels were monitored over 48 h. We presumed that the apparent half-life of myricetin could be obtained with this single-dose glucose tolerance test (GTT), and this information would be beneficial for the determination of the appropriate administration frequency for future long-term GTTs. After a period of food withdrawal, myricetin (250 μg/kg body weight) was administered orally to male Wistar rats (n = 7 per group; ~300 g) 30 min prior to glucose administration. GLP-1 and liraglutide were injected into the control animals. The rats were given 2 g glucose per kilogram body weight via intraperitoneal injections. Blood was drawn from the tail vein, and the glucose levels were measured using a glucometer 30 min after glucose administration. Chronic glucose injections (2 g/kg body weight) were administered 30 min prior to each blood glucose measurement time point during the 48-h experimental period. After the observations of the glucose clearance activity of myricetin, the dosage-effect relationship of myricetin was investigated after 12 h of treatment with dosages of 5, 50, 250, and 500 μg/kg.

**HbA1c and body weight measurement**

Based on the glucoregulatory properties exhibited by myricetin, the long-term glucose tolerances of ZDF rats were investigated to determine the antidiabetic activities of the derivatives. The HbA1c levels were assessed using a DCA 200 analyzer (Bayer Diagnostics, Tarrytown, NY, USA), and the body weights and glucose levels were monitored during the experimental period. Male ZDF rats (n = 10 per group) were treated with myricetin (250 μg/kg/12 h) for the entire experimental period (40 d). The control groups were injected with wild-type GLP-1 (100 μg/kg/12 h) and liraglutide (300 μg/kg/24 h).

**Myricetin measurements in GLP-1RKO mice**

We further characterized the effect of myricetin on GLP-1RKO mice to provide direct evidence supporting myricetin as an agonist for GLP-1R. A 24-h GTT was performed in GLP-1RKO mice (n = 7 per group; ~26 g) upon the administration of myricetin. In this experiment, myricetin was administered either orally or by intravenous injection. Glucose was given at dosage of 2 g/kg body weight 30 min before administration of myricetin. Blood glucose levels were monitored throughout the 48-h experiment.

**Real-time PCR**

RNA was isolated from the harvested INS-1 cells treated with myricetin (10 nM, 100 nM, 500 nM, 1 μM, and 3 μM) for 24 h using the RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. cDNA was produced from the extracted RNAs using the cDNA synthesis kit (Fermentas, Walltham, MA, USA). Each forward and reverse primer (10 pmol, 0.2 μl) and 2 μl cDNA were added to the PCR reaction mix (final volume, 25 μl) containing 12.5 μl of SYBR Green Master Mix (Fermentas) and 10.1 μl DNase-free water. The primer sequences of Glut-2 were as follows: forward, CAGCTGTCTTGTGCT-CTGCTGT; reverse, GCCGTCACTGCTACATAACICA. PCR amplification was done over 40 cycles using the following program: 95°C for 10 min, 95°C for 15 s, 5°C for 30 s, and 60°C for 34 s. Data were analyzed using the 2^(-DeltaDeltaCt) method. Expression values were corrected for the housekeeping genes β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The β-actin gene produced similar results to those obtained with Gapdh.

**Statistical analyses**

Student’s t tests were used to analyze the data. Unless otherwise stated, the results are reported as the means ± se. Values of P < 0.05 were considered significant.

**RESULTS**

Myricetin is a small chemical agonist for GLP-1R

As a potent agonist of GLP-1R, the receptor binding assay of myricetin against GLP-1R was performed initially. In radioligand-binding experiments, myricetin bound to the receptor in a concentration-dependent manner, and the EC_{50} was calculated to be 465.75 ± 33.24 nM (Fig. 1). GLP-1 is capable of stimulating the

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Figure 1. Binding assay of myricetin to rat GLP-1R. Binding affinity measurement was carried out in 96-well microtiter plates in buffer containing 25 mM Hapes/0.1% bovine serum albumin (pH 7.4). 125I-GLP-1(7–37) (80 kBq/pmol) was added at 50,000 cpm per well. Nonspecific binding was determined with 1 M GLP-1 solution. The plates were then incubated at 37°C for 1 h. All concentrations were tested in triplicate. The chemical structure of myricetin is depicted in the inset.
secretion of insulin after its binding to GLP-1R in the islet. Accordingly, GLP-1R agonists are presumed to act as insulin secretagogues in the presence of high glucose levels. To examine this property of myricetin (structure in Fig. 1), pancreatic islets isolated from Wistar rats were incubated in either normal-glucose (3.3 mM) or high-glucose (16.7 mM) medium. Neither GLP-1 nor myricetin stimulated the secretion of insulin into the medium after 90 min of incubation at the normal glucose level (3.3 mM) (Fig. 2A). However, in this condition, the incubation of islets with sulfonylurea glibenclamide (5 μM) led to a significant increase in the insulin concentration in the incubation mixture. As an agonist of GLP-1R in islets, GLP-1 exhibited the appropriate insulinotropic properties in the high-glucose concentration of 16.7 mM. As predicted, the treatment of the islets with myricetin in the high-glucose condition caused a 3-fold increase in the insulin level (Fig. 2B). The ability of myricetin to cause cAMP accumulation in the INS-1 cells (Fig. 3A), in combination with its glucose-dependent insulinotropic activity, supports the conclusion that myricetin is a GLP-1R agonist.

Because insulin secretion activity is considered to be an important characteristic trait of a GLP-1R agonist (31), whether a novel agonist exhibits this property is a crucial indicator in the evaluation of the success of the development of a GLP-1R agonist or GLP-1 derivatives. To explore the in vivo insulinotropic effects of myricetin, glucose-stimulated insulin secretion was measured in Wistar rats undergoing a GTT. In this study, the insulinotropic properties of myricetin were ascertained in Wistar rats, and GLP-1 (100 μg/kg body weight) was used as a control (Fig. 3B). In the rats treated with only wild-type GLP-1, the administration of oral glucose dramatically increased the insulin level to 745.30 ± 42.48 pM at 5 min, and this level returned to baseline (210.45 ± 31.94 pM) by 30 min. Myricetin, which possesses physiologic insulinotropic characteristics, exhibited similar temporal features (Fig. 3B). The insulin secretion response induced by myricetin observed in this assay was consistent with the effects demonstrated by peptide GLP-1R agonists, which supports the results obtained from the islet assays that demonstrated that myricetin is a glucose-dependent insulin secretagogue.

Blood glucoregulatory properties of myricetin
To examine the blood glucose clearance activity of myricetin, GTTs were performed 48 h after single-dose administrations of myricetin, GLP-1, or liraglutide into male Wistar rats (n = 6 per group). The peptides were injected subcutaneously, myricetin was orally administered 1 h before the first measuring point, and glucose was administered 30 min before the first measuring point. The glucose concentrations in the rats treated with GLP-1 remained at the plateau of approximately 12 mM throughout the 48-h experimental period (Fig. 4). Presumably, the GLP-1 had been degraded before the first measuring point (1 h after GLP-1 administration). However, the rats injected with myricetin exhibited better glucose tolerance in this single-dose injection experiment than those treated with GLP-1. The treatment with myricetin resulted in blood glucose levels that were maintained at 7.5–8.5 mM over 8 h, and these results were similar to those after liraglutide administration. Additionally, myricetin seemed to exhibit a similar glucoregulatory duration (0–8 h), which suggests that myricetin might possess a half-life similar to that of liraglutide (11.3 h). In a combination dose-efficacy assay, it was suggested that administration frequency of myricetin could be reduced to 2 injections each day at dosage of 250 μg/kg body weight orally (Fig. 4B).

Figure 2. Myricetin increases glucose-dependent insulin secretion from Wistar rat islets. A) Insulin concentrations from the Wistar rat islets incubated in medium containing low glucose (3.3 mM) and either GLP-1 (100 nM), myricetin (3 μM), or the sulfonylurea glibenclamide (5 μM). B) Insulin concentrations from the Wistar rat islets incubated in medium containing high glucose (16.7 mM) and either GLP-1 (100 nM) or myricetin (3 μM). All islet treatments were performed for 90 min. The results are expressed as the means ± SEM. *P < 0.05.
Long-term treatment with myricetin

To evaluate the profile of the physiologic effects of long-term treatment with myricetin, the glucose levels, HbA1c levels, and body weights of ZDF rats were monitored over 40 d in Fig. 5. In this trial, the blood glucose levels and body weights were monitored every 3 d. After 40 d of treatment with myricetin, the HbA1c levels decreased by 0.97 ± 0.01% from 9.1 ± 0.12% (blank group), whereas treatment with GLP-1 alone failed to reduce the HbA1c levels upon rapid proteolysis. Liraglutide induced a 1.01 ± 0.02% decrease, which agrees with the reports in the literature. Combined with the changes in the glucose levels and the body weight indices, these results clearly indicate that myricetin exhibits efficient antidiabetic effects while possessing the unique advantages oral administration and a natural origin.

Gene expression of Glut-2 upon the myricetin treatment in INS-1 cells

To determine whether myricetin induces changes in gene expression profiles for pancreatic cells (rat INS-1 cell), the Glut-2 gene was assessed using real-time PCR. Low expression of Glut-2 was detected in untreated...
INS-1 cells (control) (Fig. 6). However, expression of Glut-2 genes in myricetin-treated cells increased nearly 4.9-fold ($P < 0.01$).

**DISCUSSION**

Class B GPCRs are activating receptors for many endocrine peptide hormones (32, 33), including glucose-dependent insulinotropic polypeptide, glucagon, parathyroid hormone, vasoactive intestinal peptide, secretin, corticotropin-releasing factor, calcitonin, and GLP-1. The endogenous ligands of class B GPCRs are typically 30–40 amino acids in size, which limits their clinical applications owing to poor stability and the requirement of injections (32), like GLP-1 peptide. Unfortunately, traditional small-chemical molecules minimally modulate the activities of class B GPCRs because of the unique structural architectures and activation mechanisms used by these GPCRs (33). Although many efforts have been made to screen small-class B GPCR agonists, much difficulty has been encountered in identifying small organic molecules by class B GPCRs. Recently, substantial progress has been made in the generation of small-molecule agonists of GLP-1R, such as substituted quinoxalines, pyrimidine derivatives, and a cyclobutane derivative (Boc-5) (34–36). The quinoxaline derivatives exhibit chemical similarities to the pyrimidine derivatives in terms of the manner of activation of GLP-1R. Quinoxaline derivatives of GLP-1R agonists have been hypothesized to bind in a pocket formed by the first and second extracellular loops of the juxtamembrane regions of GLP-1R (34). Another GLP-1R agonist, Boc-5, has physiochemical properties that probably make it unsuitable for oral administration (34, 36).

The known physiologic functions of GLP-1 suggest that it plays a critical role in the regulation of glucose homeostasis and is thus a feasible candidate target in the treatment of T2DM (37, 38). In addition to its potential role in the treatment of T2DM, GLP-1 is also presumed to affect cardiovascular function or CVD because GLP-1R has been identified in the heart, kidneys, and blood vessels. A preclinical study of GLP-1 derivatives approved by the U.S. Food and Drug Administration provided evidence that GLP-1 favorably affects endothelial function, sodium excretion, recovery from ischemic injury, and myocardial function in animals. Preliminary data also suggest that GLP-1 reduces markers of CVD risk, such as C-reactive protein and plasminogen activator inhibitor-1. Ongoing studies are examining the effects of the administration of GLP-1 to patients who are at risk of CVD,

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**Figure 5.** Effect of multiple doses of myricetin over 40 d of treatment. Myricetin (250 μg/kg body weight) was administered twice every day during the 40-d experimental period. A glucometer was used to measure the glucose levels at various time intervals. A) The long-lasting glucose regulatory effect of myricetin in ZDF rats. The results indicate that the myricetin-treated ZDF rats maintained relatively constant and lower glucose levels than the control rats, similar to rats treated with liraglutide. Native GLP-1 failed to produce a similar glucose regulatory effect. B) Body weight changes after the myricetin treatment in ZDF rats.

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**Figure 6.** Expression of Glut-2 gene upon myricetin treatment on INS-1 cells. The low expression of Glut-2 gene was detected in untreated INS-1 cells (control). However, the expression of Glut-2 genes in myricetin-treated INS-1 cells was increased nearly 4.9-fold ($P < 0.01$).
postangioplasty patients, post-CABG patients, and patients with heart failure (39). Despite its attractive physiologic characteristics, the therapeutic potential of native GLP-1 is limited by its short lifetime \((<2 \text{ min})\) in vivo. This rapid in vivo clearance rate is due primarily to rapid enzymatic inactivation by DPP-IV (40) and a renal clearance \(<10 \text{ min}\) (41). To provide clinical utility, therapeutic derivatives of human GLP-1 require extended half-life properties or oral administration. To date, many efforts have focused on altering the pharmacokinetic properties of GLP-1 and screening novel chemical agonists of GLP-1R. Along with the development of novel drug carrier, oral preparations of GLP-1 were also developed, such as TTP-054 or TTP-273 from VTV Therapeutics (High Point, NC, USA) and Exendin-4 from Oramed (Jerusalem, Israel) (42, 43).

The findings of the present study demonstrate that the modulation of GLP-1R with myricetin is feasible. Myricetin is a bioflavonoid that is abundant in tea, berries, fruits, and vegetables (44). Myricetin has been described as a promising therapeutic agent for the treatment of T2DM, but the effects of myricetin in animal models of T2DM are not fully understood (45). Evidence has demonstrated that the injection of myricetin enhances insulin activity in rats that are receiving fructose-rich chow (46). Chang et al. (47) found that dietary myricetin decreases the body weight and improves the blood lipid profiles of rats fed a high-fat diet. We found that myricetin induced glucose-dependent insulin secretion in vitro and in vivo. The single-dose glucoregulatory assay revealed the blood glucose clearance activity of myricetin, and the time frame of this effect was likely 8 h, which suggests that this dosage of myricetin could be administered twice daily. Notably, myricetin failed to exert its glucoregulatory in GLP-1R–deficient GLP-1RKO mice, strongly demonstrating that myricetin is an agonist for GLP-1R (Fig. 7). The numerous physiologic profiles of the ZDF rats after long-term treatment with myricetin indicated that the glucose-regulating properties and body weight–controlling activities of myricetin were similar to those of other GLP-1R agonists, such as exenatide, liraglutide, and albiglutide. In addition, myricetin did not retard the proteolysis property of DPP-IV, a main endogenous protease against GLP-1. The incubation of myricetin had no effect on degradation assay of GLP-1 by DPP-IV in vitro (Fig. 8). However, the treatment of myricetin in Wistar rats did not induce the secretion of leptin in comparison with that feature of GLP-1 (Fig. 9). Together, these findings support the notion that myricetin activated GLP-1R and subsequently stimulated the secretion of insulin. The glucose- and body weight–controlling properties of myricetin make it a potent, natural-origin, noninjection antidiabetic candidate drug for T2DM treatment.

In summary, myricetin was determined to be a small-molecule chemical agent that activates GLP-1R, and its physiochemical properties suggest that myricetin could be the first natural agonist of GLP-1R that can be orally administered. However, the potency and pharmacokinetic properties of myricetin might require optimization for further clinical development. Additionally, many more structural details regarding the binding of myricetin to GLP-1R require clarification via mutation studies or computer simulations. Improved understandings of the pocket and the mechanism of activation will facilitate molecular modeling strategies for the development of more potent small-molecule GLP-1R agonists. Structural modifications of myricetin might be beneficial to our understanding of the interactions between myricetin and the receptor and thus
might be helpful for the development of chemical agonists for class B GPCRs.

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AUTHOR CONTRIBUTIONS

Y. Li performed the experiments, including islet isolation, insulin secretion, cAMP measurement, and GTT, and wrote the manuscript; X. Zheng performed the long-term GTT; X. Yi contributed to the discussion; and J. Zhang and M. Gong proofread and wrote the manuscript, including the discussion.

REFERENCES

1. Linnenkamp, U., Guariguata, L., Beagley, J., Whiting, D. R., and Cho, N. H. (2014) The IDF Diabetes Atlas methodology for estimating global prevalence of hyperglycemia in pregnancy. Diabetes Res. Clin. Pract. 103, 186–196
2. Guariguata, L. (2012) By the numbers: new estimates from the IDF Diabetes Atlas Update for 2012. Diabetes Res. Clin. Pract. 98, 524–525
3. Guariguata, L. (2013) Contribute data to the 6th edition of the IDF Diabetes Atlas. Diabetes Res. Clin. Pract. 101, 280–291
4. Kirland, K. A., Burrows, N. R., and Geiss, L. S. (2014) Diabetes interactive atlas. Prev. Chronic Dis. 11, 130500
5. Whiting, D. R., Guariguata, L., Weil, C., and Shaw, J. (2011) IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res. Clin. Pract. 94, 511–521
6. Janghorban, M., and Amini, M. (2011) Metabolic syndrome in first degree relatives of patients with type 2 diabetes: incidence and risk factors. Diabetes Metab. Syndr. 5, 201–206
7. Scott, R., Donoghoe, M., Watts, G. F., O’Brien, R., Pardy, C., Taskinen, M. R., Davis, T. M., Colman, P. G., Manning, P., Fulcher, G., Keech, A. C., and Investigators, F. S.; FIELD Study Investigators. (2011) Impact of metabolic syndrome and its components on cardiovascular disease events in 4900 patients with type 2 diabetes assigned to placebo in the FIELD randomised trial. Cardiovasc. Diabetol. 10, 102
8. Martell-Carros, N., Aranda, P., González-Albarrán, O., Dalfo-Baqué, A., Domínguez-Sardina, M., de la Cruz, J. J., Campo, C., and de Álvaro, C. (2013) Perception of health and understanding of cardiovascular risk among patients with recently diagnosed diabetes and/or metabolic syndrome. Eur. J. Prev. Cardiol. 20, 21–28
9. Park, H. K. (2015) Severe hypoglycemia and cardiovascular disease in Type 2 diabetes. Diabetes Metab. J. 39, 478–480
10. Song, S. H. (2016) Early-onset type 2 diabetes: high lifetime risk for cardiovascular disease. Lancet Diabetes Endocrinol. 4, 87–88
11. Jansson, S. P., Andersson, D. K., and Wårdhudda, K. (2016) Mortality and cardiovascular disease outcomes among 740 patients with new-onset Type 2 diabetes detected by screening or clinically diagnosed in general practice. Diabet. Med. 33, 324–331
12. Fridolf, T., Böttcher, G., Sundler, F., and Ahren, B. (1991) GLP-1 and GLP-1 (7-36) amide: influences on basal and stimulated insulin and glucagon secretion in the mouse. Pancreas 6, 208–215
13. Roth, K. A., Kim, S., and Gordon, J. I. (1992) Immunocytochemical studies suggest two pathways for enteroendocrine cell differentiation in the colon. Am. J. Physiol. 263, G174–G180
14. Eisele, R., Göke, R., Weichhardt, U., Fehmann, H. C., Arnold, R., and Göke, B. (1994) Glucagon-like peptide 1 immunoreactivity in gastrointestinal-pancreatic endocrine tumors: a light- and electron-microscopic study. Cell Tissue Res. 276, 571–579
15. Elahi, D., McAlonon-Dyke, M., Fukagawa, N. K., Meneilly, G. S., Sclater, A. L., Minaker, K. L., Habener, J. F., and Andersen, D. K. (1994) The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7-37) in normal and diabetic subjects. Regul. Pept. 51, 63–74
16. Göke, B., Herrmann, C., Göke, R., Fehmann, H. C., Berghöfer, P., Richter, G., and Arnold, R. (1994) Intestinal effects of alpha-glucosidase inhibitors: absorption of nutrients and enterohormonal changes. Eur. J. Clin. Invest. 24(Suppl 3), 25–30
17. Holst, J. J. (1994) Glucagonlike peptide 1: a newly discovered gastrointestinal hormone. Gastroenterology 107, 1848–1855
18. Brubaker, P. L., and Drucker, D. J. (2004) Minireview: glucagon-like peptides regulate cell proliferation and apoptosis in the pancreas, gut, and central nervous system. Endocrinology 145, 2633–2639
19. Drucker, D. J. (2005) Glucagon-like peptides: regulators of cell proliferation, differentiation, and apoptosis. Mol. Endocrinol. 17, 161–171
20. Nauck, M. A., Holst, J. J., Willms, B., and Schmiegel, W. (1997) Glucagon-like peptide 1 (GLP-1) as a new therapeutic approach for type 2 diabetes. Exp. Clin. Endocrinol. Diabetes 105, 187–195
21. Linn, T., Schneider, K., Göke, B., and Federlin, K. (1996) Glucagon-like-peptide-1 (7-36) amide improves glucose sensitivity in beta-cells of NOD mice. Acta Diabetol. 33, 19–24
22. Oshima, I., Yamamoto, C., and Shima, K. (1991) Half-disappearance time of endogenous GLP-1 immunoreactivity in man. Horm. Metab. Res. 23, 240–242
23. Tong, Y., Zhou, X. M., Wang, S. J., Yang, Y., and Cao, Y. L. (2009) Analgesic activity of myricetin isolated from Myrica rubra Sieb. et Zucc. leaves. Arch. Pharm. Res. 32, 527–533
24. Kwon, O., Eck, P., Chen, S., Corpe, C. P., Lee, J. H., Kruhlak, M., and Levine, M. (2007) Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. FASEB J. 21, 366–377
25. Strobel, P., Allard, C., Perez-Acute, T., Calderon, R., Aldunate, R., and Leighton, F. (2005) Myricetin, quercetin and catechin-gallate inhibit glucose uptake in isolated rat adipocytes but not glucose transport translocation. Biochem. Pharmacol. 51, 423–429
27. Manaharan, T., Ming, C. H., and Palanisamy, U. D. (2013) Syzygium aqueum leaf extract and its bioactive compounds enhances pre-adipocyte differentiation and 2-NBDG uptake in 3T3-L1 cells. Food Chem. 136, 354–363
28. Kandasamy, N., and Ashokkumar, N. (2014) Protective effect of bioflavonoid myricetin enhances carbohydrate metabolic enzymes and insulin signaling molecules in streptozotocin-cadmium induced diabetic nephrotic rats. Toxicol. Appl. Pharmacol. 279, 173–185
29. Ong, K. C., and Khoo, H. E. (2000) Effects of myricetin on glycemia and glycogen metabolism in diabetic rats. Life Sci. 67, 1695–1705
30. Lin, C. Y., Ni, C. C., Yin, M. C., and Lii, C. K. (2012) Flavonoids protect pancreatic beta-cells from cytokines mediated apoptosis through the activation of PI3-kinase pathway. Cytokine 59, 65–71
31. Sloop, K. W., Willard, F. S., Brenner, M. B., Ficorilli, J., Valasek, K., Showalter, A. D., Farb, T. B., Cao, J. X., Cox, A. L., Michael, M. D., Gutierrez-Sanfeliciano, S. M., Tebbe, M. J., and Coghlan, M. J. (2010) Novel small molecule glucagon-like peptide-1 receptor agonist stimulates insulin secretion in rodents and from human islets. Diabetes 59, 3099–3107
32. Wooten, D., Savage, E. E., Valant, C., May, L. T., Sloop, K. W., Ficorilli, J., Showalter, A. D., Willard, F. S., Christopoulos, A., and Sexton, P. M. (2012) Allosteric modulation of endogenous metabolites as an avenue for drug discovery. Mol. Pharmacol. 82, 281–290
33. Devigny, C., Perez-Balderas, F., Hoogeland, B., Caboni, S., Wachtel, R., Mauch, C. P., Webb, K. J., Deussing, J. M., and Hausch, F. (2011) Biomimetic screening of class-B G protein-coupled receptors. J. Am. Chem. Soc. 133, 8927–8933
34. Teng, M., Johnson, M. D., Thomas, C., Kiel, D., Lakis, J. N., Kercher, T., Aytes, S., Kostrowicki, J., Bhunmalark, D., Truesdale, L., May, J., Sidelman, U., Kodra, J. T., Jørgensen, A. S., Olesen, P. H., de Jong, J. C., Madsen, P., Behrens, C., Pettersson, I., Knudsen, L. B., Holst, J. J., and Lau, J. (2007) Small molecule ago-allosteric modulators of the human glucagon-like peptide-1 (hGLP-1) receptor. Bioorg. Med. Chem. Lett. 17, 5472–5478
35. Irwin, N., Flatt, P. R., Patterson, S., and Green, B. D. (2010) Insulin-releasing and metabolic effects of small molecule GLP-1 receptor agonist 6,7-dichloro-2-methylsulfonyl-3-N-tert-butylaminoquinoxaline. Eur. J. Pharmacol. 628, 268–273
36. Chen, D., Liao, J., Li, N., Zhou, C., Liu, Q., Wang, G., Zhang, R., Zhang, S., Lin, L., Chen, K., Xie, X., Nan, F., Young, A. A., and Wang, M. W. (2007) A nonpeptidic agonist of glucagon-like peptide 1 receptors with efficacy in diabetic db/db mice. Proc. Natl. Acad. Sci. USA 104, 943–948
37. Shim, K., Hirota, M., and Ohsboosi, C. (1988) Effect of glucagon-like peptide-1 on insulin secretion. Regul. Pept. 22, 245–252
38. Kreymann, B., Williams, G., Ghatei, M. A., and Bloom, S. R. (1987) Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet 2, 1300–1304
39. Okerson, T., and Chilton, R. J. (2012) The cardiovascular effects of GLP-1 receptor agonists. Cardiovasc. Ther. 30, e146–e155
40. Kieffer, T. J., McIntosh, C. H., and Pederson, R. A. (1995) Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. Endocrinology 136, 3585–3596
41. Ruiz-Grande, C., Pintado, J., Alarcón, C., Castillo, C., Valverde, I., and López-Novoa, J. M. (1990) Renal catabolism of human glucagon-like peptides 1 and 2. Can. J. Physiol. Pharmacol. 68, 1568–1573
42. Liu, Y., Krogl-Andersen, K., Pellecier, J., Marrote, H., Östenson, C. G., and Hammarström, L. (2016) Oral delivery of pentameric glucagon-like peptide-1 by recombinant Lactobacillus in diabetic rats. PLoS One 11, e0162733
43. Eldor, R., Kidron, M., Greenberg-Shushlav, Y., and Arbit, E. (2010) Novel glucagon-like peptide-1 analog delivered orally reduces post-prandial glucose excursions in porcine and canine models. J. Diabetes Sci. Technol. 4, 1516–1523
44. Harrah, J. M., Doherty, R. F., Beccher, G. R., Holden, J. M., Haytowitz, D. B., Bhagwat, S., and Gebhardt, S. (2006) Flavonoid content of U.S. fruits, vegetables, and nuts. J. Agr. Food Chem. 54, 9960–9977
45. Semwal, D. K., Semwal, R. B., Combrinck, S., and Viljoen, A. (2016) Myricetin: a dietary molecule with diverse biological activities. Nutrients 8, 90
46. Liu, I. M., Tseng, T. F., Liu, S. S., and Lan, T. W. (2007) Myricetin, a naturally occurring flavonol, ameliorates insulin resistance induced by a high-fructose diet in rats. Life Sci. 81, 1479–1488
47. Surwit, R. S., Kuhn, C. M., Cochrane, C., McCubbin, J. A., and Feinglos, M. N. (1988) Diet-induced type II diabetes in C57BL/6J mice. Diabetes 37, 1163–1167

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