Modulation of glucagon-like peptide-1 release by berberine: In vivo and in vitro studies

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A B S T R A C T S

Glucagon-like peptide (GLP)-1 is a potent glucose-dependent insulinotropic gut hormone released from intestinal L cells. Our previous studies showed that berberine increased GLP-1 secretion in streptozotocin-induced diabetic rats. The aim of this study was to investigate whether berberine affected GLP-1 release in normal rats and in NCI-H716 cells. Proglucagon and prohormone convertase 3 genes regulating GLP-1 biosynthesis were analyzed by RT-PCR. Effects of pharmacological inhibitors on berberine-mediated GLP-1 release were studied. In vivo, 5-week treatment of berberine enhanced GLP-1 secretion induced by glucose load and promoted proglucagon mRNA expression as well as L cell proliferation in intestine. In vitro, berberine concentration-dependently stimulated GLP-1 release in NCI-H716 cells. Berberine also promoted both prohormone convertase 3 and proglucagon mRNA expression. Chelerythrine (inhibitor of PKC) concentration-dependently suppressed berberine-mediated GLP-1 secretion. Compound C (inhibitor of AMPK) also inhibited berberine-mediated GLP-1 secretion. But only low concentrations of H89 (inhibitor of PKA) showed inhibitory effects on berberine-mediated GLP-1 release. The present results demonstrated that berberine showed its modulation on GLP-1 via promoting GLP-1 secretion and GLP-1 biosynthesis. Some signal pathways including PKC-dependent pathway were involved in this process. Elucidation of mechanisms controlling berberine-mediated GLP-1 secretion may facilitate the understanding of berberine's antidiabetic effects.

1. Introduction

Glucagon-like peptide-1 (GLP-1) is a gut-derived hormone secreted from intestinal L cells in response to nutrient ingestion. It is produced by a tissue-specific post-translational process of its precursor proglucagon peptide by the prohormone convertase 3 [1]. GLP-1 exerts important effects on regulating glucose metabolism, stimulating glucose-dependent insulin secretion, promoting beta-cell proliferation, as well as inhibiting glucagon release, gastric emptying and food intake [2]. The physiological properties which GLP-1 possesses make it a subject of intensive investigation as a potential treatment of diabetes mellitus.

Berberine ($[C_{20}H_{18}NO_4]^+$), a major active constituent of Rhizoma coptidis, has been successfully used for treating diabetes [3]. It was reported that berberine may stimulate glucose uptake [4], modulate lipids metabolism and scavenge free radical [5], enhance insulin sensitivity and stimulate insulin secretion [6]. However, its hypoglycemic mechanism remains unclear because of its poor absorption and low concentration in plasma. Recently, we reported that complex of Rhizoma coptidis Huang-Lian-Jie-Du-Tang [7] and its bioactive compound berberine [8] may increase GLP-1(7–36) amide secretion in STZ-induced diabetic rats, accompanied by increase of insulin levels, which indicated that enhancement of GLP-1 release may contribute to beneficial effects of berberine on diabetes mellitus.

The NCI-H716, an enteroendocrine cell line, was derived from cells present in ascites fluid of a 33-year-old Caucasian male with poorly differentiated adenocarcinoma of the colon after treatment with 5-fluorouracil [9]. The cell line was described to display some endocrine feature [10,11], including secretory granules and chromogranin A. Several neurohormonal receptors such as muscarinic receptors [12], leptin receptors [13] and Gαs-coupled receptor [14] were identified on the cell line. The NCI-H716 cell

line highly expressed GLP-1 and was widely used as a unique human model to study the regulation of GLP-1 secretion [15]. GLP-1 secretion from NCI-H716 cells is regulated by multiple extracellular secretagogues through some signal pathways [16].

The aim of this study was to investigate: (1) whether berberine treatment also increased GLP-1 secretion in normal rat; (2) berberine-mediated GLP-1 secretion in NCI-H716 cells; and (3) some intracellular signaling pathways involved in regulation of GLP-1 release induced by berberine. It is expected to obtain an improved understanding of the mechanism regulating GLP-1 secretion by berberine using NCI-H716 cells, thereby providing an alternative prospect to the use of berberine in the treatment of diabetes mellitus.

2. Materials and methods

2.1. Materials

Berberine (purity: 98%) was purchased from Nanjing Qingze Pharmaceutical Technology Ltd. CO. (Nanjing, China). Pentobarbital was bought from Sigma Chemical Co. (St. Louis, MO, USA). Both DPP-IV inhibitor and GLP-1 active ELISA kit were purchased from Linco Research (St. Charles, MI, USA). Primers for proglucagon gene, PC 3 gene and DNA Taq polymerase used in RT-PCR were provided by Xinlun Biotechnical Laboratory (Shanghai, China). Rabbit anti-GLP-1 antibody was purchased from Phoenix Pharmaceuticals (Mountain View, CA, USA). Primary antibody of insulin was obtained from Maixin Biotechnical Company (Fuzhou, China). H89 was purchased from Beyotime Institute of Biotechnology (Shanghai, China). Chelerythrine and Compound C were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Bovine serum alume (BSA) was obtained from Amresco Co. (Solon, OH, USA). Both RPMI 1640 and DMEM were purchased from Invitrogen Co. (Carlsbad, CA, USA). Matrigel was bought from Becton Dickinson Biosciences (Bedford, MA, USA). Fetal bovine serum (FBS) was bought from PAA Laboratory (Chicago, IL, USA).

2.2. Animals

Male Sprague–Dawley rats (180–220 g), purchased from B&K Universal Group Ltd. (Shanghai, China), were used in the study and housed under controlled room of humidity ($50 \pm 5\%$) and temperature ($23 \pm 1\,°C$) with a 12 h light/12 h darkness cycle. They were fed commercial stock diet and water ad libitum. The studies were approved by the Animal Ethics Committee of China Pharmaceutical University.

2.3. Berberine treatment and sample collection

Rats were acclimated for 3 days before the experiment and divided into three groups randomly. One group served as control group, received only vehicle. The other two groups served as berberine-treated groups, which orally received low dose (60 mg/kg) or high dose (120 mg/kg) of berberine once a day for 5 weeks. During the treatment, fasted blood glucose (FBG) was monitored once a week, both body weight and food intake was monitored once a day.

On day 35 of the treatment, rats were fasted for 12 h. Two hours after last treatment, portal vein cannulation was performed according to the method described previously [17] under sodium pentobarbital (60 mg/kg, i.p.) anesthesia. Then, the rats orally received 2.5 g/kg of glucose. Portal blood (300 μL) was collected in tube containing EDTA and DPP-IV inhibitor (10 μl/ml) via the cannula before and subsequently at 10, 20 and 30 min following glucose load. Plasma samples were obtained and stored at $-80\,°C$ for assessing GLP-1(7–36) amide and insulin. Then the rats were immediately sacrificed, the pancreas, segments of distal ileum (4 cm

above the junction with the caecum) and proximal colon (4 cm of intestine below the junction with the caecum) were obtained and immersed in liquid nitrogen, and stored at $-80\,°C$ for peptide analysis. Parts of tissues were used for immunohistochemistry and RT-PCR analysis. GLP-1 and insulin measurement, immunohistochemistry and RT-PCR were performed as described previously [7].

2.4. Cell culture

Human NCI-H716 cells were obtained from the American Type Culture Collection (Manassas, USA). Cells were grown in suspension at $37\,°C$, 5% CO_2. The culture medium was RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin. Endocrine differentiation was induced by seeding cells in dishes coated with Matrigel, in high-glucose DMEM, 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin [15].

2.5. Effect of berberine on secretion of GLP-1 from NCI-H716 cells

Two days before the experiments, 1.5×10^6 cells were seeded in 12-well culture plates coated with Matrigel and containing high-glucose DMEM, 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin. On the day of the experiment, medium was replaced by Krebs–Ringer bicarbonate buffer (KRB) buffer (128.8 mmol/l NaCl, 4.8 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgSO_4$, 2.5 mmol/l $CaCl_2$, 5 mmol/l $NaHCO_3$, and 10 mmol/l HEPES, pH 7.4) [18] containing 0.2% BSA and different concentrations of berberine (0 μM, 1 μM, 10 μM or 100 μM). Following incubating at $37\,°C$ for 2 h, the supernatants were collected with the addition of 50 μg/ml phenylmethylsulfonyl fluoride and stored at $-80\,°C$ for analysis. The cells were scraped off and sonicated in a homogenization buffer (1 M HCl containing 5% formic acid, 1% trifluoroacetic acid and 1% NaCl). GLP-1(7–36) amide in supernatant and cells was measured by a GLP-1 active ELISA kit, respectively, according to the manufacturer's protocol. Protein content of the cells was determined using the Bradford protein assay. The GLP-1 content was normalized for the total protein of the cells. At the same time, the cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay [19]. No damage in cells was found for all the tested agents within tested concentrations.

2.6. RT-PCR analysis of proglucagon mRNA and prohormone convertase 3 mRNA

A total of 3×10^6 cells were seeded in 6-well culture plates coated with Matrigel and incubated for 24 h. Medium was replaced by serum-free medium containing 0.2% BSA without or with 100 μM berberine. The cells were re-incubated for 24 h and were washed with cold-PBS and stored at $-80\,°C$ for RT-PCR analysis.

Total RNA was isolated from each well with Trizol reagent (Invitrogen Co., USA). RT-PCR was performed according to the protocol provided with the TwoStep RT-PCR kit (BestBio, Shanghai, China). The sequences of the forward and reverse primers were: 5′-GTAATGCTGGTACAAGGCAG-3′ and 5′-TTATAAAGTCCCTGGCGGCA-3′ for the proglucagon gene, 5′-CGCTGACCTGCACAATGACT-3′ and 5′-CAGACAACCAGGTGCTGCAT-3′ for prohormone convertase 3 gene, 5′-GCTGAGAACGGGAAGCTTGT-3′ and 5′-TCTCCATGGTGGT-GAAGACG-3′ for internal control GAPDH gene. After denaturing at $94\,°C$ for 5 min, the amplification was obtained by 33 cycles of $94\,°C$ for 30 s, $59\,°C$ for 30 s and $72\,°C$ for 30 s each. A final extension step at $72\,°C$ for 5 min was performed. 27 cycles were performed for the amplification for GAPDH gene. PCR products were subjected to electrophoresis on 2.5% agarose gel, and visualized by means of ethidium bromide staining. Densitometric quantification was

recorded using Jeda image analysis system 3.3 (Jiangsu Jeda Science-Technology Co. Ltd., Nanjing, China).

2.7. Effect of pharmacological inhibitors on berberine-mediated GLP-1 release

Differentiated cells were starved in FBS-free DMEM containing 0.2% BSA for 1.5 h, then incubated with KRB containing 0.2% BSA and 100 μM berberine co-administrated with pharmacological inhibitors chelerythrine (0.4 μM, 2 μM and 10 μM), H89 (2 μM, 10 μM and 50 μM) or Compound C (2 μM, 10 μM and 50 μM) for 2 h. GLP-1(7–36) amide in supernatant and cells was measured as described above. Effects of the pharmacological inhibitors (2 μM chelerythrine, 10 μM H89 and 10 μM Compound C) on GLP-1 secretion were also investigated.

2.8. Statistical analysis

Results were expressed as mean ± standard error (S.E.M.). Statistical differences among groups were evaluated by one-way of analysis of variance (ANOVA). If analysis was significant, the differences between groups were estimated using Student–Newman–Keuls multiple comparison post hoc test. A p value of less than 0.05 indicated a significant difference.

3. Results

3.1. Effects of berberine treatment on body weight, food intake and blood glucose concentration of normal rats

Five-week berberine treatment did not affect body weight (data not shown), but may decrease FBG levels (Fig. 1A) and food intake (Fig. 1B) of normal rats, significant decreases were found in rats treated with high dose of berberine (120 mg/kg).

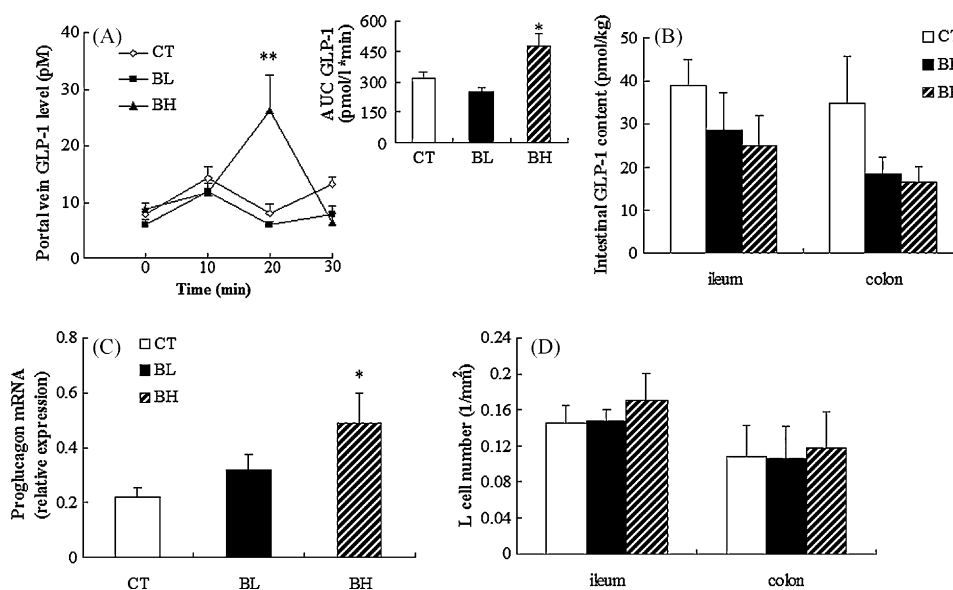### 3.2. Effects of berberine treatment on insulin level

Plasma insulin levels were measured following glucose load (Fig. 2A). It was found that glucose load may induce increase of insulin level in plasma, the peak concentration occurred at 10 min. High dose of berberine showed a trend to increase plasma insulin level, but no statistical difference was found.
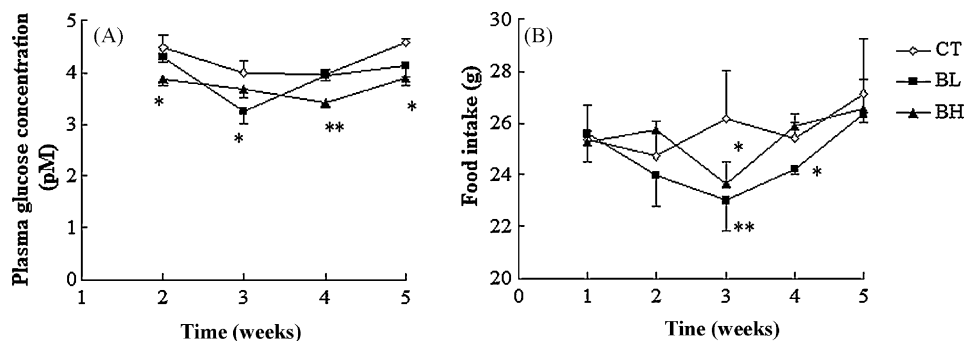
Fig. 1. Effects of berberine treatment on fasted blood glucose concentration (A) and food intake (B) during the 5 weeks of treatment. Rats received vehicle (◇, CT) and berberine at 60 mg/kg (■, BL) and 120 mg/kg (▲, BH), respectively. Values are expressed as mean ± S.E.M. (n = 5–7). *p < 0.05, **p < 0.01 vs. CT rats.
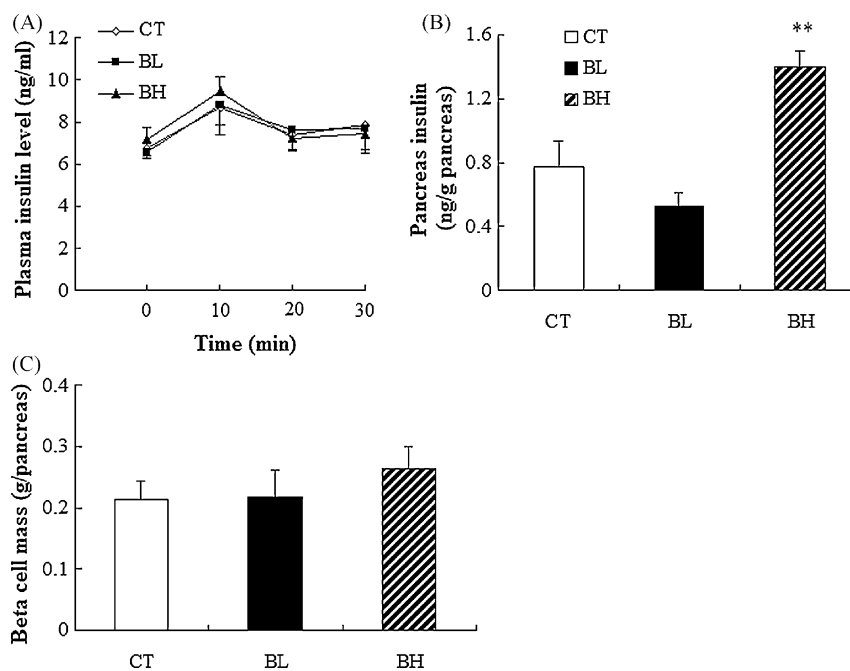
Fig. 2. Effects of 5-week berberine treatment on portal vein insulin level (A), pancreas insulin (B) and beta-cell mass (C) in normal rats. Rats received vehicle (◇, CT) and berberine at 60 mg/kg (■, BL) and 120 mg/kg (▲, BH), respectively. Plasma samples were collected before and 10, 20 and 30 min following glucose load. Pancreas samples were obtained at 30 min after glucose load. Insulin level was assessed by ELISA. Beta-cell proliferation was presented by beta-cell mass, detected by immunohistochemistry technique. Data are means ± S.E.M. (n = 4–5), **p < 0.01 vs. CT rats.

Fig. 3. Effects of 5-week berberine treatment on GLP-1(7–36) amide in normal rats. (A) Portal vein GLP-1 level, area under concentration–time curves from 0 to 30 min (AUC$_{30}$) was calculated, (B) GLP-1 content in intestinal tract of normal rats, (C) proglucagon mRNA expression in ileum, and (D) intestinal L cell proliferation. Rats received vehicle (□, CT) and berberine at 60 mg/kg (■, BL) and 120 mg/kg (▲, BH), respectively. Plasma samples were collected before and 10, 20 and 30 min following glucose load. Intestinal samples were obtained at 30 min after glucose load. GLP-1(7–36) amide level was assessed by ELISA. Proglucagon mRNA expression was operated using RT-PCR and L cell proliferation was estimated by immunohistochemistry. Data are means ± S.E.M. (n = 4–5), *p < 0.05, **p < 0.01 vs. CT rats.

The pancreas insulin levels were measured at 30 min after glucose load. It was found that high dose of berberine significantly increased pancreas insulin level, which induced a 2-fold increase compared with control rats (Fig. 2B, p < 0.01). However, low dose of berberine treatment showed a trend to decrease pancreas insulin level, although no significance was found.

The beta-cell volume density corresponds to the ratio of insulin immunoreactivity area to pancreatic parenchymal area. The beta-cell mass was calculated by multiplying the beta-cell volume density by the weight of the pancreas [20]. The data from immunohistochemistry showed a trend to increase beta-cell mass in berberine-treated rats (Fig. 2C).

3.3. Effects of berberine treatment on GLP-1(7–36) amide in normal rats

GLP-1 levels in portal plasma were investigated after glucose load (Fig. 3A) and area under concentration–time curves from 0 to 30 min (AUC$_{30}$) was estimated using linear trapezoidal rule. It was found that glucose load may induce GLP-1 release. High dose of berberine (120 mg/kg) may enhance GLP-1 release induced by glucose load, peak level occurred at 20 min, accompanied by higher values of AUC$_{30}$ (478.15 ± 54.60 pM·min in rats treated with 120 mg/kg berberine vs. 320.40 ± 27.08 pM·min in control rats, p < 0.05). However, low level of GLP-1 was observed in rats treated with 60 mg/kg berberine, even lower than that of control rats.

GLP-1(7–36) amide in ileum and colon was measured at 30 min after glucose load (Fig. 3B). Lower GLP-1 levels were found in both ileum and colon of rats treated with berberine.

It was found that berberine treatment may increase proglucagon mRNA expression in ileum in a dose-dependent manner, 60 mg/kg and 120 mg/kg of berberine treatment may induce 1.5-fold increase and 2.3-fold increase compared with control rats, respectively (Fig. 3C). A trend of increase number of GLP-1-positive L cells in intestine was found in berberine-treated rats (Fig. 3D), and no morphologic changes in intestinal L cells were observed between CT rats and berberine-treated rats (Fig. 4A–F).

3.4. Effects of berberine on GLP-1 secretion in NCI-H716 cells

To determine the effect of berberine on GLP-1 secretion, NCI-H716 cells were incubated with KRB in presence of different concentrations of berberine (0 μM, 1 μM, 10 μM and 100 μM) for 2 h. GLP-1 levels in both medium (secreted) and cells were measured (Fig. 5). It was found that berberine treatment may increase GLP-1 secretion (medium) in a concentration-dependent manner (Fig. 5A), which GLP-1 level increased from basal level 0.34 ± 0.02 pM/μg protein to 0.51 ± 0.03 pM/μg protein in presence of 100 μM berberine. Berberine also increased cellular GLP-1 level, significant increases were found in cells treated with 100 μM berberine (Fig. 5B, p < 0.01).

3.5. Effects of berberine on proglucagon mRNA and prohormone convertase 3 mRNA expression in NCI-H716 cells

NCI-H716 cells were treated with 100 μM berberine for 24 h, proglucagon mRNA and prohormone convertase 3 mRNA, which controlled biosynthesis of GLP-1 were investigated using RT-PCR analysis. Data from RT-PCR showed that berberine treatment may increase both proglucagon mRNA expression and prohormone convertase 3 mRNA expression, which induced 1.5-fold increase for the two genes compared with cells without berberine treatment (Fig. 6).

3.6. Effects of pharmacological inhibitors on berberine-mediated GLP-1 release in NCI-H716 cell

To determine whether some cell signaling pathways were involved in the regulation of berberine-mediated GLP-1 secretion, NCI-H716 cells were incubated in presence of 100 μM berberine co-administrated with different pharmacological inhibitors. It was found that chelerythrine inhibited berberine-mediated GLP-1 release in a dose-dependent manner, and presence of 10 μM chelerythrine resulted in 50% decrease of GLP-1 release (p < 0.01, Fig. 7A). Compound C also inhibited berberine-mediated GLP-1 release within the concentration range tested, but no concentration-dependent manner was found. Only low concentrations of
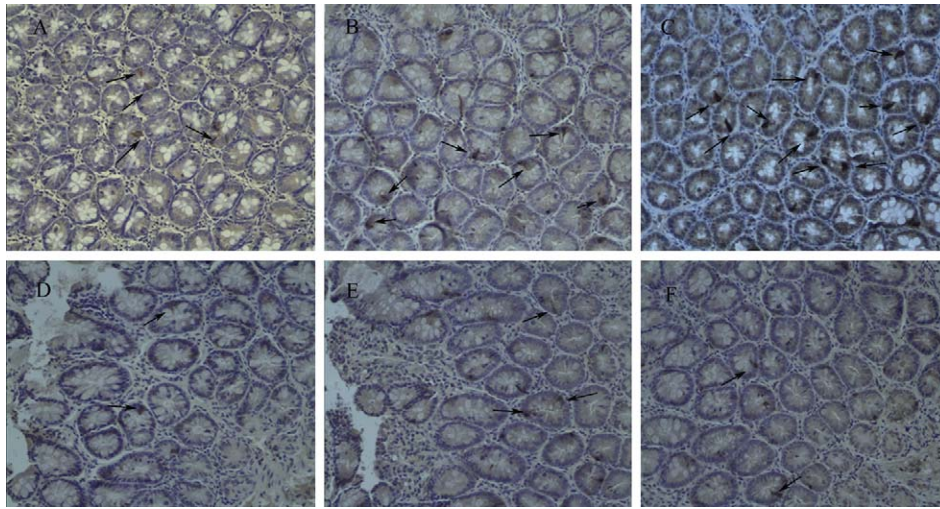
Fig. 4. The morphology of immunohistochemistry about intestinal L cell (final magnification 200×). (A)–(C) are sections from ileum, CT, BL and BH rats respectively; (E)–(G) are sections from colon, CT, BL and BH rats respectively. Rats received vehicle (CT) and berberine at 60 mg/kg (BL) and 120 mg/kg (BH) for 5 weeks, respectively. Intestinal samples were obtained at 30 min after glucose load.
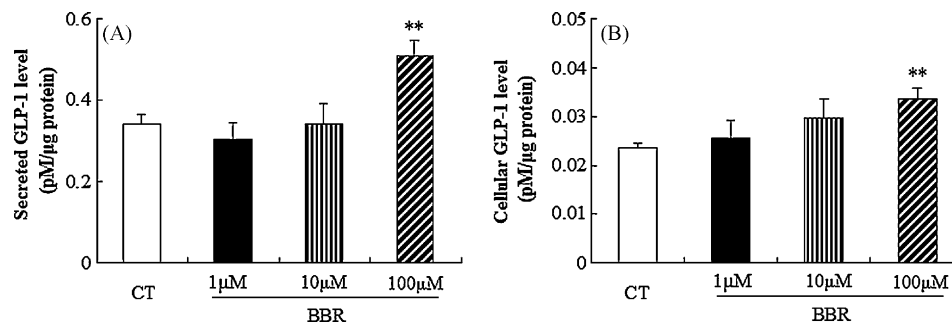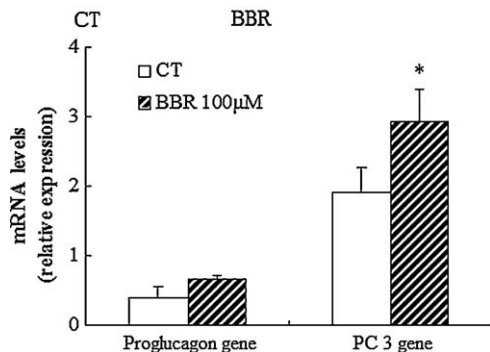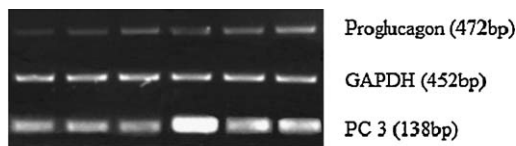

Fig. 5. Release of GLP-1(7–36) amide from NCI-H716 cells in response to 2-h incubation with medium alone, berberine (1 μM, 10 μM, and 100 μM). (A) Secreted GLP-1 level, (B) cellular GLP-1 level. GLP-1 concentration was measured by ELISA. Data are means ± S.E.M. ($n = 5$), $^{**}p < 0.01$ vs. CT.

H89 (2 μM and 10 μM) may suppress berberine-mediated GLP-1 secretion. In contrast, high concentration (50 μM) of H89 showed a trend to enhance berberine-mediated GLP-1 secretion.

Effects of the pharmacological inhibitors themselves on GLP-1 release were investigated. The results demonstrated that similarly


Fig. 6. Proglucagon gene and prohormone convertase 3 (PC3) gene expression in NCI-H716 cells. Cells were treated with medium alone (CT) and berberine (100 μM, BBR) for 24 h. Proglucagon and PC3 mRNA levels were operated using RT-PCR. Data are means ± S.E.M. ($n = 5$), $^{*}p < 0.05$ vs. CT.

to berberine, tested concentrations (2 μM chelerythrine, 10 μM H89 or 10 μM Compound C) of the pharmacological inhibitors themselves may induce GLP-1 release, significant increase were found in H89-treated cells and Compound C-treated cells. However, co-administration of the same concentration of the pharmacological inhibitors and berberine may significantly suppress GLP-1 release (Fig. 7B).

4. Discussion

Berberine has been widely used as an antidiabetic agent, but its mechanism of action is still obstacle because of poor absorption and low concentration in plasma. Our previous studies showed that berberine may increase GLP-1 secretion in experimental diabetic rats [8], suggesting that berberine showed antidiabeitc effect partly via promoting GLP-1 secretion. The present study was focused on extending the previous findings and further providing direct evidences using in vivo and in vitro experiments.

In vivo studies showed that berberine treatment may lower fasted blood glucose, accompanied by increase of insulin levels in plasma and pancreatic tissue, as well as beta-cell mass. 120 mg/kg of berberine treatment may significantly increase GLP-1 release induced by glucose load, accompanied by higher AUC$_{30}$ of GLP-1 in portal plasma. Peripherally released GLP-1 enter brain areas and participate in the regulation of anorexic response [21], which indicated that suppression of food intake induced by berberine may partly due to enhancement of GLP-1 release. It was well-known that GLP-1 exerts important effects on regulating glucose
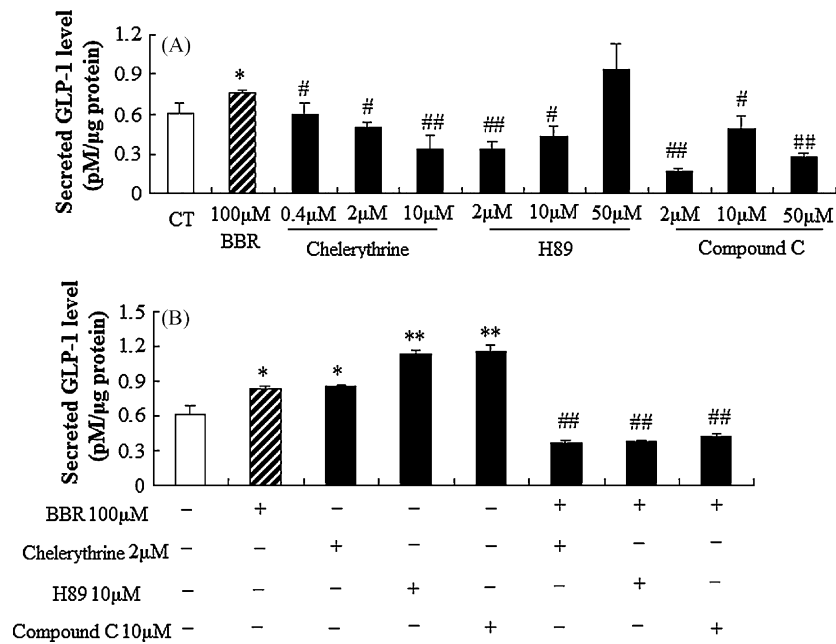
Fig. 7. (A) Effects of berberine (100 μM) with chelerythrine (0.4 μM, 2 μM, and 10 μM), H89 (2 μM, 10 μM, and 50 μM) and Compound C (2 μM, 10 μM, and 50 μM) on GLP-1 secretion in NCI-H716 cells. (B) Effects of chelerythrine (2 μM), H89 (10 μM) and Compound C (10 μM) with or without berberine on GLP-1 secretion in NCI-H716 cells. GLP-1 concentration is measured by ELISA. Data are means ± S.E.M. (n = 5), $^*p < 0.05$, $^{**}p < 0.01$ berberine vs. CT. $^\#p < 0.05$, $^{\#\#}p < 0.01$ vs. BBR.

homeostasis via stimulating insulin secretion, beta-cell proliferation, inhibiting food intake [2]. All these results further supported our previous findings that berberine showed its antidiabetic effects partly via increasing GLP-1 release [8]. It was noticed that berberine promoted proglucagon mRNA expression and L cell proliferation in intestine of normal rats as well as diabetic rats [8]. It is known that proglucagon gene is precursor gene of GLP-1. These findings further indicated the possibility of enhancement of GLP-1 biosynthesis by berberine.

The present studies also showed that GLP-1 content in ileum and colon of berberine-treated rats at 30 min following glucose load was lower than that in control rats, although increase of proglucagon mRNA expression and L cell proliferation was showed. The mechanism resulting in this phenomenon was unclear. These decreases may partly come from large amount of GLP-1 release from tissue into blood, which was in agreement with lower level of GLP-1 in portal plasma at 30 min after glucose load. Similar phenomenon was found in previous result [8].

NCI-H716 cells were served as in vitro models of the intestinal L cell to further investigate the effect of berberine on regulating GLP-1 release. In vitro study demonstrated that berberine may stimulate GLP-1 secretion from the NCI-H716 cells in a dose-dependent manner, accompanied by increased both cellular GLP-1 level and total GLP-1 content, which indicated that berberine may increase GLP-1 release as well as promote GLP-1 biosynthesis.

Effects of berberine on proglucagon gene and prohormone convertase 3 gene in NCI-H716 cells, which regulate GLP-1 biosynthesis, were further investigated to verify the deduction. Data from RT-PCR analysis showed that berberine treatment may promote proglucagon mRNA expression, which supported in vivo results. GLP-1 is derived from tissue-specific post-translational processing of the proglucagon peptide by prohormone convertase 3 in L cells [1,22]. The present study also showed that berberine treatment may up-regulate expression of prohormone convertase 3 mRNA. These findings indicated that up-regulation of the two upstream genes may be one of the reasons that berberine promoted GLP-1 biosynthesis.

Some reports showed that the PKC-dependent pathways were involved in the regulation of GLP-1 secretion in NCI-H716 cells [15,23]. Our present study showed that chelerythrine, inhibitor of PKC-dependent pathway, suppressed berberine-mediated GLP-1 secretion in NCI-H716 cells in a concentration-dependent fashion. No study to date reported on the direct interaction between berberine and PKC pathway in GLP-1 secretion. A study using cultured human liver cell model showed that berberine-induced insulin receptor expression via activating the PKC-dependent pathway [24], which indicated that berberine was connected with PKC-dependent pathway, to some extend at least. These findings revealed that activation of PKC-dependent pathway may be involved in regulation of GLP-1 secretion mediated by berberine, although a report showed inhibitory effect of berberine on the activation of PKCα using glioma cells [25]. Chelerythrine itself stimulated GLP-1 release, which indicated that PKC-mediated regulation of GLP-1 secretion from the intestinal L cell is complex process, multiple isoforms of PKC may be involved in. It was reported that PKCζ was required in oleic acid-induced GLP-1 secretion from GLUTag L cells and primary rat intestinal L cells [23]. However, our results still provided a clue to the involvement of PKC pathway in berberine-induced GLP-1 secretion.

cAMP-dependent GLP-1 secretion was observed in the human, murine and rat L cell [8,26,27], but whether this was regulated through a PKA-dependent pathway has not been identified [16]. In our study, the effect of H89 (PKA inhibitor) on berberine-mediated GLP-1 secretion in NCI-H716 was ambiguous. Although a report showed that 10 μM H89 may suppress glucose-induced GLP-1 release in GLUTag cells [28], the present study gave a contrast result that 10 μM H89 itself enhanced GLP-1 release in NCI-H716. We also found that low concentrations (2 μM or 10 μM) of H89 significantly inhibited the berberine-mediated GLP-1 secretion from NCI-H716 cells. However, the high concentration (50 μM) of H89 showed enhanced berberine-mediated GLP-1 secretion. H89 is marketed as a selective and potent inhibitor of PKA, but its mode of specific inhibition of PKA is still unclear. There was reports that H89 inhibited at least 8 other kinases [29].

AMPK is a major intermediate in facilitating the beneficial effects of berberine [30,31]. Several reports showed that AMPK lay on upstream of p38 MAPK [4] and MAPK pathway is involved in GLP-1 secretion in NCI-H716 cells [16]. We supposed that there exist some correlations among berberine, AMPK and GLP-1. As results in the present study showed that Compound C, inhibitor of AMPK-dependent pathway, inhibited berberine-mediated GLP-1 secretion in NCI-H716 cells, no concentration-dependent response was found. Similarly to two other pharmacological inhibitors chelerythrine and H89, Compound C-induced GLP-1 release suggested the effect of AMPK on GLP-1 secretion was also complex process.

The main finding in the present study was that berberine modulated GLP-1 release and biosynthesis in rats, which were supported by data from NCI-H716 cells. Berberine enhanced GLP-1 biosynthesis partly via up-regulating both proglucagon and prohormone convertase 3 gene expression; and PKC-dependent pathways was involved in GLP-1 release induced by berberine although roles of other signal pathways were not excluded. Understanding the mechanisms controlling berberine-mediated GLP-1 alteration allowed development of recognition of poor absorbed berberine exerts good effects on hyperglycemia.

Acknowledgements

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