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The absorption characteristics of bifendate solid dispersion in rat intestinal tissue

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Abstract

Aim: Research on bifendate intestinal absorption. *Method*: The single passed intestinal backflow method was used. Bifendate with different concentrations, bifendate with and without 2,4-dinitrophenol, verapamil, and probenecid, and solid state of bifendate in different systems were studied. *Result*: Change of concentration and the presence of energy inhibitor, P-glycoprotein inhibitor and multidrug-resistant protein inhibitor did not affect the K_a of bifendate intestinal absorption; there were obvious differences among intestinal absorption of native drug, solid dispersion, and physical mixtures. *Conclusion*: The result indicated that the intestinal absorption mechanism of bifendate is passive transport. Solid state of bifendate in different systems could affect the intestinal absorption.

Key words: *Bifendate; intestinal absorption; single-pass intestinal backflow; solid dispersion*

Introduction

Bifendate was first synthesized in China. It is an effective liver aid and has potential in clinical setting. The chemical formula of bifendate is $C_{20}H_{18}O_{10}$ with a molecular weight of 418.36 Da and melting point in the range 180–183°C; the structural formula is shown in Figure 1. Bifendate has poor solubility in water and ethanol but good solubility in $CHCl_3$. It appeared to be absolutely liposoluble.

The dosage forms on sale are pills and conventional tablets. The main disadvantage with bifendate is its poor solubility in water. Pills improved its water solubility but it caused a worse patient compliance. The patients have to take the pills 3 times per day and 5 pills per time. Hence, searching for a new dosage form is necessary for bifendate and the design should be based on the intestinal absorption mechanism of bifendate.

There are various experimental methods of intestinal absorption[1], for example, single-pass intestinal backflow in vivo, intestinal loops, isolated mucosa, everted sacs, and Caco-2 cell model. Each method has its advantages and disadvantages. Considering single-pass intestinal backflow in vivo as an example, the physiologic influence of elimination of gastric contents and spontaneous movement of alimentary tube can be avoided, but the administration, the pH, the drug concentration in intestine, and site of absorption are its limitations. Different from single-pass intestinal backflow in vivo, the advantage of intestinal loops is in its simple operation; however, the complicated sample processing as well as the poor accuracy of the experimental data are the main disadvantages. Isolated mucosa carried out with few influence factors as well as high accuracy and performance, but there is difficulty in practical operation caused by mucosa damage. Everted sacs could be used for not only observing absorption but also for studying transport mechanism[2,3]. The system provides information on drug absorption mechanisms by testing the drug content in the intestinal tissue and during transport through the intestinal tissue. It reflects the uptake and absorption of tested drug quantitatively[4]. Caco-2 cell is derived from human colon adenocarcinoma cell line. This cell line spontaneously differentiates during culture into polarized cells with many enterocyte-like properties of transport-related epithelia[5–9]. Caco-2 cells retain various transporters expressed in the intestines such as *P*-glycoprotein (*P*-gp), multidrug-resistant protein (MRP), and sodium glucose

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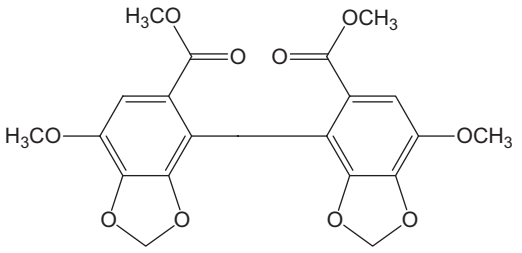

Figure 1. The structural formula of bifendate.

cotransporters[10]. It has been widely used in studies to determine the transport kinetics, absorption characteristics, and metabolism of dietary polyphenols.

Considering the merits and the demerits of each method, single-pass intestinal backflow in vivo was chosen for investigating the absorption of bifendate in rats. For this approach there are two methods: phenol red and simple gravimetric method. The phenol red may interfere with the transport or analytical measurement of some compounds (data on file). However, the gravimetric method appeared to be as accurate as the more elaborate 'nonabsorbed' marker method, and it is acceptable for single-pass intestinal backflow studies[11].

Some transport proteins play an important role in drug accumulation and transport in the human intestines. *P*-gp and MRP are constitutively expressed and abundant in the apical membrane of many epithelial and endothelial barriers[12]. Therefore it is necessary to test whether bifendate is the substrate of *P*-gp or MRP.

Mechanism of absorption can be divided into passive transport, active transport, facilitated diffusion, pinocytosis, and phagocytosis. Passive transport could improve absorption and bioavailability by increasing the drug concentration: this study determined whether intestinal absorption mechanism of bifendate was a passive transport. Single-pass intestinal backflow was used to determine (1) the relationship between the bifendate concentration and its intestinal absorption, (2) the influence of energy inhibitors, *P*-gp inhibitor and MRP inhibitor, on the absorption of bifendate, and (3) the influence of the solid state of bifendate on the absorption of bifendate in different systems.

Intestinal absorption of bifendate could affect the PK parameters and the drug plasma concentration directly; therefore, at the end, the PK parameters of solid state of bifendate in different systems were determined.

Materials and methods

Materials

Bifendate was supplied by Hangzhou Tea Polyphenols Biochemistry Pharmacy Ltd. (Hangzhou, China). Polyvinylpyrrolidone (PVP) was obtained from Yonghua Chemicals (Shanghai, China) and Tablettose from Meggle (Munich, Germany). Verapamil was purchased from Hengrui Pharmaceuticals (Lianyungang, China). Probenecid was purchased from Shanghai Pharmaceuticals Co., Ltd. Magnesium stearate was purchased from Merck (Darmstadt, Germany). Tween 80 was purchased from Nanjing Chemical Regent Co., Ltd. (Nanjing, China) All other reagents and solvents used were of analytical grade; the reagents used in high-performance liquid chromatography (HPLC) were of HPLC grade.

Krebs–Ringer solution (pH 7.4; per 1000 mL) contains NaCl 7.8 g, KCl 0.35 g, CaCl 0.37 g, $NaHCO_3$ 1.37 g, NaH_2PO_4 0.22 g, $MgCl_2$ 0.22 g, and glucose 1.4 g.

Preparation of solid dispersion and physical mixture

Solid dispersion (SD) of bifendate and PVP_{K30} was prepared at the ratio of 1:3 (w/w) by solvent evaporation method. Bifendate and PVP_{K30} were dissolved in minimum amount of ethanol and acetone (1%, w/v).

The solvent was evaporated under continuous stirring in water bath maintained at 60 ± 1°C. The dried mass was crushed, dried, ground gently with a mortar and pestle and sieved; the 100–250 μm particle size fractions were used throughout the study.

Physical mixture (PM) was prepared by homogeneous blending of previously sieved and weighed bifendate and PVP in tumble mixer for 15 minutes.

Assay of bifendate

A HPLC assay method was developed to determine the concentration of bifendate[13]. The HPLC system consisted of a pump (model LC-10A; Shimadzu, Kyoto, Japan), a Shim-pack CLC-ODS column (250 × 4.6 mm i.d., 5 μm; Shimadzu) maintained at 30°C, a UV detector (model SPD-10A, Shimadzu) at 278 nm, and a data station (model SCL-10A, Shimadzu). The composition of the mobile phase was methanol/water (80:20, v/v). The mobile phase was delivered at a flow rate of 1 mL/min and the injection volume was 20 μL.

Single-pass intestinal backflow

After being fasted for 16 hours, SD rats weighing 200–250 g (provided by Central Animal Laboratory of China Pharmaceutical University) were anesthetized with 20% ethyl carbamate solution by two 0.7 mL i.p. injections with a gap of 15 minutes. About 5 cm of duodenum, 10 cm of jejunum, 10 cm of ileum, and 10 cm of colon were quickly inserted into a glass tube on the two sides, fixed by tying with a cotton thread. The apparatus consisted of a pump and a water bath maintained at 37°C. The intestine was cleaned with normal saline for about

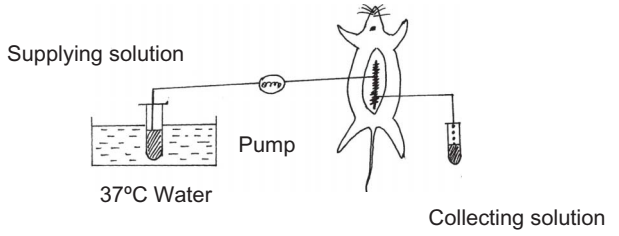

Figure 2. Rat single-pass intestinal perfusion.

15 minutes before the experiment. The perfusate was pumped (HL-2B pump; Huxi Apparatus Inc., Shanghai, China) at a nominal flow rate of 0.2 mL/min for 15 minutes before connecting to the intestine. It had been previously determined that in the rare occasion where nonspecific binding of compound to apparatus had occurred, it was essentially saturated during this perfusion. A sample from the stock drug solution ('supplying') and from the apparatus (at the end of the 15-minute initial equilibration period, 'collecting') was collected and assayed. Every 15 minutes, the solution was changed and the whole process lasted for about 120 minutes. During the experiment, the temperature of four intestines were maintained at 37°C. The solution was collected with a weighed flasket and the volume from the weight loss was calculated. The flow rate was adjusted at 0.2 mL/min and the supplying solution tube and the collecting solution tube were changed[11,14,15]. The perfusion process is shown in Figure 2.

At the end of the 2-hour experiment, the animals were killed and the samples were collected. The samples were rinsed with Krebs–Ringer solution three times and blotted dry. The area of each sac was measured.

Stability of bifendate in 37°C blank intestinal juice

Bifendate was weighed accurately and dissolved in blank intestinal juice with 0.3% Tween 80 (v/v) to prepare solutions with concentrations of 3, 30 and 63 μg/mL. Samples were placed in a constant temperature water bath maintained at 37°C. Mixed O_2 was passed through the samples; samples were filtered through 0.45 μm Millipore filter to obtain clear solutions for HPLC assay, and then sampled at 0, 30, 60, 90, and 120 minutes. The 0-hour peak area was taken as 100% and the content of each sample was calculated. Tween 80 was added to improve the solubility of bifendate in Krebs–Ringer solution, as in the absence of it, the maximum solubility of bifendate is 10.6 μg/mL. With this solubility, the influence of concentration on intestinal absorption could not be studied accurately.

Physical adsorption of intestines wall

A segment of intestine was taken after cleaning, the length equaling the one used in single-pass intestinal backflow in vivo. All of the four intestines were tested. The test solution (30 μg/mL) consisted of 50 ml blank intestinal juice with 0.3% Tween 80 (v/v) solution and q.s. bifendate. The mucous layers were transferred into the test solution at 37°C using a glass rod. Samples were filtered through 0.45 μm Millipore filter to obtain clear solutions for HPLC assay and sampled at 0, 30, 60, 90, and 120 minutes. The remaining content was calculated and 0-hour content was taken as 100% reference.

Intestinal absorption mechanism

Effect of bifendate in different concentrations

The experiment was carried out with the following groups: the blank group of Krebs–Ringer solution with 0.3% Tween 80 (v/v) and the test groups of bifendate solution with concentrations of 20, 40, and 60 (the blank group solution making the solvent and the bifendate making the solute). The net water flux (NWF), K_a, and P_{app} values were calculated according to the data obtained from the experiment.

Effect of 2,4-dinitrophenol (energy inhibitor)

The experiment was carried out with the following groups: the blank group of Krebs–Ringer solution with 0.3% Tween 80 (v/v), the negative test group of bifendate solution (the blank group solution making the solvent and the bifendate making the solute) (30 μg/mL), and the positive test group of bifendate solution (30 μg/mL) with 2,4-dinitrophenol (DNP; 1 mmol/L)[16] (the blank group solution making the solvent while bifendate and DNP make the solute). NWF, K_a, and P_{app} values were calculated according to the data obtained from the experiment.

Effect of verapamil (*P*-gp inhibitor)

The experiment was carried out with the following groups: the blank group of Krebs–Ringer solution with 0.3% Tween 80 (v/v), the negative test group of bifendate solution (the blank group solution making the solvent and the bifendate making the solute) (30 μg/mL), and the positive test group of bifendate solution (30 μg/mL) with verapamil (300 μmol/L)[17] (the blank group solution making the solvent while the bifendate and verapamil making the solute). NWF, K_a, and P_{app} values were calculated according to the data obtained from the experiment directly.

Effect of probenecid (MRP inhibitor)

The influence of MRP was studied with the following groups: the blank group of Krebs–Ringer solution with

0.3% Tween 80 (v/v), the negative test group of bifendate solution (the blank group solution making the solvent and the bifendate making the solute) (30 μg/mL), and the positive test group of bifendate solution (30 μg/mL) with probenecid (1 mmol/L)[18] (the blank group solution making the solvent while the bifendate and probenecid making the solute). NWF, K_a, and P_{app} values were calculated according to the data obtained from the experiment.

The comparison of the solid state of bifendate in different systems on intestinal absorption and PK

Effect of the solid state of bifendate in different systems on intestinal absorption

The single-pass intestinal backflow in vivo was used with solutions with bifendate, SD, and PM of the same concentration. Data were collected and analyzed to calculate NWF, K_a, and P_{app} values of each sample.

Effect of the solid state of bifendate in different systems on PK

After being fasted for 16 hours, 18 SD rats weighing 180–220 g (provided by Central Animal Laboratory of China Pharmaceutical University) were divided into three groups at random: i.g. tablets prepared with bifendate (2 mg), SD, and PM. The blood samples were taken after 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hours of administration.

Plasma sample was transferred into a 15 mL test tube, and 2.0 mL methanol was added. The solution was vortex-mixed for 3 minutes. After centrifugation (4000 rpm) for 10 minutes, the supernatant was transferred to another conical tube for evaporation until dry at room temperature. The residue was redissolved in 200 μL of mobile phase, and 20 μL was injected into the HPLC.

Calculation method

The NWF, K_a, and P_{app} values are calculated using the following equations:

$$\mathrm{NWF} = \frac{Q_{in} - Q_{out}}{l}$$

$$K_a = \left(1 - \frac{C_{out}}{C_{in}}\frac{Q_{out}}{Q_{in}}\right)\frac{Q}{V}$$

$$P_{app} = \frac{-Q\ln\left(\frac{C_{out}}{C_{in}}\frac{Q_{out}}{Q_{in}}\right)}{2\pi rl},$$

where Q_{out}, Q_{in} are the perfusate volumes (mL) of the intestinal tract exit and entry, respectively, and C_{out}, C_{in} are the perfusate concentrations (μg/mL) of the intestinal tract exit and entry, respectively. The length and transverse section radii of intestinal tract used in the experiment are l (cm) and r (cm), Q is the perfusing rate (~0.2 mL/min), t is the perfusing time (~0.25 hours), and V is the volume of the perfusing intestinal tract.

All of the blood drug concentration data were fitted by Kinetica 4.4 (Thermo Electron Corporation). The results are all expressed by $\bar{x} \pm s$. For each pair of groups, a t-test was made and a p-value of less than 0.05 was considered statistically significant.

Results

Stability of bifendate in 37°C blank intestinal juice

The change in bifendate content after incubation in 37°C Krebs–Ringer solution for 120 minutes is shown in Table 1. Data obtained from the experiment in Table 1 suggested that bifendate was steady in 37°C Krebs–Ringer solution for 120 minutes. This period of time is sufficient to conduct the experiment and to ensure the data utility. At the end of the experiment (120 minutes), the bifendate remaining was 97.03, 96.76, and 96.52% at concentrations of 3, 30, 63 μg/mL, respectively.

Physical adsorption of intestinal wall

Table 2 showed the influence the physical absorption of the intestinal wall on the experiment. The results in Table 2 indicated that physical absorption of intestinal wall had little influence to bifendate and experiment, and could be ignored. Cross group t-test between the data of 0 hour and 2 hours showed there was no significant differences ($p > 0.05$). After the 2 hours incubation, the remaining bifendate was at an average value of 98.73 ± 0.55% compared to that of 0 hour.

Intestinal absorption mechanism

Table 3 showed the relationship between NWF, K_a, and P_{app} values of intestinal absorption and bifendate

Table 1. Remaining ratio of bifendate after incubation in Krebs solution at 37°C ($n = 6$).

	Remaining ratio (%)		
Time (minutes)	3 μg/mL	30 μg/mL	63 μg/mL
0	100.00	100.00	100.00
30	99.74	98.45	98.39
60	98.43	97.84	97.77
90	97.86	96.43	97.09
120	97.03	96.76	96.52

Table 2. The influence of physical absorption from intestinal wall ($n = 6$).

Time (h)		1	2	3
Duodenum	0 hour	31.1 μg/mL	32.4 μg/mL	30.6 μg/ml
	2 hours	30.9 μg/mL	31.9 μg/mL	30.1 μg/ml
	$C_{2\,h}/C_{0\,h}$	99.36%	98.46%	98.37%
	$\bar{x} \pm s$		98.73 ± 0.55%	
Jejunum	0 hour	30.8	31.4	31.6
	2 hours	30.3	30.5	30.7
	$C_{2\,h}/C_{0\,h}$	98.38%	97.13%	97.15%
	$\bar{x} \pm s$		97.55 ± 0.71%	
Ileum	0 hour	30.5	30.2	30.9
	2 hours	30.1	29.7	30.3
	$C_{2\,h}/C_{0\,h}$	98.69%	98.34%	98.06%
	$\bar{x} \pm s$		98.36 ± 0.32%	
Colon	0 hour	31.4	31.8	30.6
	2 hours	30.8	31.2	30.0
	$C_{2\,h}/C_{0\,h}$	98.09%	98.11%	98.04%
	$\bar{x} \pm s$		98.08 ± 0.03%	

concentration. The data shown in Table 3 indicated that the concentration of solution had no effect on intestinal absorption K_a of bifendate of the four intestines. Cross group *t*-test showed there were no significant differences in intestinal absorption among different bifendate concentrations ($p > 0.05$). It is initially inferred that bifendate appeared to have passive transport in vivo, and the intestinal absorption could be improved by increasing the bifendate concentration in intestine. This result suggests that it is possible to increase the bioavailability by increasing the concentration of bifendate. The results obtained from this experiment could be a guide to design new dosage form.

Table 4 showed the change in NWF, K_a, and P_{app} values of intestinal absorption after the addition of energy inhibitor DNP. The data in Table 4 suggested that there was little difference between negative and positive groups, and it can be therefore concluded that energy had no effect on bifendate intestinal absorption of the four intestines. Based on cross group *t*-test results, there was no significant effect of adding DNP on the intestinal absorption ($p > 0.05$). Hence, it is clear that bifendate did not display active transport.

Table 5 showed the change in NWF, K_a, and P_{app} values of intestinal absorption after adding *P*-gp inhibitor verapamil. It is obvious from Table 5 that bifendate did not play the substrate of *P*-gp, as cross group *t*-test indicated that there was no significant difference between the negative group and positive group ($p > 0.05$).

Table 4. Intestinal absorption of bifendate in the absence or presence of DNP ($\bar{x} \pm s$, $n = 9$).

	Index	With DNP[a]	Without DNP[b]
Duodenum	NWF (μL/h/cm)	67.21 ± 24.32	65.31 ± 23.67
	K_a ($\times 10^{-2}$/min)	19.42 ± 3.31	18.41 ± 2.73
	P_{app} ($\times 10^{-3}$cm/min)	25.33 ± 4.57	22.36 ± 3.75
Jejunum	NWF (μL/h/cm)	79.37 ± 27.21	71.31 ± 23.471
	K_a ($\times 10^{-2}$/min)	42.56 ± 5.83	38.63 ± 4.56
	P_{app} ($\times 10^{-3}$ cm/min)	43.64 ± 5.97	46.54 ± 5.43
Ileum	NWF (μL/h/cm)	84.52 ± 23.54	84.31 ± 24.39
	K_a ($\times 10^{-2}$/min)	31.26 ± 4.32	34.75 ± 5.83
	P_{app} ($\times 10^{-3}$ cm/min)	35.52 ± 5.47	30.43 ± 4.32
Colon	NWF (μL/h/cm)	7.45 ± 2.45	9.45 ± 2.34
	K_a ($\times 10^{-2}$/min)	8.54 ± 1.22	7.57 ± 1.13
	P_{app} ($\times 10^{-3}$ cm/min)	13.62 ± 2.42	10.46 ± 1.53

[a]The intestinal absorption parameter of bifendate solution with DNP.
[b]The intestinal absorption parameter of bifendate solution without DNP.

Table 3. Intestinal absorption of bifendate in different concentrations ($\bar{x} \pm s$, $n = 9$).

	Index	20 μg/mL[a]	40 μg/mL[b]	60 μg/mL[c]
Duodenum	NWF (μL/h/cm)	65.65 ± 22.42	64.25 ± 23.24	62.51 ± 21.32
	K_a ($\times 10^{-2}$/min)	14.53 ± 1.85	17.35 ± 1.93	15.44 ± 1.93
	P_{app} ($\times 10^{-3}$ cm/min)	23.63 ± 2.32	25.43 ± 2.36	21.34 ± 2.57
Jejunum	NWF (μL/h/cm)	73.52 ± 24.56	75.43 ± 26.63	77.38 ± 25.38
	K_a ($\times 10^{-2}$/min)	38.68 ± 5.46	39.62 ± 5.37	41.75 ± 6.35
	P_{app} ($\times 10^{-3}$/cm/min)	44.54 ± 7.45	42.34 ± 6.33	44.46 ± 7.23
Ileum	NWF (μL/h/cm)	77.37 ± 25.54	78.83 ± 26.54	78.28 ± 24.82
	K_a ($\times 10^{-2}$/min)	33.73 ± 4.17	31.17 ± 5.16	32.37 ± 5.37
	P_{app} ($\times 10^{-3}$ cm/min)	36.72 ± 4.77	35.38 ± 4.27	37.53 ± 6.26
Colon	NWF (μL/h/cm)	8.45 ± 2.64	8.93 ± 2.82	8.35 ± 2.86
	K_a ($\times 10^{-2}$/min)	8.32 ± 1.29	7.94 ± 1.26	8.67 ± 1.33
	P_{app} ($\times 10^{-3}$ cm/min)	11.64 ± 1.65	12.28 ± 1.92	11.82 ± 2.09

[a]The intestinal absorption parameter of 20 μg/mL concentration for bifendate.
[b]The intestinal absorption parameter of 40 μg/mL concentration for bifendate.
[c]The intestinal absorption parameter of 60 μg/mL concentration for bifendate.

Table 5. Intestinal absorption of bifendate in the absence or presence of verapamil ($\bar{x} \pm s$, $n = 9$).

	Index	With verapamil[a]	Without verapamil[b]
Duodenum	NWF (μL/h/cm)	68.31 ± 22.68	64.73 ± 21.28
	K_a ($\times 10^{-2}$/min)	16.83 ± 2.34	18.32 ± 2.83
	P_{app} ($\times 10^{-3}$/cm/min)	26.79 ± 4.62	22.52 ± 3.79
Jejunum	NWF (μL/h/cm)	77.82 ± 25.45	72.51 ± 24.67
	K_a ($\times 10^{-2}$/min)	41.03 ± 4.47	39.48 ± 4.73
	P_{app} ($\times 10^{-3}$/cm/min)	42.16 ± 5.23	47.33 ± 5.64
Ileum	NWF (μL/h/cm)	71.53 ± 22.53	78.46 ± 23.85
	K_a ($\times 10^{-2}$/min)	35.93 ± 3.77	35.42 ± 3.51
	P_{app} ($\times 10^{-3}$ cm/min)	33.56 ± 5.45	36.37 ± 4.77
Colon	NWF (μL/h/cm)	8.57 ± 2.36	9.42 ± 2.84
	K_a ($\times 10^{-2}$/min)	9.35 ± 1.29	8.25 ± 1.11
	P_{app} ($\times 10^{-3}$ cm/min)	13.56 ± 1.95	11.16 ± 1.88

[a] The intestinal absorption parameter of bifendate solution with verapamil.
[b] The intestinal absorption parameter of bifendate solution without verapamil.

Table 6. Intestinal absorption of bifendate in the absence or presence of probenecid ($\bar{x} \pm s$, $n = 9$).

	Index	With probenecid[a]	Without probenecid[b]
Duodenum	NWF (μL/h/cm)	65.13 ± 21.43	68.63 ± 21.74
	K_a ($\times 10^{-2}$/min)	18.42 ± 2.32	16.56 ± 2.21
	P_{app} ($\times 10^{-3}$ cm/min)	23.64 ± 4.32	26.63 ± 4.82
Jejunum	NWF (μL/h/cm)	79.41 ± 24.311	74.53 ± 21.47
	K_a ($\times 10^{-2}$/min)	39.62 ± 4.52	41.56 ± 4.46
	P_{app} ($\times 10^{-3}$/cm/min)	46.67 ± 6.68	42.19 ± 7.43
Ileum	NWF (μL/h/cm)	80.14 ± 25.34	75.96 ± 22.53
	K_a ($\times 10^{-2}$/min)	31.45 ± 3.97	29.83 ± 3.63
	P_{app} ($\times 10^{-3}$ cm/min)	36.52 ± 5.77	34.53 ± 6.17
Colon	NWF (μL/h/cm)	8.64 ± 2.82	9.24 ± 2.86
	K_a ($\times 10^{-2}$/min)	7.94 ± 1.06	8.45 ± 1.29
	P_{app} ($\times 10^{-3}$ cm/min)	10.45 ± 1.75	12.46 ± 2.17

[a]The intestinal absorption parameter of bifendate solution with probenecid.
[b]The intestinal absorption parameter of bifendate solution without probenecid.

Table 6 showed the change in NWF, K_a, and P_{app} values of intestinal absorption after using *P*-gp inhibitor probenecid. The results in Table 6 and cross group *t*-test indicated that probenecid had no significant influence on bifendate intestinal absorption ($p > 0.05$), which suggested that bifendate was not the substrate of MRP.

The comparison of the solid state of bifendate in different systems on intestinal absorption and PK

The NWF, K_a, and P_{app} values were calculated according to the experimental data and compared with the bifendate intestinal absorption data of solid state of bifendate in different systems. Table 7 showed the relationship between the NWF, K_a, and P_{app} values of intestinal absorption and the solid state of bifendate in different systems. It is indicated that the absorption of SD was better than that of PM and native bifendate in all of the four intestines. There was significant difference in intestinal absorption between SD and bifendate as well as PM ($p < 0.05$), and there was no significant difference in intestinal absorption between bifendate and PM ($p > 0.05$). It is inferred that in the SD, bifendate dispersed and was easy to absorb. But for PM, its absorption was even worse than native bifendate. This may be caused by the free PVP, as it might inhibit the absorption of bifendate. Results of intestinal absorption showed that the improvement of intestinal absorption was attributed to the physical characteristic of bifendate-SD, but not because of the simple effect of excipients. The new state of bifendate was the reason for the improvement in its solubility, its absorption, and its bioavailability. Simple physical mixtures were useless[19].

Figure 3 showed the relationship between the bifendate blood plasma concentration and the solid state of bifendate in different systems. Table 8 showed the PK parameters of bifendate in rats after a single dose of solid-state bifendate in different systems. Results shown in Figure 3 and Table 8 indicated that SD improved the absorption appreciably compared to native bifendate. There was significant difference in PK parameters between SD and bifendate as well as PM ($p < 0.05$), and for some parameters there was significant difference in PK parameters between bifendate and PM ($p < 0.05$). After prepared into SD, the solubility of bifendate increased greatly; this was the reason for the increase of intestinal absorption and the drug blood plasma concentration. In Table 7, SD had better intestinal absorption than native bifendate and PM; and in Table 8 SD had higher drug blood plasma concentration than the other two correspondingly. It could be seen clearly that the drug blood plasma concentration increased from 0.225 μg/mL (C_{max} for bifendate) to 0.531 μg/mL (C_{max} for SD) and this was caused by the intestinal absorption improvement shown in Table 7. The drug blood plasma concentration of PM was poorer than native bifendate and this may have been caused by PVP_{K30}. Addition of PVP_{K30} might prevent the diffusion of bifendate and its intestinal absorption, hence, the decrease in the drug blood plasma concentration. The data of AUC showed that the bioavailability of SD was about 126% of native bifendate and 120% of PM; the C_{max} of SD was about 228% of native bifendate and 303% of PM.

Discussion

The small intestine is the primary site of absorption for many drugs administered orally and so is the target

Table 7. Intestinal absorption of bifendate in different physical states ($\bar{x} \pm s$, $n = 9$).

	Index	Bifendate[a]	Physical mixture[b]	Bifendate-SD[c]
Duodenum	NWF (μL/h/cm)	63.16 ± 23.41	58.64 ± 18.37	89.47 ± 26.65*#
	K_a ($\times 10^{-2}$/min)	17.92 ± 2.23	16.73 ± 2.88	30.25 ± 3.12*#
	P_{app} ($\times 10^{-3}$/cm/min)	21.38 ± 3.32	20.23 ± 3.21	47.43 ± 3.97*#
Jejunum	NWF (μL/h/cm)	75.75 ± 25.13	70.15 ± 25.63	122.31 ± 31.21*#
	K_a ($\times 10^{-2}$/min)	39.98 ± 4.83	36.41 ± 4.99	71.24 ± 5.41*#
	P_{app} ($\times 10^{-3}$ cm/min)	44.19 ± 4.23	41.54 ± 4.64	79.14 ± 5.32*#
Ileum	NWF (μL/h/cm)	78.43 ± 26.54	75.26 ± 21.35	135.36 ± 30.15*#
	K_a ($\times 10^{-2}$/min)	32.52 ± 3.97	33.81 ± 3.32	67.89 ± 4.52*#
	P_{app} ($\times 10^{-3}$ cm/min)	36.37 ± 3.77	33.44 ± 3.26	70.32 ± 4.47*#
Colon	NWF (μL/h/cm)	8.32 ± 2.82	6.21 ± 2.28	14.56 ± 3.79*#
	K_a ($\times 10^{-2}$/min)	8.74 ± 1.29	7.14 ± 1.13	16.37 ± 2.17*#
	P_{app} ($\times 10^{-3}$ cm/min)	11.36 ± 1.95	10.07 ± 1.64	19.55 ± 2.69*#

[a]The intestinal absorption parameter of bifendate in native drug state.
[b]The intestinal absorption parameter of bifendate in physical mixture state.
[c]The intestinal absorption parameter of bifendate in solid dispersion state.
*$p < 0.05$, versus group bifendate.
#$p < 0.05$, versus group physical mixture.

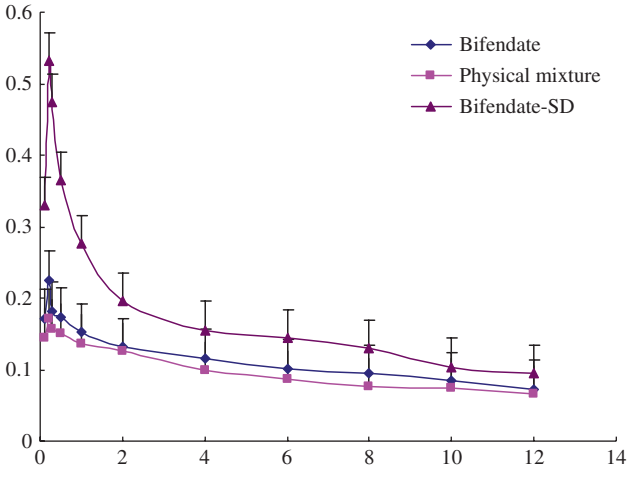

Figure 3. Blood drug concentration value after single use of bifendate in different physical states in rats ($\bar{x} \pm s$, $n = 6$).

tissue for pharmacotherapeutic strategies to control the oral absorption of drugs[20]. Perhaps the most used classic technique employed in the study of intestinal absorption of compounds had been the single-pass intestinal backflow in vivo[21–23]. In this model, a solution containing the compound of interest was perfused through a section of intestinal, with the blood supply left intact. However, water absorption and secretion during the perfusion may introduce errors in the calculated absorption. Various water flux correction methods have been published, and phenol red is the traditional method at this site. This method involves a nonabsorbent marker to correct the data, even though the added complexity was often not trivial. For example, phenol red may interfere with the transport or with the analytical measurement of some compounds. Gravimetric method is introduced to overcome these problems by calculating the volume of the solution by determining its weight, and therefore, there is no error caused by the weak absorption of the maker.

Drug absorption is the first step for expression of pharmacological effects, and has many difficulties and unknown factors[24]. The study on bifendate intestinal absorption indicated that concentration did not change the K_a value of bifendate, that is bifendate intestinal absorption was relative to the concentration, the higher the concentration, the more it absorbed. This fitted the condition of passive transport. Therefore, it was possible for us to improve the bioavailability of bifendate by preparing it into SD to increase its solubility[25]. As the experiment of effect on DNP suggested that energy had no effect on bifendate intestinal absorption, which strongly proved this process was not an active absorption. *P*-gp is a plasma membrane glycoprotein of about 170 kDa that belongs to the superfamily of ATP-binding cassette transporters[26]. It can actively pump drugs out of cells, thus reducing the oral bioavailability of a wide range of drugs such as digoxin, vinca alkaloids, and β-adrenergic agonists[27,28]. In our experiments, addition of *P*-gp inhibitor such as verapamil did not prevent the absorption of bifendate, which indicated that the intestinal absorption of bifendate did not rely on the presence of *P*-gp. That is to say, the transport of bifendate in the body did not need pump like *P*-gp. This is a characterstic of passive transport[29,30]. MRPs are a subfamily of the ATP-binding cassette superfamily of transport protein. They are separate and distinct from the well-known family of transporter proteins called

Table 8. Pharmacokinetics parameters of bifendate in rats after single use of bifendate in different physical states ($\bar{x} \pm s$, $n = 6$).

Index	$t_{1/2K_a}$(hours)	$t_{1/2\alpha}$(hours)	$t_{1/2\beta}$(hours)	AUC (μg/mh)	AUMC (μg/mL)	MRT (hours)	t_{max} (hours)	C_{max} (μg/mL)
Bifendate	0.078 ± 0.008	0.110 ± 0.149	11.0 ± 0.25	2.46 ± 0.072	38.8 ± 2.02	15.7 ± 0.367	0.203 ± 0.003	0.208 ± 0.002
Physical mixture	0.034 ± 0.001*	1.09 ± 0.096	15.4 ± 0.342*	2.60 ± 0.125	56.0 ± 3.62*	21.5 ± 0.489*	0.220 ± 0.003	0.163 ± 0.003*
Bifendate-SD	0.160 ± 0.027*#	0.123 ± 0.015*#	8.59 ± 0.236*#	3.05 ± 0.072*#	36.2 ± 1.76*#	11.6 ± 0.292*#	0.248 ± 0.001*#	0.488 ± 0.003*#

*$p < 0.05$, versus group bifendate.
# $p < 0.05$, versus group physical mixture.

P-glycoproteins. The overexpression of MRPs is, in part, responsible for the drug resistance seen in many tumors. Some of the MRPs are found ubiquitously in normal human tissue and thus may affect drug distribution, metabolism and efficacy. Probenecid was added as the MRP inhibitor and the result showed there was no effect on bifendate intestinal absorption. Results of these parts showed bifendate intestinal absorption was neither active transport as the process was independent of energy nor facilitated diffusion as the process was not related to carriers. Considering the results gained above, it could be inferred that bifendate intestinal absorption was passive transport. All of the intestinal absorption experiment indicated that ileum and jejunum had better absorption, while colon did the poorest. It is showed that the best absorb sites of bifendate were ileum and jejunum; however, these two parts of intestines were longer than the other two. Therfore, it is suitable to prepare sustained-release dosage form to extend the time bifendate remains in ileum and jejunum. The longer the bifendate stayed in the two parts of the intestine, the more it was absorbed[31,32]. Moreover, experiment on solid state of bifendate in different systems showed that, at the same concentration, SD played better intestinal absorption. It is indicated that the structure of the SD contributed to the intestinal absorption and not the simple addition of PVP_{K30}[33,34]. As in the SD system, bifendate dispersed in the carrier greatly; however, the distance between bifendate molecules in SD was farther than that in native bifendate. More bifendate molecules could get in contact with water molecules in a higher dispersed state; therefore, SD had better solubility and dissolubility[35–37]. As the bifendate intestinal absorption was passive transport, it could be easily concluded that SD had better intestinal absorption compared to native bifendate and the PM. For PM, the intestinal absorption was even worse compared with native bifendate; this may be attributed to the free PVP_{K30} that prevented the bifendate dissolution and intestinal absorption. The better intestinal absorption caused the higher drug blood plasma concentration directly; the intestinal absorption data of solid state of bifendate in different systems were consistent with the corresponding pharmacokinetics data.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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