

Sennoside A induces GLP-1 secretion through activation of the ERK1/2 pathway in L-cells

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Research

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Abstract

Background Glucagon-like peptide-1 (GLP-1) is secreted from the intestinal L-cells to stimulate insulin secretion in the control of blood glucose. Sennoside A (SA), derived from Rhubarb extract of traditional Chinese medicine, is often used to treat constipation and lose weight. Our previous study suggests that SA can increase the plasma GLP-1 level in a mouse model of type 2 diabetes. However, the mechanism of SA activity remains unknown. This issue was addressed in this study.

Methods C57bl/6 mice were divided randomly into four groups at n = 12. Group one was used as a control group without drug treatment. The other three groups were treated with (SA) at three dosages: a low dose (15 mg/kg/day), medium dose (30mg/kg/day) or high dose (45 mg/kg/day). SA was delivered into the mice through drinking water. Bodyweight was monitored. After treatment, blood glucose was assayed by OGTT. Plasma GLP-1 and insulin were determined at 15 mins of oral glucose challenge. Colon tissues were collected for mRNA or western blot analysis. Immunofluorescence staining assays was used to evaluate the number of β -cells and L-cells. NCI-H716 cells were employed to investigate the mechanism of SA-induced GLP-1 secretion, and the cells were subjected to western blot analysis. In the study of extracellular signal-regulated kinases 1/2 (ERK1/2) pathway, NCI-H716 cells were pretreated with ERK1/2 inhibitors (PD98059, 50 μ M) for 30 min in the presence of SA (100 μ M).

Results In the current study, SA can reduce body weight during 5 weeks of weight monitoring and improve OGTT in C57BL/6 mice on the Chow diet. Furthermore, plasma GLP-1 was significantly elevated in the mouse treated by SA at the dosage of 45 mg/kg/day. The SA activity was supported by improving glucose-induced insulin secretion. Meanwhile, increased expression of EKR1/2 and prohormone convertase 1/3 (PC1/3) proteins was observed in the large intestine of SA-treated mice. The number of L-cells was not altered in each group. In the NCI-H716 cells, GLP-1 secretion was induced by SA with activation of the ERK1/2 pathway and elevation of PC1/3 protein. The SA effect was blocked by the ERK1/2 inhibitor. These data suggest that SA induced GLP-1 secretion in L-cells through activation of the ERK1/2 pathway in the mouse intestine.

Conclusion Our study provides direct evidence that SA interacts with L cells for GLP-1 secretion. The data suggest that the SA effect is dependent on the ERK1/2 signaling pathway. Therefore, SA is a new drug candidate for the treatment of type 2 diabetes by induction of GLP-1 secretion.

1. Introduction

Glucagon-like peptide-1 (GLP-1) is encoded by the proglucagon (GCG) gene in the intestinal L cells. GLP-1 secretion is induced by nutrients in the control of blood glucose and food intake [1]. GLP-1 reduces blood glucose through multiple mechanisms, which include the promotion of glucose-induced insulin secretion from the pancreatic β cells. A decrease in plasma GLP-1 contributes to the hyperglycemia in type 2 diabetes (T2DM) [2–4]. The induction of GLP-1 activity is an effective strategy in the treatment of T2DM with a variety of GLP-1 analogues. The GCG gene encodes the hormone glucagon in the pancreatic α -

cells, a hormone elevated in the fasting condition for the induction of gluconeogenesis. The GCG gene products, GLP-1, and glucagon are derived from alternate posttranslational processing of prohormone by convertase PC1/3 in L-cells, and convertase PC2 in α -cells, respectively [5]. The active form of GLP-1 (7–36 amide) is inactivated by Dipeptidyl peptidase-4 (DPP4) through peptide degradation to an inactive form (9–36 amide) [6, 7]. The active form of GLP-1 is essential in the control of blood glucose [8, 9].

Sennoside A (SA), a major active constituent of *Rhizoma Coptis*, has been widely used in the treatment of constipation. SA is also used in the treatment of obesity for its activity in the reduction of energy absorption through an acceleration of intestine transmits [10, 11]. SA was reported to restore GLP-1 level in the diet-induced obese (DIO) mice, through an improvement of the gut microbiota profile, the short-chain fatty acids (SCFAs) levels, the mucosal structure and mitochondrial function in the colon of obese mice [12]. Also, SA improved hepatic steatosis with an impact in mitochondrial structure and function [13]. These studies suggest that SA may control obesity through the restoration of GLP-1 level in the obese mice. However, the effect of SA activity remains to be characterized in the regulation of GLP-1. SA has a very low ability to enter the bloodstream [14].

In this study, SA was tested in the regulation of GLP-1 secretion in lean mice fed on the Chow diet. SA was found to induce GLP-1 secretion from L-cells through activation of the ERK1/2 pathway. The results suggest that SA may directly act on L-cells to induce GLP-1 secretion.

2. Materials And Methods

2.1 Chemicals and reagents

Sennoside A (98% in purity) was purchased from Desite biotechnology CO. Ltd. (Chengdu, China). The antibodies to GCG (ab200474, Abcam), PC1/3 (ab220363, Abcam), pERK1/2 (Thr202/Tyr204) (4370T, Cell Signaling Technology), ERK1/2 (4370S, Cell Signaling Technology) were purchased from the China Divisions of the corresponding companies. The ERK1/2 inhibitor PD98059 was purchased from MCE (HY-12028, shanghai, China). Other chemicals were purchased from Sigma-Aldrich Co. Ltd. (Shanghai, China) unless stated otherwise.

2.2 Animals

Male C57BL/6 mice (6-week-old, SPF grade) were purchased from the Shanghai Xipuer Bikai Laboratory Animal Co. Ltd. (Shanghai, China). The mice were kept in the animal facility of Shanghai Jiao tong University with controlled temperature (22 ± 2 °C), humidity ($60 \pm 5\%$) and a 12-h dark/light cycle. They were fed on Chow diet ad libitum in the study. The mice were divided randomly into four groups at $n = 12$. Group one was used as a control group without drug treatment. The other three groups were treated with Sennoside A (SA) at three dosages: a low dose (15 mg/kg/day), medium dose (30 mg/kg/day) or high dose (45 mg/kg/day). SA was delivered into the mice through drinking water. The treatment was administrated for five weeks. At the end of treatment, the mice were subject to tissue collection under anesthesia following an intraperitoneal injection of sodium pentobarbital (35 mg/kg). The procedures

were approved by the Institutional Animal Care and Use Committee (IACUC) of the Shanghai Jiao tong University.

2.3 Oral glucose tolerance test (OGTT), plasma GLP-1 and insulin assay

OGTT was conducted in the mice after overnight (12 hrs.) fasting. Glucose was administrated into the mice at 2 g/kg body weight by oral gavage in the OGTT assay. Plasma GLP-1 was determined in the mice using the ocular vein blood collected with heparin tubes containing dipeptidyl peptidase IV inhibitor (10 µl/mL), which was conducted at 15 min after the glucose administration. The plasma was obtained by centrifugation of the blood at 3000 g and stored at -80 °C before the GLP-1 assay. Plasma GLP-1 and insulin were determined individually using corresponding ELISA kits according to the manufacturer's instructions. The biologically active form of GLP-1 (7–36) and insulin were measured with the GLP-1 ELISA Kit (BMS2194, Invitrogen, CA, USA) and insulin ELISA Kit (90080, Crystal Chem, Downers Grove, USA).

2.4 Immunofluorescence staining

In the tissue collection, the proximal colon was excised, washed with ice-cold saline, and stored at -80 °C. Pancreas and colon tissues were also collected and fixed in 4% paraformaldehyde for analysis of β-cells and L-cells with immunofluorescence assays. The antibody-based staining was performed to determine the numbers of L cells in the colonic tissue and β-cells in the pancreas. Briefly, the tissue slides were deparaffinized, rehydrated, and subjected to antigen retrieval with EDTA (pH 9.0). The slides were preincubated with 0.5% bovine serum albumin (BSA) to block the nonspecific interaction between antibody and samples. The GLP-1 antibody (diluted 1:800, Servicebio, Shanghai, China) and the insulin antibody (diluted 1:200, Servicebio, Shanghai, China) were applied to the slide for overnight at 4 °C, respectively. The slides were incubated with a secondary antibody (diluted 1:400, Servicebio, Shanghai, China) at room temperature for 50 min after washing out the first antibody. The nuclei were stained with DAPI for 10 min at room temperature to determine the cell density. The fluorescent images were captured under a fluorescent microscope (Olympus, Tokyo, Japan).

2.5 Cell culture

Human NCI-H716 cells were obtained from the Cell Bank of the China Science Academy (Shanghai, China). The cells were grown in suspension at 37.8 °C, 5% CO₂. The culture medium was RPMI 1640 (11875-093, Gibco, USA) supplemented with 10% FBS, 2 mM L-glutamine, 100 IU/mL penicillin, and 100 mg/mL streptomycin. Cell adhesion and differentiation were induced by seeding the cells in dishes coated with Matrigel in high-glucose DMEM, 10% FBS, 2 mM L-glutamine, 100 IU/mL penicillin, and 100 mg/mL streptomycin as described in a previous study [15].

2.6 Cell viability assay by CCK8

NCI-H716 cells (5.0×10^4) were seeded in a 96-well culture plate to induce cell differentiation for two days before the GLP-1 experiments. When the cells were grown to 80% confluence, the culture medium was

replaced with a serum-free DMEM supplemented with 0.2% BSA, and the cells were treated with SA at different concentrations for 24 hrs. in the medium. Cell viability was measured using the cell counting kit - CCK8 assay Kit(Dojindo, Japan)(n = 6/group). The percentage of living cells was calculated as previously described in a study [16].

2.7 GLP-1 secretion in NCI-H716 cells

The NCI-H716 cells (5.0×10^5 /well) were seeded in a 24-well culture plate to induce cell differentiation as described above. On the day of the experiment, medium was replaced with the Krebs–Ringer bicarbonate buffer (KRB,128 mmol/L NaCl, 4.8 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 5 mM NaHCO₃, and 10 mM HEPES, pH 7.4) containing 0.2% BSA and different concentrations of SA (0 μM, 1 μM, 10 μM or 100 μM) in the presence of 1 mM diprotin A. Following incubation at 37.8 °C for 2 hrs., the supernatants were collected, treated with 50 mg/mL phenylmethylsulphonyl fluoride and stored at -80 °C for analysis of GLP-1 with the ELISA kit. In the study of the ERK pathway, NCI-H716 cells were pretreated with ERK1/2 inhibitors (PD98059, 50 μM) for 30 min in the presence of SA (100 μM). The cells were harvested by scraping and the protein content of the cells was determined using the enhanced BCA protein assay kit. The GLP-1 content was normalized for the total protein of the cells.

2.8 mRNA of GLP-1

Total RNA was extracted from the large intestine tissues using TRIzol reagent (Invitrogen Life Technologies, CA, USA), and cDNAs were generated using a first-strand cDNA synthesis kit (Takara, Tokyo, Japan). qRT-PCR was performed as described in a study [17]. The primers of GLP-1 were listed as followed: GLP-1 forward, 5'-GAGGACCCTGATGAGATGAATG-3'; and reverse, 5'-GGAGTCCAGGTATTTGCTGTAG-3'.

2.9 Statistical analysis

The results are expressed as the mean ± SEM. The statistical analyses were performed using one-way ANOVA. All statistical analyses were performed using GraphPad Prism 8.0 (La Jolla, CA, USA) with a statistical significance set at $P < 0.05$.

3. Results

3.1 Sennoside A improved OGTT in mice

C57BL/6 mice were fed on Chow diet and treated with SA for 5 wks at 3 dosages (15, 30, and 45 mg/kg/day) through the drinking water. The body weight was significantly decreased by SA in the group of 45 mg/kg/day relative to the untreated mice in the control group (Fig. 1A). OGTT was conducted in the mice to test the impact of SA on GLP-1. Oral intake of glucose induces GLP-1 secretion in the intestine. In OGTT, the blood glucose did not exhibit a significant change at the basal condition in all groups of mice. The blood glucose was significantly reduced in the mice of 45 mg/kg/day group after 15 min of glucose administration. The glucose level was significantly lower in the group at 30 min and

60 min compared with the control group (Fig. 1B). No significant effect was observed for SA at the low or medium dosages. The data suggest that SA at 45 mg/kg/day can improve OGTT and reduce the body weight in the lean mice on the Chow diet.

3.2 Sennoside A induced increase of plasma GLP-1

The plasma GLP-1 was examined in the mice to investigate the mechanism of improved OGTT by SA. The improvement of OGTT suggests that SA may promote GLP-1 secretion in the lean mice. Blood GLP-1 was determined in the plasma at 15 min of oral glucose challenge, which was often used to test GLP-1 secretion. GLP-1 exhibited a trend of up-regulation in the mice treated with SA and the increase was in a dose-dependent manner (Fig. 2A). A significance elevation was detected in the dosage group of 45 mg/kg/day (Fig. 2A). The number of L-cells was examined in the large intestine for the mechanism of GLP-1 induction by SA with immunofluorescence staining against GLP-1. No significant alteration was observed in the SA-treated groups as indicated by the mean value of optical density (MOD) of fluorescence (Fig. 2B). The GCG protein was examined in the homogenization of large intestine, and no alteration was observed in the SA-treated group (Fig. 2C). There was no alteration in GLP-1 mRNA in the large intestine of SA-treated mice (Fig. 2D). These data suggest that SA promotes the elevation of plasma GLP-1 in the SA-treated mice without affecting the L-cell numbers in the large intestine. Current evidence supports that other mechanisms may be implicated in the increase of plasma GLP-1 levels by SA.

3.3 Sennoside A induced increase of plasma insulin

Plasma insulin was examined in the mice to evaluate the GLP-1 function. A significant increase was observed in the plasma insulin in the group of 45 mg/kg/day (Fig. 3A). To further evaluate the number of islet β cells, we performed insulin immunofluorescence staining in mouse pancreatic tissue. The number of β -cells was increased in the pancreatic islet in the group of 45 mg/kg/day (Fig. 3B and C). An increase in the biological activity of GLP-1 is supported by the elevation of plasma insulin and β -cell numbers.

3.4 Sennoside A induced activation of ERK1/2 pathway and expression of PC1/3 in colon tissue

GLP-1 is produced from PC1/3-mediated cleavage of proglucagon protein. The activity of PC1/3 was examined with its protein abundance. A significant increase in PC1/3 protein was observed in the large intestine of the SA-treated group (Fig. 4A). In the search about the signaling pathway of SA in the induction of GLP-1 secretion, we examined several signaling molecules. An increase in ERK1/2 activity was found with the change in phosphorylation status in the SA-treated mice (Fig. 4B). The results suggest that SA may increase ERK1/2 activity in the intestinal L-cells to induce GLP-1 secretion.

3.5 Sennoside A induced GLP-1 secretion in NCI-H716 cells

To verify the active role of SA in inducing GLP-1, we employed the cellular model of GLP-1 secretion. In NCI-H716 cells, GLP-1 secretion was induced by SA in the culture supernatant, and a significant increase was observed at the dosage of 1 μ M and above of SA (Fig. 5A). SA did not exhibit any toxicity in the cell model for no reduction in the cell viability (Fig. 5B), which was examined with the CCK8 assay in a 24 hr SA-treatment. The protein level of GCG was not significantly increased in the SA-treated cells (Fig. 5C),

suggesting that SA does not induce GLP-1 expression in the cells. Consistent with in vivo experiments, PC1/3 expression was induced by SA in the cells (Fig. 5D).

3.6 Sennoside A-induced GLP-1 secretion is blocked by ERK1/2 inhibitors (PD98059)

The above data suggest the protein level of GCG and L cell number was not significantly increased in the SA-treated cells (Fig. H). To further confirm the mechanism of SA-induced GLP-1 secretion, we investigated the protein expression of ERK1/2 by western blot analysis in NCI-H716 cells. Similar to the findings in vivo experiments, the protein expression of ERK1/2 phosphorylation significantly increased in a 2 hr SA-treatment (Fig. 6A). In vivo and in vitro experiments suggested that the ERK1/2 signaling pathway played an important role in SA-regulated GLP-1 secretion. To test the possibility, we treated NCI-H716 cells with media containing ERK1/2 inhibitors (PD98059, 50 μ M) in the presence of SA 100 μ M. It was shown that the sole PD98059 did not affect the GLP-1 secretion as compared with control (Fig. 6B). However, the elevation of GLP-1 levels induced by SA was abolished by preincubation with PD98059 (Fig. 6B). The data suggest that SA induced GLP-1 secretion from L-cells in an ERK1/2-dependent manner.

4. Discussion

The current study demonstrates that SA may induce GLP-1 secretion through direct interaction with L-cells. SA is a single compound derived from Chinese herbal medicine such as Rhizoma Rhei (RR). SA is believed to reduce adiposity through inhibition of nutrient absorption in the intestine through stimulation of intestine transition, which decreases the time of food digestion and nutrient absorption in the intestine. However, the impact of SA in GLP-1 was observed in obese mice in our early study [12]. However, the SA activity remains to be characterized in the regulation of GLP-1 secretion, especially a direct effect of SA on L-cells. In the current study, SA was found to directly stimulate L-cells in the intestine to induce GLP-1 secretion in nonobese mice. SA did not alter the L-cell density in the large intestine. Since SA is not absorbed into the blood [14], it is unlikely that SA acts on L-cells through the circulation. SA increased the production of short-chain fatty acids (SCFAs) in obese mice through an impact on gut microbiota [12]. SCFAs can induce GLP-1 secretion [18]. However, the cell culture data of the current study demonstrated that SA might act on L-cells directly without the involvement of SCFAs, which suggests that the increased level of plasma GLP-1 in the SA-treated mice is a result of enhanced secretion of L-cells upon SA stimulation. The SA effect was observed at 45 mg/kg/day. The elevated GLP-1 was observed with enhanced β -cell function in insulin secretion and an increased number of β -cells in the pancreatic islet, which supports an increase in the biological activity of GLP-1.

Our data suggest that SA may act through induction of protein abundance of PC1/3 in the L-cells. PC1/3 promotes GLP-1 secretion through the processing of the proglucagon peptide. The SA effect on PC1/3 was observed in the intestine tissue of SA-treated mice. The effect was confirmed in NCI-H716 cells, suggesting that the direct interaction of SA and L-cells leads to the PC1/3 elevation. The up-regulation of

PC1/3 protein abundance by SA provides a molecular mechanism for the SA activity in the induction of GLP-1 secretion.

The current study suggests that the ERK1/2 pathway may mediate the SA activity in the induction of GLP-1 secretion. The ERK1/2 pathway involves cell growth, development, proliferation, and differentiation [19]. ERK1/2 participates in various physiological and pathological processes through the regulation of a variety of downstream molecules [20, 21]. Insulin was reported to induce GLP-1 secretion through activation of ERK1/2 in NCI-H716 cells. An impairment of ERK1/2 activity made the cells secrete less GLP-1 upon insulin stimulation [22]. SCFA also stimulates GLP-1 secretion via the GPR43 [18], and GPR43 is also linked to activation of ERK1/2 [23]. In the current study, the ERK1/2 activity was induced by SA, and the induction of GLP-1 secretion by SA was blocked by inhibition of ERK1/2 with PD98059. It is not clear if ERK1/2 is involved in the regulation of PC1/3.

It was reported that cAMP-activating agents forskolin and IBMX promoted GLP-1 secretion [24], suggesting that the cAMP/PKA signaling pathway may participate in the regulation of GLP-1 release. In addition, an increase in the cytosolic Ca^{2+} concentration is another mechanism for the GLP-1 secretion [25]. The cytosolic Ca^{2+} elevation is a result of an increase in the intracellular ATP levels, which leads to the closure of K_{ATP} channels. Cell depolarization occurs after the closure of K_{ATP} channels, which causes calcium influx [26, 27]. The Ca^{2+} elevation drives GLP-1 secretion by induction of exocytosis. However, whether the cAMP signal and intracellular calcium ion flow are involved in the SA regulation of GLP-1 remains to be studied.

In summary, we demonstrated that SA directly acted on L-cells to induce GLP-1 secretion in this study. The SA activity was observed with an elevation of GLP-1 in the plasma of the lean mice and the culture supernatant of the L-cell line. SA improved protein expression of PC1/3 and induced activation of ERK1/2. The SA activity in the induction of GLP-1 secretion was blocked by the ERK1/2 inhibitor. The data suggest that SA may induce GLP-1 secretion in L-cells through activation of the ERK1/2 pathway. However, the mechanism of ERK1/2 activation remains to be explored, and its impact on PC1/3 is not clear. SA is a new bioactive component of Chinese herbal medicines that induces GLP-1 secretion through activation of the ERK1/2 pathway and PC1/3 [18–20].

5. Conclusion

Our study provides direct evidence that SA interacts with L cells for GLP-1 secretion by activation ERK1/2 and PC1/3. The effect of SA on GLP-1 secretion is dependent on the ERK1/2 signaling pathway. Therefore, SA is a new drug candidate for the treatment of type 2 diabetes and inhibition of obesity by induction of GLP-1 secretion.

Declarations

Acknowledgments

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Authors' contributions

YongningSun and Jianping Ye contributed to the study conception and design. Li Ma carried out all the experiments and finished the whole paper. All authors contributed to the discussion.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article or are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the ethics committee of Shanghai Jiao Tong University.

Consent for publication

All authors consent to the publication of the data.

Competing interests

The authors declare they have no competing interests.

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Figures

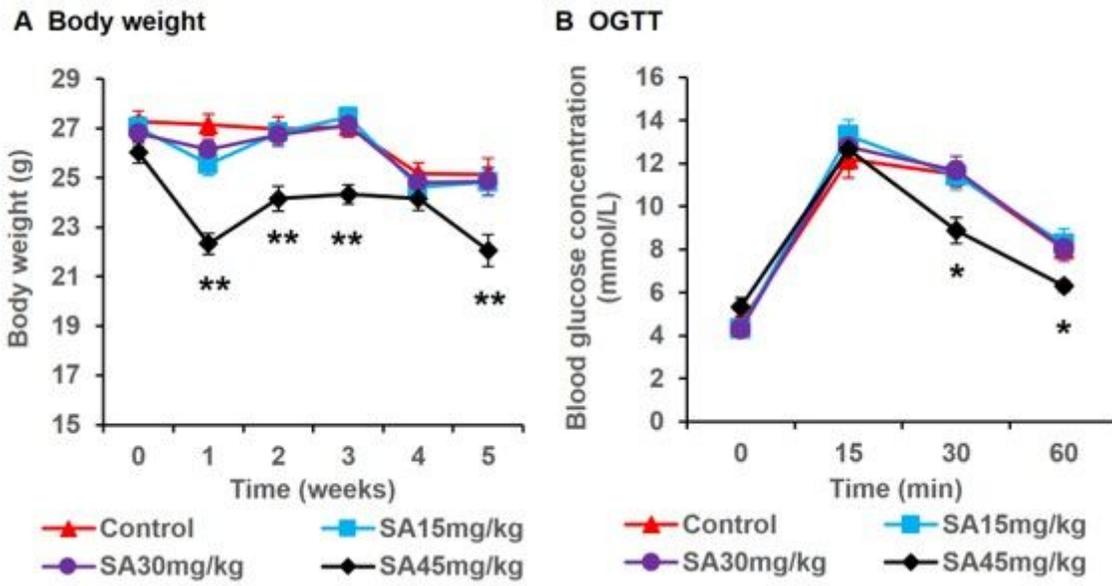


Figure 1

. Sennoside A treatment improved OGTT. (A) Bodyweight. (B) Blood glucose at t = 0 min, 15 min, 30 min, and 60 min after the glucose load. Data are presented as the mean \pm SEM; n = 12. *P < 0.05 vs. the normal control group. **P < 0.01. vs. the normal control group.

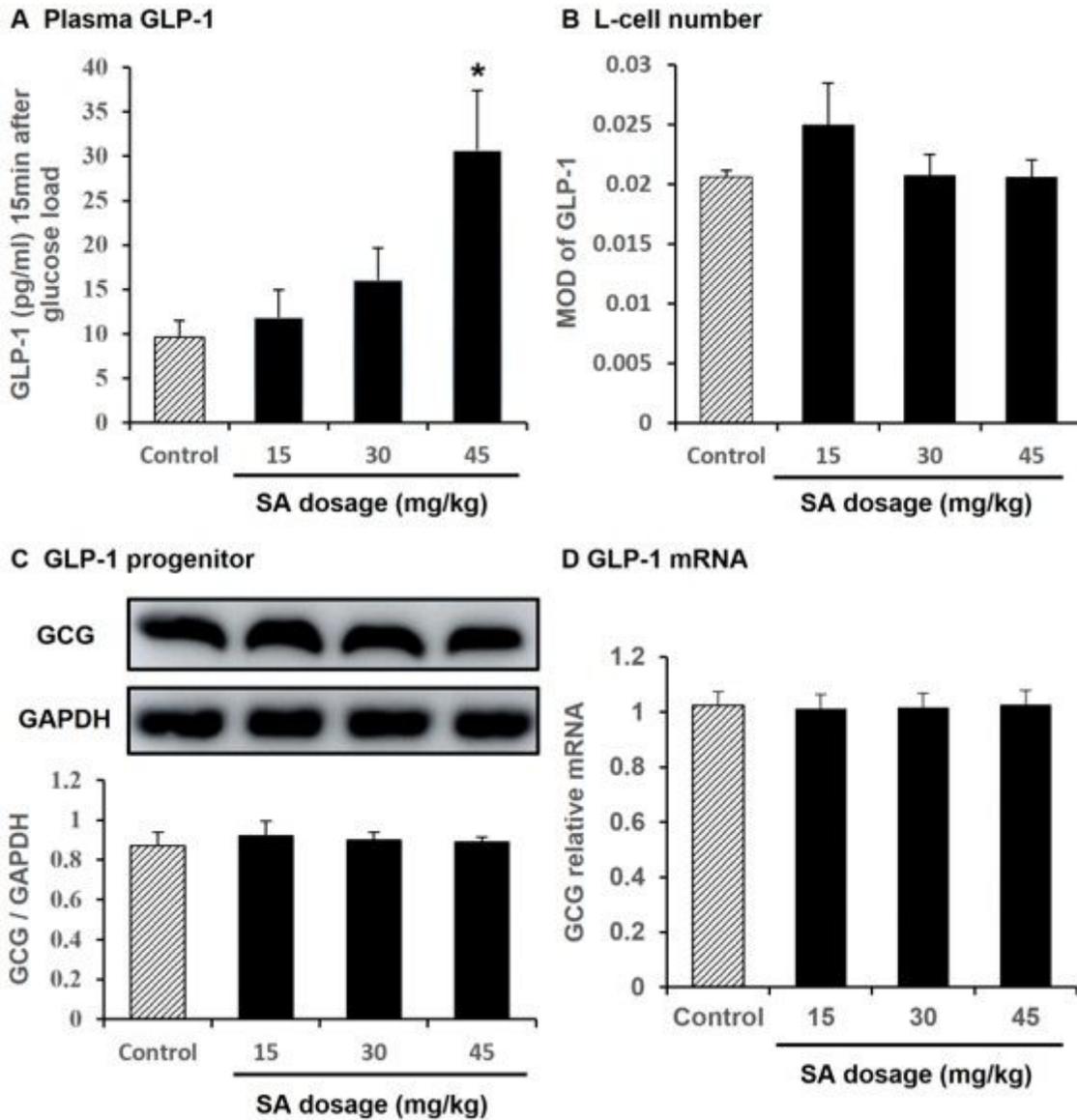
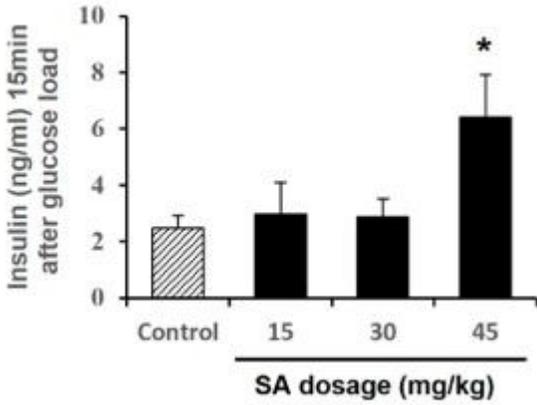


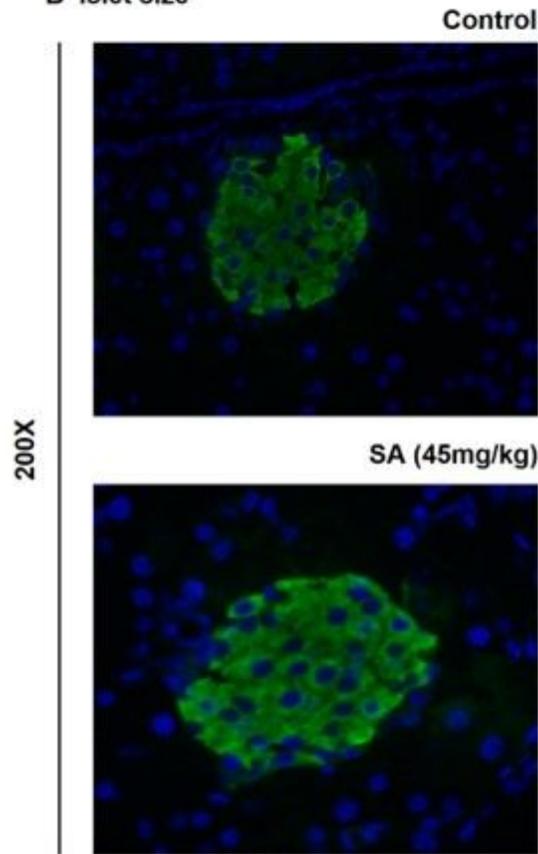
Figure 2

Sennoside A induced increase of plasma GLP-1. (A) Plasma GLP-1. (B) L-cell number: evaluation of mean optical density (MOD) in the GLP-1 immunofluorescence staining for different groups. (C) The expression of GLP-1 progenitor in the colon tissue. (D) mRNA expression of GLP-1 in the colon tissue. Data are presented as the mean \pm SEM; n = 10. *P < 0.05 vs. the normal control group.

A Plasma insulin



B Islet size



C β cell number

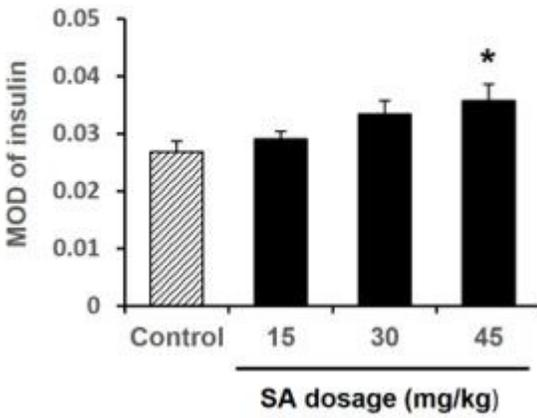
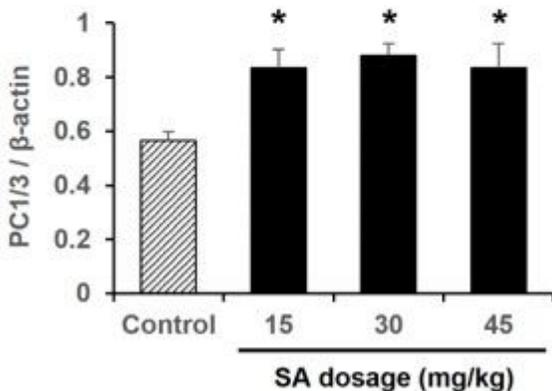
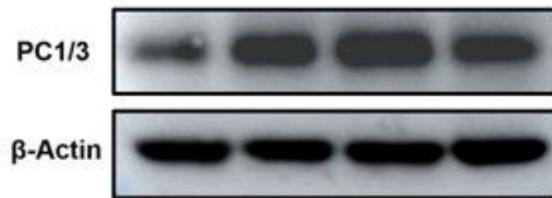


Figure 3

Sennoside A induced increase of plasma insulin (A) Plasma insulin. (B) Islet size by immunofluorescence staining with insulin (green). Nuclei were stained with DAPI (blue). (C) Mean optical density (MOD) of insulin. Data are presented as the mean \pm SEM; n = 6. *P < 0.05 vs. the normal control group.

A PC1/3 expression in colon



B p-ERK1/2 expression in colon

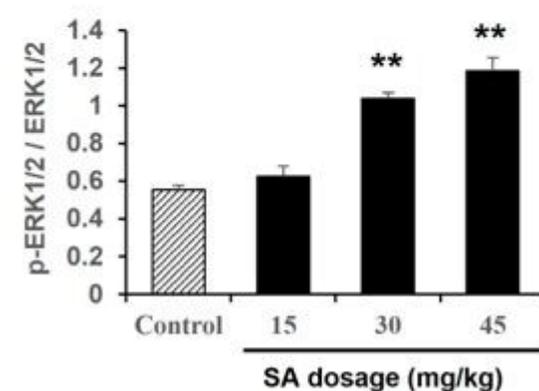
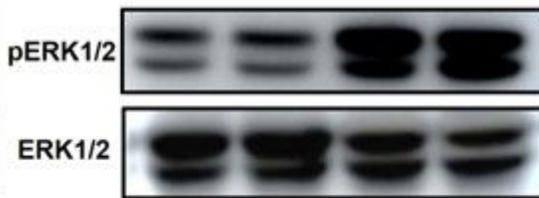


Figure 4

Senoside A induced activation of ERK1/2 pathway and expression of PC1/3 in colon tissue (A, B) The PC1/3, ERK1/2 expression influenced by SA in the colon of normal mice. Data are presented as the mean \pm SEM; n = 6. *P < 0.05 vs. the normal control group. **P < 0.01. vs. the normal control group.

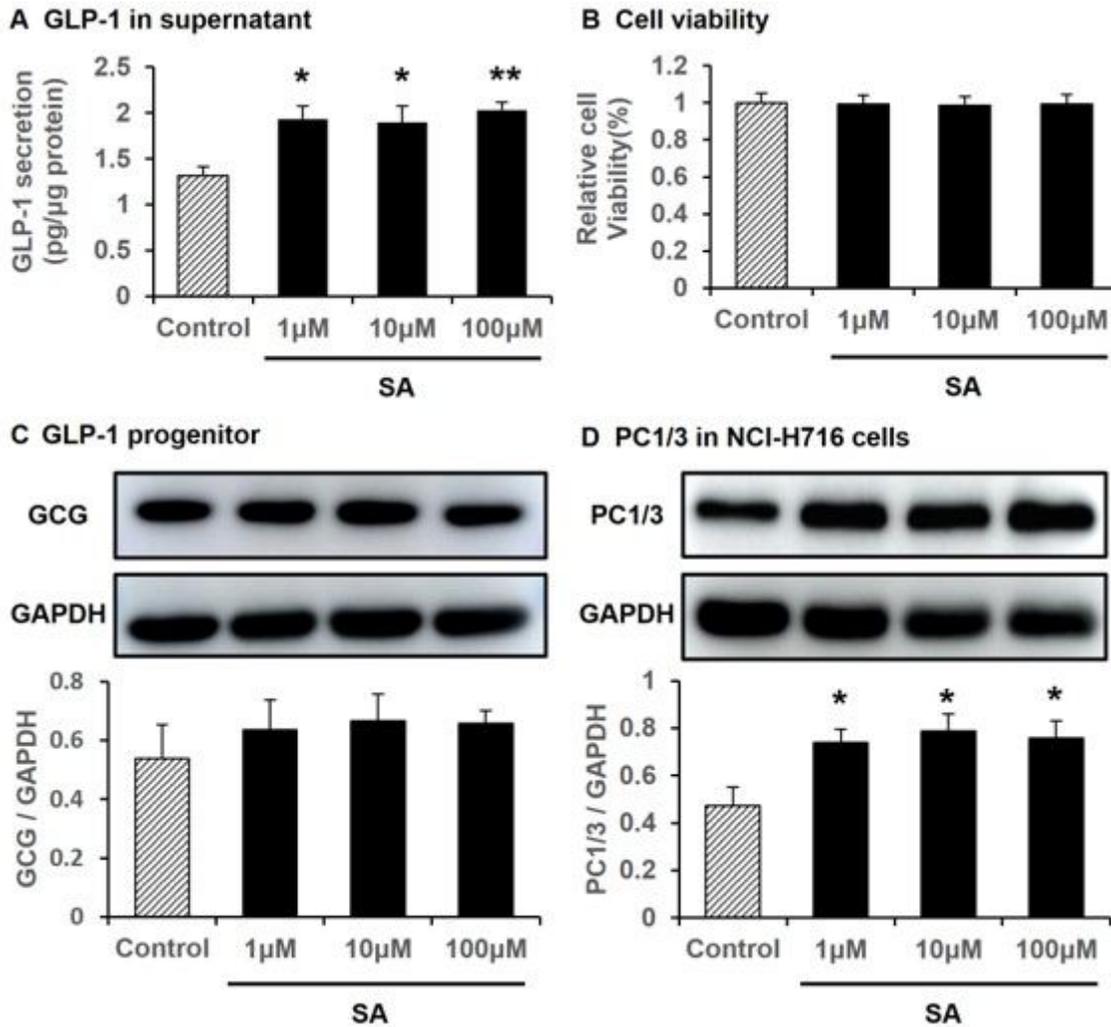


Figure 5

Senoside A induced GLP-1 secretion in NCI-H716 cells (A) GLP-1 levels treated by different concentrations of SA in NCI-H716 cells. (B) Cell viability assay by CCK8. (C, D) GLP-1 progenitor, PC1/3 expression influenced by SA in NCI-H716 cells. Data are presented as the mean \pm SEM; n = 6. *P < 0.05 vs. the normal control group. **P < 0.01. vs. the normal control group.

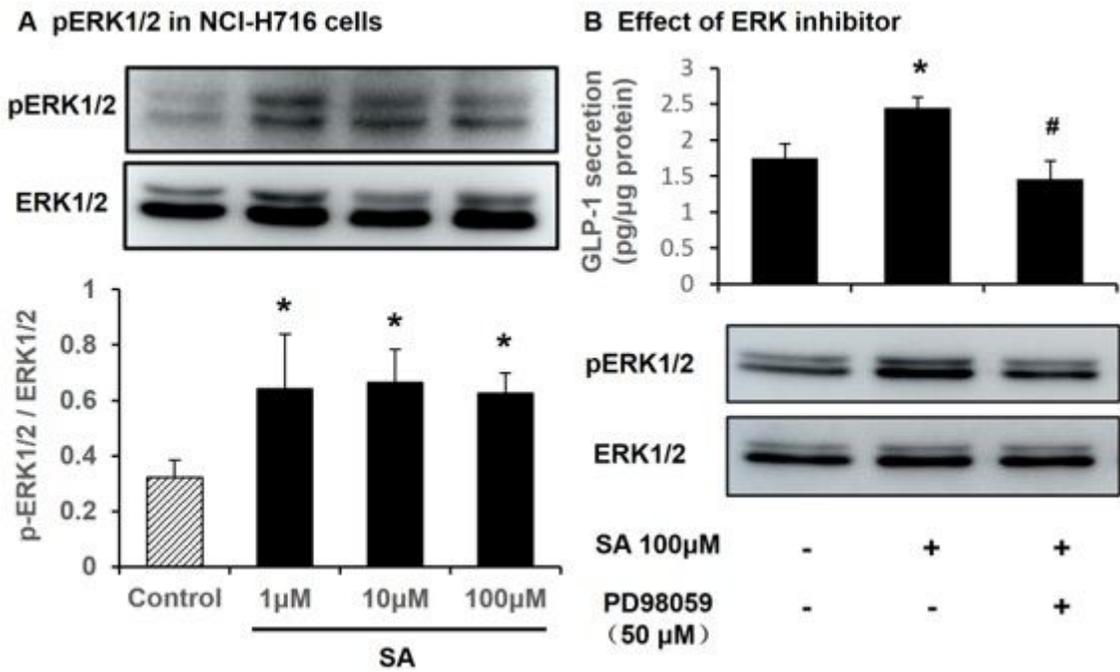


Figure 6

Sennoside A-induced GLP-1 secretion is blocked by ERK1/2 inhibitors (PD98059) (A) ERK1/2 expression influenced by SA in NCI-H716 cells. (B) Effect of ERK1/2 inhibitor(PD98059) on GLP-1. Data are presented as the mean \pm SEM; n = 6. *P < 0.05 vs. the normal control group. #P < 0.05. vs. the SA100 μ M group.