Effect of Bak Foong Pills on Exocrine Pancreatic-Bile Secretion

Ning Tang, Jin Xia Zhu, Wen Chao Zhao, Ying Xing, Yu Lin Gou
Dewi Kenneth Rowlands, Yu Wa Chung, and Hsiao Chang Chan*

*To whom correspondence should be addressed. E-mail: hsiaocchan@cuhk.edu.hk © 2003 Pharmaceutical Society of Japan

We have recently demonstrated that Bak Foong Pills (BFP), a well-known Chinese medicine widely used for treating gynecological disorders, stimulates human colonic epithelial anion secretion, which was mediated by intracellular cAMP and Ca²⁺. The present study further investigated the effect of BFP on exocrine pancreatic-bile secretion using in vivo and in vitro approaches. Duodenal infusion of BFP ethanol extract (1 g/kg) in rats produced increases in the volume and protein output of pancreatic-bile juice, but did not affect its pH. Surgical ablation of vagal neural pathway slightly reduced the effect of BFP on the protein output and volume, indicating that the vagal nerve pathway was not the major player in mediating the effect of BFP on exocrine pancreatic-bile secretion. Using CAPAN-1 cell line, a human pancreatic duct cell line, in conjunction with the short-circuit current (I(sc)) measurements, we further demonstrated that BFP could directly stimulate pancreatic HCO₃⁻ secretion. Basolateral addition of BFP (600 µg/ml) produced averaged charges transported of 2100 ± 382.5 µC/cm², which was blocked by apical addition of Cl⁻ channel blocker. Removal of HCO₃⁻ from the Krebs–Henseleit (K–H) solution inhibited the BFP-induced I(sc) by more than 95%. The present results suggest that BFP could improve digestive function by stimulating pancreatic protein and HCO₃⁻ secretion.

Key words Bak Foong Pills; Bai Feng Wan; pancreas; vagotomy; CAPAN-1; rat

Bak Foong Pills (BFP, China registration #Z980035, also known as Bai Feng Wan) is a well-known traditional Chinese medicine widely used for treating gynecological disorders. It has also been used to treat hepatitis, for improvement of immunological function, blood circulation and overall body functions. We have recently reported that BFP ethanol extract exerted a stimulatory effect on gastrointestinal Cl⁻ secretion, indicating possible beneficial effect of BFP on digestive function.

The exocrine pancreas, one of the most important digestive organs, is divided into two functional parts, the acinar and duct cells. Acinar cells secrete enzymes and fluid high in NaCl and low in NaHCO₃ while duct cells secrete NaHCO₃-rich fluid, which neutralizes the acid coming from the stomach for optimal function of digestive enzymes. Pancreatic-bile juice measurement in rats is a useful in vivo model for studying pancreatic protein output and the secretion of pancreatic-bile juice. The pancreatic-bile juice consists of fluid and electrolytes, which come from both the bile and exocrine pancreas. The protein content contained in pancreatic-bile juice is mainly from pancreatic acinar cells. On the other hand, CAPAN-1, the pancreatic duct cell line, provides a useful in vitro model for the study of the human pancreatic ductal secretory mechanisms since it has been shown to possess most of the properties of pancreatic ductal epithelial cells and contain apical Cl⁻ channels that are involved in HCO₃⁻ secretion. HCO₃⁻ secreted by CAPAN-1 cells could be measured using the short-circuit current technique. The present study investigated the effect of BFP on digestive function using both of the in vivo and in vitro models by measuring the pH, fluid volume and protein content of pancreatic-bile secretion in rats and anion secretion in CAPAN-1 pancreatic duct cells.

MATERIAL AND METHODS

Materials and Solutions d-Mannitol, xylymine (2%), ketamine (10%), d-glucose, sodium bicarbonate, calcium gluconate, N-2-hydroxypiperazine-N’-2-ethanesulfonic acid (HEPES), penicillin–streptomycin (P/S), Bradford agent, calcium chloride, magnesium sulfate, potassium chloride, sodium chloride, potassium gluconate and sodium gluconate were purchased from Sigma Chemical Co., U.S.A. Millipore filter was purchased from Collaborative Biochemical Products (Bedford, MA, U.S.A.). RPMI 1640 and fetal bovine serum (FBS) were purchased from Gibco (Grand, NY, U.S.A.). BFP was obtained from Eu Yan Sang (Hong Kong) Limited.

Krebs–Henseleit (K–H) solution had the following composition (mm): NaCl, 117; KCl, 4.5; CaCl₂, 2.5; MgCl₂, 1.2; NaHCO₃, 24.8; KH₂PO₄, 1.2; Glucose, 11.1. The solution was gassed with 95% O₂ and 5% CO₂, and kept the pH at 7.4. In some experiments gluconate was used to replace anions Cl⁻ or/and HCO₃⁻. For HCO₃⁻-free K–H solution, HEPES and Tris were used and the solution was gassed with 100% O₂.

Extraction of Bak Foong Pills The extraction procedure has been described previously. In brief, 1 kg of BFP powder in 70% ethanol at a ratio of 1 to 10 (g/ml) was put in round-bottom flask and boiled under reflux for two hours. The mixture was filtered and the residue of BFP was subjected to the same treatment for a second time. The filtrates from the two treatment procedures were collected and put in the vacuum rotary evaporator for concentration. The extract was collected and lyophilized by freeze dryer. The BFP was resuspended to desired concentrations in K–H solution.

Animal Preparation Experiments were performed on adult male Sprague–Dawley rats weighting 250—280 g. After overnight fasting with free access to water the animals were anesthetized with a mixture of xylazine and ketamine.
(13—22 and 87—140 mg/kg body weight, respectively). Supplemental doses of the anesthetic agents were administered as needed to maintain a suitable level of anesthesia. The rats were placed on a warm operating table to maintain their body temperature at 37°C. A polyethylene catheter (PE-10) was inserted into the common bile pancreatic duct at the ampulla to collect pancreatic-bile juice. A second polyethylene cannula (PE-50) was placed into the duodenum slightly above the sphincter of Oddi for infusion of 25% BFP (1 g/4 ml/kg body weight) or vehicle (13.7% D-mannitol solution, of which osmolarity, pH and volume were the same as BFP) and return of pancreatic-bile juice. The abdomen was closed with Michel clips and covered with moistened saline-gauze and pancreatic-bile juice was returned to the duodenum every 15 min.

**Subdiaphragmatic Vagotomy** Subdiaphragmatic vagotomy was performed on a separate group of rats under anesthesia. The oesophagus was exposed by a midline laparotomy. Subdiaphragmatic vagal trunks were exposed halfway between the diaphragm and the gastric cardia, and both anterior and posterior trunks were transected. Animals were allowed to recover for 7 d before treatment with BFP or vehicle.

**Collection and Measurement of Exocrine Pancreatic-Bile Secretions** After a 30-min stabilization period, bile-pancreatic secretions were collected every 15 min. The volume was measured, and an aliquot was taken and diluted with distilled water for protein determination. The remainder of the undiluted bile-pancreatic juice was pumped back into the rat via the duodenal cannula during the next collection period. According to the method of Bradford11) and Fryer et al.,12) protein measurement in the bile-pancreatic juice was carried out using 96-well microtiter plates and an automatic microplate spectrophotometer (Spectra MAX-250, purchased from Molecular Devices. U.S.A.). Data were collected and analyzed by a computer. BSA (100 to 1400 μg/ml) was used as a standard to plot the standard curve from which protein contents of the pancreatic-bile juice were derived.

**Cell Culture** Human pancreatic duct cell line, CAP-1 was purchased from American Type Culture Collection (Maryland, U.S.A.). Culture procedures for CAPAN-1 cells, grown in RPMI 1640 medium with 15% FBS, have been described previously.9,10) A volume of 0.2 ml of the cell suspension (1.2 x 10⁶ cells/ml) were plated on to each floating permeable support, which was made of a Millipore filter with a silicone rubber ring attached on top of it for confining the cells (area of 0.45 cm²). Cultures were incubated at 37°C in 95% O₂—5% CO₂ for 5—7 d till the monolayers reached confluence and were ready for short-circuit current (I_sc) measurement.

**I_sc Measurement** The measurement of I_sc has been described previously.3) Monolayers epithelium, CAPAN-1 cells grown on permeable supports, which were clamped vertically between of the Ussing chambers and bathed in K–H solution, which was maintained at 37°C by a water jacket enclosing the reservoir and maintained at 7.4 pH with 95% O₂—5% CO₂. The basal potential difference of the Monolayer’s transepithelial was measured by the Voltage/current clamp (DVC-1000, was purchased from World Precision Instruments (Inc. Sarasota, FL, U.S.A.). I_sc (μA/cm²) was defined as the maximal rise in I_sc following agonist stimulation. In most of the experiments, the change in I_sc was defined as the maximal rise in I_sc or the area under the I_sc response curve following agonist stimulation and they were normalized to current change per unit area of the epithelial monolayer (μA/cm² or μC/cm²). In each experiment, a transepithelial potential difference of 0.1 mV was applied. The change in current in response to the applied potential could be used to monitor the transepithelial resistance of the monolayer.

**Statistics** The results were expressed as means ± S.E.M. In I_sc studies, comparisons between groups of data were made by Student’s t-tests. In bile-pancreatic secretion studies, two-way ANOVA was used to determine the differences between treatment and time course followed by one-way ANOVA with Newman-Keuls post-hoc test to determine the significant difference at individual time point. Differences were considered significant when p values were less than 0.05.

**RESULTS**

Rat pancreatic-bile juice was collected at different time points after treatment with BFP or vehicle, and its protein content, volume and pH were measured. To investigate possible involvement of vagal cholinergic pathway in mediating the BFP effect, subdiaphragmatic vagotomy was performed on a separate group of rats 7 d before BFP treatment. BFP solution was measured and found to be hypertonic; therefore, D-mannitol was used to match the hyperosmolarity of BFP in the vehicle solution.

**Effect of BFP on Pancreatic Protein Secretion** The protein contents were measured from pancreatic-bile juice collected at different time points after intraduodenal infusion of 25% BFP (1 g/kg body weight) or vehicle (13.7% D-mannitol). Compared with the basal secretion, the pancreatic protein output in normal rats (n = 16) was increased by 55±18% (p < 0.01), 69±17% (p < 0.01), 88±22% (p < 0.01), 92±27% (p < 0.05) and 67±34% (p < 0.05) at 15, 30, 45, 60 and 75 min, respectively (Fig. 1A). In vagotomized rats (n = 12), the BFP-induced protein output was increased by 14±10% (p < 0.05), 59±16% (p < 0.01), 57±14% (p < 0.05), 65±13% (p < 0.01) and 63±14% (p < 0.001) at 15, 30, 45, 60 and 75 min, respectively (Fig. 1B). Surgical ablation of vagal neural pathway appeared to reduce BFP-induced pancreatic protein secretion although the difference was not statically significant. However, in both normal (n = 22) and vagotomized control groups (n = 8), intraduodenal infusion of vehicle solution did not affect the pancreatic protein output (Fig. 1).

**Effect of BFP on the Volume of Pancreatic-Bile Juice** Slight increases in the volume of pancreatic-bile juice with a maximum increase of about 10% of the basal value were steadily measured after 15 min (n = 16, Fig. 2A). In vagotomized rats (n = 12), BFP-induced volume increase was reduced with a maximum increase of only 5% observed 30 min after treatment (Fig. 2B). In contrast, intraduodenal infusion of D-mannitol-containing vehicle solution significantly reduced the volume of pancreatic-bile juice in normal (n = 22, Fig. 2A) and vagotomized rats (n = 8, Fig. 2B).

**Effect of BFP on the pH of Pancreatic-Bile Juice** The values of the pH measured in pancreatic-bile juice collected at different time points after drug treatment are shown in Fig.
Fig. 1. Effect of BFP on Pancreatic Protein Secretion
Protein content in pancreatic-bile juice collected before (basal) and 15, 30, 45, 60 and 75 min after duodenal infusion of 25% BFP (1 g/kg) and vehicle in normal rats (A) and vagotomized rats (B). The results were expressed as mean±S.E.M.; *p<0.05, **p<0.01, ***p<0.001. * As compared to basal values.

Fig. 2. Effect of BFP on the Volume of Pancreatic-Bile Secretion
The volume of pancreatic-bile juice secretion collected before (basal) and 15, 30, 45, 60 and 75 min after duodenal infusion of 25% BFP (1 g/kg) and vehicle in normal (A) and vagotomized rats (B). The results were expressed as mean±S.E.M.; *(#)p<0.05, **(##)p<0.01. * As compared to basal values; # as compared to vehicle group.

Fig. 3. Effect of BFP on the pH of Pancreatic-Bile Juice
The pH values of pancreatic-bile juice collected before (basal) and 15, 30, 45, 60 and 75 min after duodenal infusion of 25% BFP (1 g/kg) and vehicle in normal (A) and vagotomized rats (B). The results were expressed as mean±S.E.M.; *p<0.05, **p<0.01. * As compared to basal values.

3, showing similar values for BFP-treated and vehicle-treated normal rats. In vagotomized rats, basal pH was elevated and remained high in vehicle-treated animals but decreasing trends in pH with time was observed in BFP-treated animals (n=12, Fig. 3B), similar to that observed in normal rats (n=16, Fig. 3A).

Effect of BFP on HCO$_3^-$ Secretion by CAPAN-1 Cells
In normal K–H solution, basolateral addition of BFP (600 μg/ml) induced an increase in the short circuit current (I$_{SC}$) which lasted about 10 min, with an averaged total charge transferred of about 2100±382.5 μC/cm$^2$ (n=9, Figs. 4A, D). Apical addition of DPC (2 mM), an anion channel blocker, inhibited the BFP-induced current increase by more than 90% (n=5, Fig. 4B). Removal of HCO$_3^-$ from K–H solution almost completely abolished BFP-induced I$_{SC}$ increase (n=5, Figs. 4C, D).

DISCUSSION
The exocrine pancreas represents one of the most impor-
tant digestive organs in our body for its production of pancreatic juice containing digestive enzymes (proteins) and HCO₃⁻, derived from the acinar and duct cells, respectively. The present study has demonstrated the effect of BFP, an over-the-counter herbal medicine, on digestive function of the exocrine pancreas using both in vivo⁴ and in vitro¹⁰ models. BFP appears to stimulate both pancreatic juice and protein secretion, and its effect may involve vagal–vagal reflex but certainly not to a significant extent since subdiaphragmatic vagotomy did not alter the effect of BFP significantly. The effect of BFP on pancreatic juice and protein secretion may be mediated by endogenous CCK release⁵ or by direct action of BFP on the acinar through the blood. It should be noted that the effect of BFP was unlikely to be due to hyperosmolarity since D-mannitol-containing vehicle did not increase the pancreatic juice and protein output.

BFP does not seem to affect the pH in pancreatic-bile juice obtained from normal rats since the values obtained from both the BFP and vehicle-treated groups were similar. The slight difference between BFP and vehicle values in vagotomized rats may be due to a difference in juice volume, larger for pancreatic-bile juice and thus lower in HCO₃⁻ content and pH value. In general, we find it difficult to interpret the pH values in pancreatic-bile juice since so many factors, such as CO₂ in air and fluctuations in fluid volume, could affect the readings. Therefore, we conducted further studies in vitro, measuring BFP effect on HCO₃⁻ secretion by pancreatic duct cells, CAPAN-1. The results of the IₛC experiments suggest that BFP can directly stimulate HCO₃⁻ secretion from CAPAN cells since removal of HCO₃⁻ from bathing solution abolished the BFP response. Inhibition of the BFP-induced HCO₃⁻ secretion by Cl⁻ channel blocker suggests that BFP effect is mediated by apical Cl⁻ channels, activation of which may be through either cAMP or Ca²⁺ pathways since ingredients of BFP are capable of activating both.¹³ The discrepancy between the results obtained in vivo and in vitro with regard to HCO₃⁻ secretion may be due to a couple of reasons. First, changes in pancreatic HCO₃⁻ secretion cannot be reflected by the pH measured in pancreatic-bile juice since most of the juice comes from the bile. The volume of pancreatic juice alone is only about 1/40—50 of pancreatic-bile juice.¹⁶ Second, the difference in BFP effect on HCO₃⁻ secretion observed in vivo and in vitro in the present study could be due to species difference. It has been reported that in rats, indirect comparison of maximal HCO₃⁻ response to exogenous secretin plus CCK with maximal gastric acid response to exogenous gastrin show that pancreatic HCO₃⁻ output is only one-tenth that of acid output; however, in humans maximal pancreatic HCO₃⁻ output after exogenous secretin is at least as great as maximal gastric acid secretion.¹⁷ It has also been reported that the HCO₃⁻ concentration of pancreatic juice in rats is only 70 mM,¹⁸ which is much lower than that in human (145 mM).¹⁹ Therefore, the effect of BFP could be measured in human derived pancreatic duct cells but not in pancreatic-bile juice of rats.

Taken together, the present study has demonstrated effects of BFP on the digestive system. By stimulating pancreatic-bile juice with enhanced output of digestive enzymes and HCO₃⁻, BFP may exert beneficial effect on improving digestive function, in addition to its other well-known effects. The major effect of BFP on the exocrine pancreas does not seem to involve vagal–vagal reflex. The detailed mechanisms underlying the direct action of BFP require further investigation.

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REFERENCES