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Original Research Article

Effect of berbamine hydrochloride on the absorption of berberine hydrochloride in an *in situ* single-pass intestinal perfusion system in rats

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Abstract

Purpose: *To investigate the intestinal absorption characteristics of berberine hydrochloride (BBH) under different perfusion conditions in rats.*
Methods: *Based on the in situ single-pass intestinal perfusion model of rats, HPLC was used to determine the content of berberine hydrochloride in solution after perfusion under different conditions. The absorption rate constant (Ka), effective permeability coefficient (Papp) and cumulative absorption per unit area (Q) under different perfusion conditions were analyzed by one-way ANOVA.*
Results: *The Papp and Ka of BBH in perfusion solution at pH 7.4 were greater than those in perfusion solution at pH 6 and 8. There was no significant difference ($p > 0.05$) in Papp and Ka of duodenum, jejunum and ileum at high, medium and low concentrations of berberine hydrochloride perfusion solution. The Q increased linearly with increase of mass concentration of perfusion solution. The Ka and Papp of BBH in duodenum, jejunum, and ileum of BBH and berbamine hydrochloride (BAH) combined at different ratios were higher than those of BBH control group at the same BBH concentration, but absorption of BBH in the ratio B40:A50 and B30:A20 groups was highest. In the ratio of B40:A50 ratio, B30:A20 ratio group or the same concentration's BBH group, Ka and Papp of BBH decreased in the order of jejunum > duodenum > ileum.*
Conclusion: *Berberine hydrochloride is absorbed in neutral environment of pH 7.4. The intestinal absorption mechanism of BBH is passive diffusion, and jejunum is the best intestinal segment for absorption. BAH promotes the absorption of BBH.*

Keywords: *Berberine, Berbamine, P-glycoprotein, One-way intestinal perfusion*

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INTRODUCTION

Tibetan medicine is an important part of China's traditional medicine which is the medical and health experience accumulated under extreme environment of the plateau. The unique theoretical system of "three causes" in Tibetan medicine holds that the balance state of “three causes” (rLung, mKhrls-pa, Bad-kan), “seven essences” (food, flesh, blood, fat, bone, marrow

and sperm) and “three impurities” (urine, sweat, excrement) explain the occurrence and development of diseases [1]. Under health condition, between the three factors, seven essences, and three impurities that form human body material foundation, the human body and each viscera organization, and the human body and the environment, all maintain a dynamic balance. Once these balances are destroyed for some reasons, the disease will occur (Figure 1). Diabetes mellitus (DM) and diabetic retinopathy (DR) belong to the category of "Genny Saku diseases" (name of diabetes mellitus and its complications in Tibetan medicine). In Tibetan medicine, the Berberis cortex, which was first recorded in “Four-part Medical Canons” known as the encyclopedia of Tibetan medicine, is commonly used for treating "Genny Saku diseases" [1]. Previous research of the research group shows that the extract of Berberis cortex could reduce blood sugar of diabetic rats and prevent and treat diabetic retinopathy [2-3]. Modern research shows that alkaloids in Berberis cortex including berberine, berbamine, jatrorrhizine, magnoflorine and palmatine are the main active components [4].

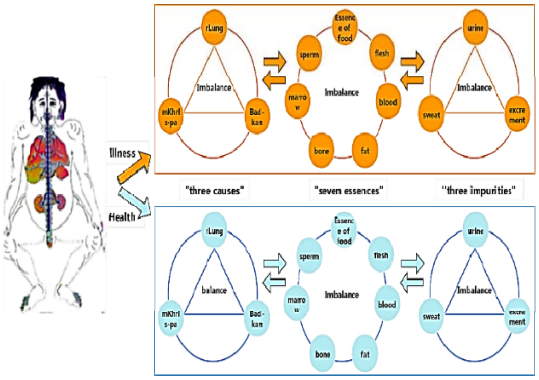

Figure 1: The balance and imbalance of the “three causes,” “seven essences,” and “three impurities” in Tibetan medicine

Barberine hydrochloride (BBH) and berbamine hydrochloride (BAH) are the hydrochloride forms of berberine and berbamine (Figure 2). The hydrochloride salts of berberine and berbamine has much higher water solubility. Modern clinical applications show that BBH can protect pancreatic islet cells, promote insulin secretion, stimulate glycolysis of surrounding tissue cells, inhibit gluconeogenesis in liver, reduce intestinal glucose absorption, which has a good effect in the treatment of diabetes and its complications [5]. However, the oral absorption rate of BBH is low [6], which affects its clinical use to a certain extent. Berberine is the substrate of P-glycoprotein (P-gp). P-glycoprotein in intestinal epithelial cells limits the transport of BBH from intestinal cavity to blood and affects the oral bioavailability of BBH [7]. Studies have shown that BBH is used in combination with P-gp inhibitors, it can improve the absorption of BBH [8]. Berbamine has calcium antagonistic effect under certain conditions [9], which is similar to the effect of verapamil, it can be used as a calcium channel inhibitor to inhibit the expression of P-gp. To a certain extent, BAH can inhibit the efflux of BBH by P-gp, and then increase the absorption of BBH.

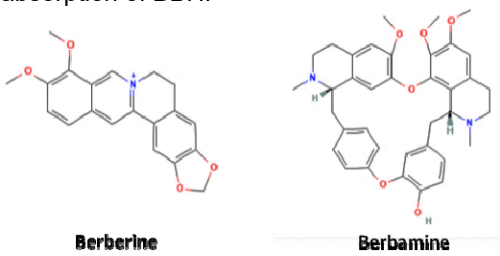

Figure 2: Chemical structures of berberine and berbamine

In view of the low bioavailability of BBH, this experiment used the *in situ* single-pass intestinal perfusion model in rats to study the effects of different pH and different mass concentrations of BBH, and combination of BBH and BAH at different ratios on BBH absorption in different intestinal segments of rats, providing reference for further improving oral absorption and bioavailability of BBH.

EXPERIMENTAL

Chemicals and reagents

Berberine hydrochloride reference standard (Chengdu Ruifensi Biological Technology Co., Ltd., batch number: Y-035-171216), berberine hydrochloride active pharmaceutical ingredient (National Pharmaceutical Standards: H51021627, batch number: 111101), and berbamine hydrochloride active pharmaceutical ingredient (National Pharmaceutical Standards: H51023712, batch number: 110402) (Chengdu Longquan High-Tech Natural Pharmaceutical Co., Ltd.) were purchased. Acetonitrile (chromatography-grade, US Fisher) and methanol (chromatography-grade, US Fisher) were also purchased. Dimethyl sulfoxide (DMSO), sodium chloride, potassium chloride, anhydrous calcium chloride, sodium bicarbonate, potassium dihydrogen phosphate, magnesium chloride, glucose hexahydrate, chloral hydrate, physiological saline, and phosphoric acid were all analytical grade. The water used is ultrapure.

The preparation of the solutions

The Krebs-Ringer (K-R) buffer consisted of 7.8 g NaCl, 0.35 g KCl, 1.37 g $NaHCO_3$, 0.22 g KH_2PO_3, and 1.40 g glucose dissolved in 1000 mL of distilled water [10].

To prepare 6 different concentrations of intestinal perfusion solution composed of BBH (B) and BAH (A), different amounts of BBH and BBA, were dissolved with K-R solution containing 1 % dimethyl sulfoxide. Concentration ratios of 60:40, 50:10, 40:50, 30:20, 20:60 and 10:30 μg/mL intestinal perfusion solutions were obtained. These solutions were categorized as high, medium and low concentrations of BBH intestinal perfusion solutions. The BBH intestinal perfusion solution of 60.47, 30.16 and 10.02 μg/mL were prepared while BBH reference solutions of 0.25, 0.5, 1, 2, 4 and 8 μg/mL were obtained using K-R solution containing 1 % dimethyl sulfoxide.

The chloral hydrate solution was prepared by dissolving 500 mg of chloral hydrate in 0.9 % physiological saline to obtain 10 % chloral hydrate solution. The injection volume to rats was about 0.35 mL of 10 % chloral hydrate solution per 100 g.

Handling of samples

A volume of 0.5 mL of intestinal fluid was mixed with 0.5 mL of methanol and swirled for 3 min. The supernatant was centrifuged for 10 min at 11,000 rpm and filtered through a 0.22 μm microporous membrane.

Determination of index components, BBH and BAH, in intestinal irrigation

High-performance liquid chromatography (HPLC) was performed with a 150 × 4.6 mm, 5 μm Swell Chromplus C_{18} column at 30 °C with a flow rate of 0.6 mL/min. The injection volume was 10 μL per sample. The mobile phase consisted of 0.02 mol/L acetonitrile (A) and potassium dihydrogen phosphate (B) pH = 2.8. The gradient elution started with 22 % A in B and increased to 28 % A in 35 min. The eluents were detected at 270 nm.

The adsorption of BBH in the rat intestinal wall was investigated. Furthermore, the specificity, stability, and precision of adsorption, the blank recovery rate, and the preparation of the standard curve were investigated.

Animals and experimental procedures

The animal experiments were conducted following the guideline approved by the Experimental Animal Commission of Chengdu University of Traditional Chinese Medicine (ref no. SYXK(Chuan)2020-124), and the animal studies followed internal guidelines for animal studies.

Healthy and Specific Pathogen Free (SPF)-grade Sprague Dawley (SD) male rats (Chengdu Dashuo Experimental Animal Breeding Co., Ltd., certificate number: SCXK (Chuan) 2015-030) weighing 220 ± 20 g were used after quarantine for two weeks.

An Eppendorf (EP) tube was filled with 0.9 % normal saline intestinal perfusion solution and preheated and maintained in a 37 ± 0.5 °C water bath. Animals were divided into different experimental groups and fasted for 18 h with water ad libitum before the experiment. After the experiment, the rats were anesthetized with 10 % chloralhydrate (3.5 ml/kg) intraperitoneally and fastened to a plate with a constant temperature of 37 ± 0.5 ℃.

***In vivo* unidirectional intestinal perfusion studies**

This is illustrated in Figure 3. Each intestinal segment was cut at both ends to achieve the final length of 10 – 15 cm. A silicone tubing was inserted into the intestinal segment and tied tightly with a surgical thread. The wound was covered with degreased gauze soaked in saline to maintain moisture, and the temperature was maintained with a lamp. The micro peristaltic device pumped normal saline through the tubing and intestinal segment at 5 ml/min to clean and drain them. An intestinal perfusion solution containing reagents was pumped through the intestine at a fast flow rate of 1 ml/min and everything was maintained at 37 ± 0.5 ºC.

At the end of the experiment, the rats were euthanized with an overdose of anesthetic. The perfused intestinal segments were quickly removed to measure its length (L) and cross-sectional radius (r). Different bowel segments were sampled. The duodenum was a 10-cm segment about 1 cm down from the pylorus. The jejunum was a 10-cm segment about 15 cm down from the pylorus. The ileum was a 10-cm segment about 20 cm down from the cecum. The whole bowel segment was about 1 cm down from the pylorus and 10 cm up the cecum.

Physical adsorption studies of berberine hydrochloride solution on rat intestinal wall

The adsorption of BBH in the intestinal wall of rats was investigated. Sprague Dawley (SD)

male rats fasted for 18 h and then anesthetized. Next, each intestinal segment of the rat was quickly incised, and the mice were euthanized by an overdose of anesthesia. In 0.9 % saline, the surface tissue was carefully peeled off the intestine segment. The mucosa was carefully turned out with a self-made silicone rod, rinsed with 0.9 % saline, and incubated in an intestinal perfusion solution with 30.16 μg/mL BBH at 37 ± 0.5 ℃ for 3 h. Then, the content of BBH in the intestinal perfusate after incubation was determined to calculate the residual rate of duodenum, jejunum, and ileum.

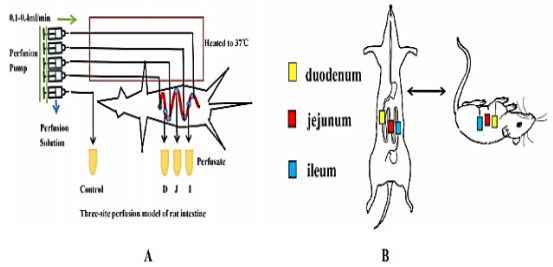

Figure 3: Illustration of *in vivo* unidirectional intestinal perfusion. A. The experimental diagram of unidirectional intestinal perfusion in rats. The perfusion rate of intestinal perfusate was 0.35 ml/min. After a 30-min perfusion, the perfusate EP tube and collecting fluid EP tube of known quality were quickly changed in 15-min intervals. The perfusate was collected at the exits: D, J, and I at 45, 60, 75, 90, and 105 min before the experiment was stopped. B. The three locations of one-way intestinal perfusion: duodenum (D, yellow), jejunum (J, red), and ileum (I, blue)

Determination of the effect of different pH of perfusate on BBH and BAH absorption in rat intestine

The perfusate with a BBH and BAH concentration ratio of 30:20 was selected to identify the optimal pH for rat intestinal absorption. A NaOH solution of 0.1 mol/L was used to adjust the pH to 6.0, 7.4 and 8.0; also, blank K-R solutions with different pH were obtained. The intestinal absorption of the perfusate solutions of BBH and BAH at a concentration ratio of 30:20 in different pH was investigated.

Evaluation of the effect of different concentrations of BBH on absorption of various intestinal segments

The K-R solution with 1 % DMSO at pH 7.4 was used as a solvent to prepare intestinal perfusion solution with high, medium, or low concentrations of BBH at 60.47, 30.16, and 10.02 μg/mL, respectively. The effects of different concentrations of BBH on intestinal absorption in rats were investigated.

Assessment of the effect of different ratios of BBH and BAH on absorption of various intestinal segments

The K-R solution containing 1 % DMSO at pH 7.4 was selected as the solvent for studying the effect of different concentrations of BAH on the intestinal absorption of BBH. The concentration ratio (μg/mL) of BBH (B) and BAH (A) with 4 factors and 6 levels was designed to be 60:40, 50:10, 40:50, 30:20, 20:60, and 10:30. The intestinal absorption of BBH and BAH mixture at different concentration ratios in rats was investigated.

Calculation of experimental parameters of *in situ* intestinal perfusion in rats

During the *in situ* intestinal perfusion test, the volume of the inflow and outflow perfusion solution was corrected by the weight method to eliminate the effect of volume change. The 0.5 mL of perfusion fluid and the EP tube were weighed to obtain the total mass and calculate the density (ρ), assuming that the density did not change before and after perfusion. Eqs 1 - 3 were used to calculate the drug absorption rate constant (Ka), effective permeability coefficient (Papp), and cumulative absorption per unit area per hour (Q). These were obtained using the volume of perfusion fluid infused (Vin) and collected (Vout), the drug concentration in the perfusion fluid in (Cin) and out (Cout) of the inlet, the length (L) and radius (r) of the intestine segment under investigation, and the perfusion velocity (u).

$$Ka = \left(1-\frac{C_{out}V_{out}}{C_{in}V_{in}}\right) \times \frac{u}{\pi r^2 L} \quad \text{..... (1)}$$

$$Papp = \frac{-\ln\left(\frac{C_{out}V_{out}}{C_{in}V_{in}}\right)}{2\pi rL} \quad \text{..... (2)}$$

$$Q = \frac{C_{in}V_{in}-C_{out}V_{out}}{2\pi rL} \quad \text{..... (3)}$$

Statistical analysis

The intestinal absorption results were expressed as mean ± standard deviation (mean ± SD), and the difference between groups was analyzed by one-way (ANOVA) with the SPSS 21.0 software. The absorption rate constant Ka of BBH and the BBH and BAH mixture in different concentrations, different pH, and different intestinal segments were considered statistically significant at $P < 0.05$.

RESULTS

HPLC result of BBH and BAH in intestinal perfusion solution

The specificity experiment (Figure 4) revealed the peak time of BAH and berberine hydrochloride (BHH) at about 7.2 min and 26.5 min, respectively; the peak shape of the index peak is good. The retention time of the index components in the sample was similar to that in the controls, indicating that the chromatographic condition had high specificity. The mass range with a linear relationship between BBH and peak area was 2.5 – 80 μg. The blank recovery experiment showed that the blank recovery rates of high-, medium-, and low-concentrations of BBH were 98.77, 95.17 and 95.49 %, respectively, indicating that the blank recovery rate met the requirements. The precision test results showed that the range of relative standard deviation (RSD) of inter-day precision and intra-day precision of high, medium, and low concentration of BHH was 0.33 and 0.88 %, indicating that the HPLC instrument was precise. The stability test results show that the RSD value is 0.97 %, thus confirming the test's stability.

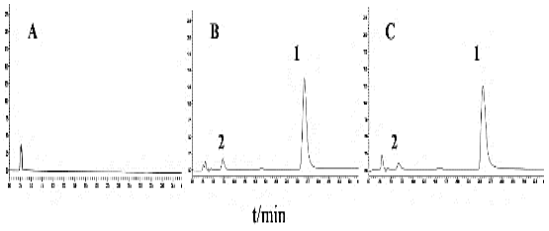

Figure 4: HPLC analysis of BBH and BAH. A. Blank K-R solution with 1 % dimethyl sulfoxide. B. Blank KR solution with 1 % dimethyl sulfoxide as the reference substance of the test reagent. C. Blank with 1 % dimethyl sulfoxide as the test reagent after perfusion KR solution. Peak 1: BBH. Peak 2: BAH

Physical adsorption of berberine hydrochloride solution

The relative standard deviation values of BBH residual rate after duodenum, jejunum and ileum incubation were 1.70, 1.91 and 1.46 %, respectively. The results showed no significant adsorption ($p > 0.05$) of BBH in the small intestine wall of rats

The effect of perfusate with different pH on the absorption of BBH and BAH

The intestinal absorption of BAH (A) and BBH (B) mixture at a concentration ratio of B30:A20 at pH 6.0, 7.4, and 8.0 is reported in Figure 5. The Ka, Papp of BBH in pH 7.4 and 8 perfusate was significantly different from pH 6 ($p < 0.05$). Still, there was no significant difference between pH 7.4 and 8 (Figure 5). The Papp and Ka of BBH in the perfusion solution of pH 7.4 are higher than those of pH 6 and 8, indicating that the intestinal absorption of BBH is relatively efficient in a neutral environment. Therefore, the optimal pH for the perfusion experiments was determined to be 7.4.

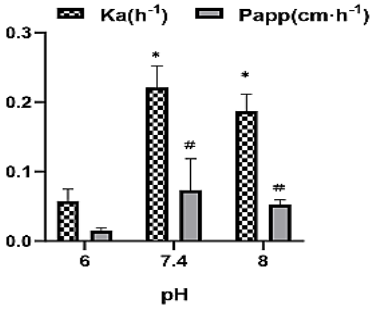

Figure 5: Effect of different pH on the absorption of BBH and BAH in (n = 6) Ka, Papp has a significant difference between the pH 7.4 perfusion solution and the pH 6 ratio, $^{*}P < 0.05$. Ka, Papp has a significant difference between the pH 8 perfusion solution and the pH 6 ratio, $^{\#}P < 0.05$. Ka, Papp has no significant difference between the pH 7.4 perfusion solution and the pH 8 ratio

Effect of high, medium, and low concentrations of BBH on its absorption

The absorption of BBH at different concentrations of BBH by different intestinal segments reported in Figure 6. The one-way ANOVA reveal that, when the concentration of the BBH perfusion solution was 10 – 60 μg/mL, there was no significant difference in Ka among the three different concentrations in duodenum, jejunum, and ileum. The cumulative absorption of drugs in the duodenum, jejunum, and ileum increased linearly with increasing concentration of BBH in the perfusate, showing that there was no saturated absorption of BBH in the concentration range of 10 – 60 μg/mL.

Absorption of BBH in the duodenum, jejunum, and ileum may be driven by passive diffusion [11], which is consistent with a first-order absorption kinetic process (Figure 6C). When the concentration of BBH perfusate was 10 – 60 μg/mL, at the same perfusion concentration, the Ka of duodenum, jejunum and ileum were significantly different from each other ($^{*}P < 0.05$, $^{\#}P < 0.05$), and the Papp and Q of jejunum were significantly higher than those of duodenum and ileum (Figure 6). The results showed that BBH was absorbed by the whole intestine. The

absorption of BBH was highest in the jejunum, followed by duodenum and ileum.

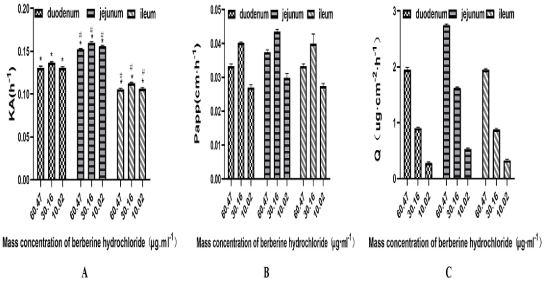

Figure 6: The absorption of different concentrations of BBH in different intestinal segments. A. The comparison of the rate constant (Ka) in different intestinal segments at different BBH concentrations. B. The comparison of the effective permeability coefficient (Papp) in different intestinal segments at different BBH concentrations. C. The cumulative absorption per unit area per hour (Q) in different intestinal segments at different BBH concentrations. *At the same concentration, the Ka of the duodenum compared to that of the jejunum and ileum, $p < 0.05$. #At the same concentration, the Ka of the jejunum compared to that of the ileum, $p < 0.05$

Effect of different concentrations of BBH and BAH on absorption of BBH

The effect of BAH on the absorption of BBH by different intestinal segments was investigated (Figure 7). Then, the Q-t curves of BBH adsorption in each intestinal segment perfused with a mixture of BBH (B) and BAH (A) at different concentration ratios were plotted (Figure 8). Through the analysis of variance, the Ka of B40:A50 and B30:A20 in duodenum, jejunum, and ileum in the BBH and BAH group were significantly different from the BBH and BAH mixtures with other concentration ratios in the same intestinal segment. There was no significant difference between the Ka of B40:A50 and B30:A20, also the Ka of BBH and BAH mixtures with other concentration ratios in the same intestinal segment.

The Papp and Q of BBH and BAH in the duodenum, jejunum, and ileum were also higher than those in other groups in the same intestinal segment. The results showed that when the concentration of BBH was 10 – 60 μg/mL and the concentration ratio of BBH to BAH was B40:A50 and B30:A20, which was better than that of other groups. In the ratio of B40:A50 or B30:A20 groups, as in same concentration's BBH group, the Ka and Papp of BBH decreased in the order of jejunum, duodenum and ileum.

The absorption of BBH in the BBH and BAH group was compared to the BBH group at the same intestinal segment and the same BBH concentration (Figures 6A, B, and C; Figures 7A, B, and C). The Ka, Papp, and Q in the duodenum, jejunum, and ileum of the BBH and BAH group were greater than those of the BBH group, indicating that the absorption of BBH in the BBH and BAH group was increased to varying degrees in each intestinal segment. BAH promoted the absorption of BBH in the small intestine, possibly via the inhibition of P-gp. Therefore, BAH and BBH may have a synergistic effect on intestinal absorption.

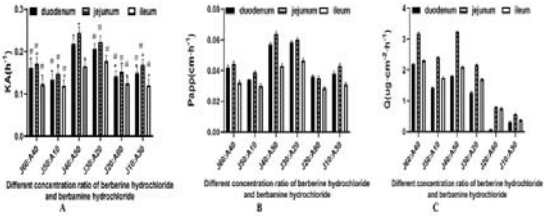

Figure 7: The absorption parameters of BBH and BAH mixtures at different concentrations ratios in each intestinal segment. A. Rate constant (Ka). B. Effective permeability coefficient (Papp). C. Cumulative absorption per unit area per hour (Q). The BBH and BAH mixture with a concentration ratio of B40:A50 or B30:A20 had the highest absorption among the groups. *The Ka of the B40:A50 group compared to other groups in the same intestinal segment; $p < 0.05$. #The Ka of the B30:A20 group compared to other groups in the same intestinal segment; $p < 0.05$.

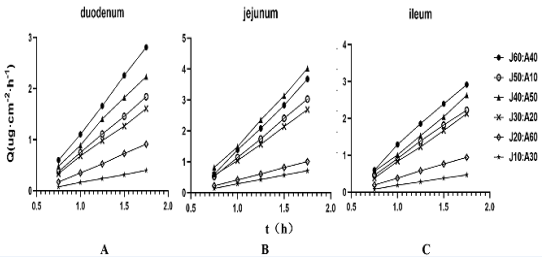

Figure 8: The Q-t curves of the intestinal absorption of BBH in intestinal segments perfused with BBH and BAH mixtures with different concentration ratios. A: duodenum. B: jejunum. C: ileum

DISCUSSION

The bioavailability of drug is affected by pH, and the pH value of the drug is closely related to the intestinal absorption of the drug. In this paper, three pH values were tested based on previous literature [12-13] and preliminary experiments. The results show that the Ka at pH 7.4 and 8 for the BBH group was significantly different from the Ka at pH 6; also, the Ka and Papp at pH 7.4 were higher than pH 8. Therefore, the optimal pH for the perfusion experiment was determined to be 7.4.

This experiment shows that when BBH concentration is in the range of 10 – 60 μg/mL, the Ka, Papp, and Q of BBH and BAH groups are higher than that of the BBH groups. In particular, the intestinal absorption of BHH in segments perfused with B40: A50 and B30: A20 was higher than other groups, indicating that BAH can indeed promote the absorption of BBH and improve the bioavailability of BBH, possibly related to the P-gp transport efflux. P-gp is a substrate drug that can be discharged from the intestinal epithelial cells back into the intestine. P-gp affects the intestinal absorption of drugs. Because P-pg binds to specific substrates, inhibitors, and inducers, P-pg inhibitors and substrates can be used to inhibit P-pg via competitive or non-competitive interactions [14].

A drug's physical and chemical characteristics, the intestinal wall structure, the intestinal enzymes, and the intestinal epithelial cells all affect drug absorption and metabolism; these factors also determine whether the drug can be administered orally [15]. The transport membrane protein P-gp in the small intestine's epithelial cells can transport the substrate drug from the intestinal epithelial cells back to the intestine. Therefore, the efflux of P-gp protein is certain to affect the bioavailability of drugs.

It has been proven that BBH is the substrate drug of P-gp, and the inhibitor of P-gp can promote the absorption of BBH [7,15]. BAH is a calcium antagonist [9] with the same effect as verapamil, a recognized inhibitor of P-gp. It can inhibit the expression of P-gp, suggesting that BAH can cooperate with BBH to improve the bioavailability of BBH. In future experiments, we can compare the effect of BAH to that of common P-gp inhibitors, such as verapamil, on the intestinal absorption of BBH to determine which inhibitor can increase the absorption of BBH. It will also be interesting to study the materials that promote the absorption and transport of the orally administered BBH to provide a basis for the development and research of BBH preparations.

CONCLUSION

This work provides a basis for improving the bioavailability of BBH, which effectively reduces blood sugar and blood lipids, and also enhancement of BAH as a calcium antagonist, which can increase the bioavailability of BBH. The combination of BBH and BAH has significant potential in preventing and treating DM and its complications.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Xi Tang: wrote the paper; Xianrong La, Hui Feng conceived and designed the study; and Jie Gao, Lin Pan, Ruohong Bi, Yi Shi, Yunfeng Han: amended the paper.

REFERENCES

1. *Yuan RY, Zhuo MDZ, Ze RLM, Xiu CJ, Ci WZM. Research progress of Tibetan medicine on the therapeutic effect of diabetes mellitus and its complications. Plateau Sci Res 2021; 5(2): 72-79.*
2. *Zhang Y, Meng XL, Yue LJ, Xiang L, Lai XR. Preliminary studies on Tibetan medicine Berberis Cortex on blood glucose level in diabetic mellitus mice. Prog in Mod Biomed 2013; 13(19): 3619-3622+3607.*
3. *Ye F, Yue LJ, Fan G, Meng XL, Lai XR. Effects of water extract from cortex of Berberi dictyophylla on diabetic retinopathy in spontaneous type 2 diabetic db /db mice (I). Chin J Exp Tradit Med Formul 2016; 22(2): 82-86.*
4. *Li Q, Du H, Weng HS, Xu T, Xu T, Wang YJ, Lai XR, Fan G. Determination of six compounds in Berberidis Cortex and comparative study of its different species. Chin J Chin Mater Med 2019; 44(5): 968-974.*
5. *Li P, He JZ, Zhou XD, Xu X. Berberine regulates type 2 diabetes mellitus related with insulin resistance. Chin J Chin Mater Med 2017; 42(12): 2254-2260.*

6. Wang YL, Yang L, Lu Z, Zhang J, Li HQ. Research progress of new dosage forms of Berberine. Lishizhen Med Mater Med Res 2016; 27(4): 942-944.
7. Fratter A, Servi BD. New oral delivery system to improve absorption of berberine: likely interaction of cationized chitosan with PG-P pump. Int J Drug Deliv Tec 2014; 1(5): 33-42.
8. Liu CS, Zheng YR, Zhang YF, Long XY. Research progress on berberine with a special focus on its oral bioavailability. Fitoterapia 2016; 109: 274-282.
9. Dai XH, Liu R, Zhou Y, Yang M, Fan DM, Xiong DS, Qi J, Yang CZ, Zhu HF. effect of calmodulin antagonist o-(4-ethoxy)-butyl berbamine on the proliferation of human breast cancer cell line MCF-7 and its relevant mechanism. Acta Acad Med Sin 2009; 31(3): 326-329+391-392.
10. He X, Song ZJ, Jiang CP, Zhang CE. Absorption properties of luteolin and apigenin in Genkwa flos using in situ single-pass intestinal perfusion system in the rat. Am J Chin Med 2017; 45(8): 1745-1759.
11. Chen JL, Zhang YL, Dong Y, Cai GZ. Absorption and efflux mechanism of berberine in Caco-2 cell monolayer model. Chin Tradit Pat Med 2014; 36(4): 719-723.
12. Li W, Yang X, Zhang J, Fu C, You Y, Lin Y, Chen H. Effect of radix aconiti lateralis preparata combined with glycyrrhiza on intestinal absorption characteristics of aconite alkaloids in rats. Chin J Exp Tradit Med Formul 2018; 24(22): 11-16.
13. Gao H, Wang M, Sun D, Sun S, Sun C, Liu J, Guan Q. Evaluation of the cytotoxicity and intestinal absorption of a self-emulsifying drug delivery system containing sodium taurocholate. Eur J Pharm Sci 2017; 106: 212-219.
14. McInerney MP, Pan Y, Volitakis I, Bush AI, Short JL, Nicolazzo JA. The effects of clioquinol on p-glycoprotein expression and biometal distribution in the mouse brain microvasculature. J Pharm Sci 2019; 7(108): 2247-2255.
15. Pan GY, Wang GJ, Liu XD, Fawcett JP, Xie YY. The involvement of P-glycoprotein in berberine absorption. Pharmacol Toxicol 2002; 4(91): 193-197.