Study on the Binding Rate of Oral Drugs with Pepsin and Trypsin

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Abstract

In order to find a research method to quickly predict the effects of oral drugs on the human digestive system, in this paper, a mathematical model of the binding rate of pepsin and trypsin was established by using the binding constant Ka and the number of binding sites n (n=1) of digestive protease (pepsin, trypsin) and common oral drugs reported under the simulative human physiological conditions. Using this model, the drug binding rate and protein binding rate of different drugs with pepsin and trypsin were calculated, which could predict the influence of drugs on human digestive function. The results showed that the effect of the drug speculated by the method on the digestive function of the human body was basically consistent with the side effects of the drug described in the drug manual. The method is simple and rapid, and provides an auxiliary means for studying the influence of oral drugs on human digestive function. This method has found a new way of thinking about the effect of oral drugs on human digestive function.

Introduction

All over the world, oral drugs show a stable development trend. Oral drugs have the advantages of easy to carry, oral, easy to preserve and so on, and are widely accepted by people. Nowadays, there are more and more kinds of oral new drugs, but most of the prediction of the side effects of new drugs can only be completed by clinical trials. The process of drug absorption by the human body is a very complex process. The way of oral administration is safe and convenient, but after taking it, it may have a certain impact on the intestines and stomach, for the new drug that has just been put into production. The effect of medication on gastrointestinal function is often difficult to determine. Therefore, it is particularly important to find a suitable method to simply predict the side effects of oral drugs on gastrointestinal function.

Pepsin (PEP) is a digestive protease released by the main cells in the stomach and degraded into peptides by cleavage hydrophobicity and preferred peptide bonds between aromatic amino acids [1]. It has a single polypeptide chain with 324 amino acid residues with a molecular weight of 35000 Da [2]. PEP is stored as pepsinogen and released only when food protein enters the stomach [3]. PEP is a monomer L-protein composed of two

homologous domains, and its most suitable pH is 1.5~2.0 [4]. Two aspartic acid residues Asp32 and Asp215 form the catalytic site of PEP, one of which must be protonated and the other deprotonated to make the protein active. The optimum temperature of PEP was between 37 $^{\circ}$C and 42 $^{\circ}$C [5]. The digestion of food in the stomach is mainly the preliminary decomposition of protein, dietary protein is difficult to absorb macromolecules. In order to let digestive tract cells, absorb nutrition in the protein, it is necessary to decompose the protein into smaller particles called peptides. PEP is a digestive enzyme that plays a major role in the digestion of proteins into polypeptides. At pH 1.5~5.0, it decomposes the ingested proteins into peptides, and the other part is broken down into amino acids necessary for human activity, such as tyrosine, phenylalanine and so on. Without PEP, the ability of the human body to metabolize protein will be severely inhibited.

Trypsin (TRP) is an important proteolytic enzyme secreted by the pancreas. It plays an irreplaceable role in protein digestion in the small intestine and contains 150-600 μg/ml in duodenal fluid. It can hydrolyze amino acid compounds linked by peptide chains, has esterase activity, and has little content in normal serum. TRP not only plays an important role as digestive enzyme in physiological process, but also plays an important role in hemostasis, apoptosis, signal transduction, reproduction and immune response [6,7]. The molecular weight of TRP is 23300, which is composed of 223

amino acid residues [8], of which 4 tryptophan, 10 tyrosine and 6 phenylalanine are its inherent fluorescent groups [9,10]. TRP is composed of two domains of almost the same size. the two domains are connected by disulfide bonds, and each domain is composed of six reverse parallel β-folds. Among them, His residue, Asp residue and Ser residue are the catalytic active centers of TRP, which constitute the structure of catalytic triplet [11]. TRP is excreted from the pancreas into the small intestine and is involved in the digestion of proteins in food and it is usually used as an important digestive protease model to study the interaction between drugs and proteins [12].

There are many reports on the interaction between small molecular drugs and digestive proteases by spectroscopy, but most of them focus on the binding mechanism between drugs and proteins. The binding constant and number of binding sites between small molecular drugs and digestive protease were used to quantitatively study the effect of the binding of small molecular drugs and digestive protease on the free concentration of digestive protease. Furthermore, it is inferred that the effect of combination on the efficacy and gastrointestinal digestive function of the human body has not been reported in the literature. In this paper, the binding constants K_a and binding sites n of some oral drugs with PEP and TRP were obtained by spectroscopy and the drug binding rate and protein binding rate were calculated by the established mathematical model. The effect of oral drugs combined with digestive protease on the efficacy of drugs and the effect of drugs on gastrointestinal digestive function of human body were quantitatively studied. The method is simple and practical, and the conclusions are basically consistent with the side effects shown in the drug instructions.

Mathematical Model

The binding constant and the number of binding sites between the protein and the drug were determined by spectroscopy. When the drug-protein binding reached a dynamic equilibrium, one part of the protein was a free protein and the other part was a binding protein. There is the following relationship between drug concentration Q, protein concentration B, binding constant K_a and number of binding sites n: $[B] + n[Q] \rightarrow [BQ_n]$, where n represents the number of equivalent and independent drug binding sites. If the interaction between B and Q accords with Langmuir monolayer adsorption model [13], the equilibrium constant K_a is:

$$K_a = \frac{[BQ_n]}{[Q]^n[B]} \quad (1)$$

When drug and protein are combined at 1:1, then:

$$K_a = \frac{[BQ]}{[Q][B]} \quad (2)$$

Assuming that the total concentration of drug is Q, the total concentration of protein is B, and the concentration of drug-protein complex is x, then K_a is also expressed as:

$$K_a = \frac{x}{(Q-x)(B-x)} \quad (3)$$

By solving the univariate quadratic equation, the following results are obtained:

$$x = \frac{K_a(Q+B)+1-\sqrt{K_a^2(Q-B)^2+2K_a(Q+B)+1}}{2K_a} \quad (4)$$

The drug combination rate can be expressed as:

$$W(Q) = \frac{x}{Q} \times 100\% = \frac{K_a(Q+B)+1-\sqrt{K_a^2(Q-B)^2+2K_a(Q+B)+1}}{2K_aQ} \times 100\% \quad (5)$$

$$W(B) = \frac{x}{B} \times 100\% = \frac{K_a(Q+B)+1-\sqrt{K_a^2(Q-B)^2+2K_a(Q+B)+1}}{2K_aB} \times 100\% \quad (6)$$

Results and Discussion

Under simulated human physiological conditions, the pH of PEP was about 2.0 and the pH of TRP was about 7.4. According to the commonly used measurement of the drug recorded in the drug instruction of different oral drugs (the drugs and digestive proteases involved in the literature report are from Sigma) and binding constant K_a calculated from fluorescence data at λ_{ex}=280 nm (n of the corresponding common oral drugs and digestive proteases reported in the literature was 1). The drug binding rate and protein binding rate were calculated by **Equation (5)** and **Equation (6)**. The results were shown in Table 1 and Table 2. Based on the data, the effects of the combination of drugs and digestive proteases on the efficacy of these drugs and the digestive function of the stomach and small intestine were inferred.

Drug; K_a (L/mol) ; Common amount (mol/L)	W_B/100%- W_B; Prediction of digestive function in the stomach	W_Q/100%- W_Q; Prediction of drug efficacy	Drug instruction
Levofloxacin 7.50×10^4[14] $2.7 \times 10^{-4} \sim 5.4 \times 10^{-4}$	95.3%~97.6%/4.7%~2.4% The effect on the digestive function of the stomach is extremely significant.	0.012%~0.006%/99.988%~99.994% Does not affect the efficacy.	Abdominal discomfort or pain, diarrhea, nausea or vomiting.
Gatifloxacin 1.01×10^6[15] $5.3 \times 10^{-4} \sim 1.1 \times 10^{-3}$	99.8~99.9%/0.2~0.1% The effect on the digestive function of the stomach is extremely significant.	0.006%~0.003%/99.994%~99.997% Does not affect the efficacy.	Abdominal pain, constipation, indigestion.
Baclofen 1.56×10^2[16] $4.7 \times 10^{-5} \sim 1.2 \times 10^{-4}$	0.73%~1.80%/99.27%~98.20% The effect on the digestive function of the stomach is extremely significant.	0.006%~0.004%/99.994%~99.996% Does not affect the efficacy.	Occasionally mild gastrointestinal dysfunction.
Oxacillin sodium 7.01×10^3[17] $1.2 \times 10^{-3} \sim 2.4 \times 10^{-3}$	89.2%~94.3%/10.8%~5.7% The effect on the digestive function of the stomach is extremely significant.	0.025%~0.013%/99.975%~99.987% Does not affect the efficacy.	Gastrointestinal dysfunction, nausea or vomiting.
Epicatechin 2.04×10^4[18] $8.6 \times 10^{-4} \sim 3.4 \times 10^{-3}$	94.6%~98.6%/5.4%~1.4% The effect on the digestive function of the stomach is extremely significant.	0.0036%~0.0010%/99.9964%~99.999% Does not affect the efficacy.	Abdominal discomfort or pain, heating, nausea, and indigestion.
Gallocatechin gallate 1.78×10^3[18] $8.2 \times 10^{-4} \sim 3.3 \times 10^{-3}$	59.3%~85.4%/40.7%~14.6% The effect on the digestive function of the stomach is extremely significant.	0.025%~0.008%/99.975%~99.992% Does not affect the efficacy.	Abdominal pain, heating, nausea, and indigestion.
Gallocatechin gallate 1.33×10^5[18] $5.5 \times 10^{-4} \sim 2.2 \times 10^{-3}$	98.7%~99.7%/1.3%~0.3% The effect on the digestive function of the stomach is extremely significant.	0.0060%~0.0015%/99.994%~99.9985% Does not affect the efficacy.	Abdominal discomfort or pain, heating, nausea, and indigestion
β-carotene 1.88×10^5[19] $6.7 \times 10^{-6} \sim 8.4 \times 10^{-6}$	55.7%~61.2%/44.3%~38.8% It has a certain influence on the digestive function of the stomach.	0.28%~0.21%/99.72%~99.79% Does not affect the efficacy.	Headache, occasional gastrointestinal discomfort, hepatosplenomegaly.
Astaxanthin 1.90×10^6[19] $6.7 \times 10^{-6} \sim 2.0 \times 10^{-5}$	92.7%~97.4%/7.3%~2.6% The effect on the digestive function of the stomach is extremely significant.	0.48%~0.16%/99.9940%~99.9985% Does not affect the efficacy.	Digestive disorders.
Tolvaptan 1.57×10^4[20] $3.3 \times 10^{-5} \sim 6.7 \times 10^{-5}$	34.4%~51.2%/65.6%~48.8% It has a certain influence on the digestive function of the stomach.	0.035%~0.025%/99.965%~99.975% Does not affect the efficacy.	Occasionally dry mouth, thirst, dizziness, nausea.
Daidzein 1.40×10^5[21] $9.8 \times 10^{-5} \sim 2.0 \times 10^{-4}$	93.2%~96.5%/6.8%~3.5% The effect on the digestive function of the stomach is extremely significant.	0.032%~0.016%/99.968%~99.984% Does not affect the efficacy.	Gastrointestinal reactions such as nausea, bloating, and indigestion.
Metformin 1.50×10^2[22] $3.9 \times 10^{-3} \sim 5.8 \times 10^{-3}$	36.9%~46.5%/63.1%~53.5% It has a certain influence on the digestive function of the stomach.	0.0032%~0.0027%/99.9968%~99.9973% Does not affect the efficacy.	Occasionally diarrhea, abdominal pain, bloating, indigestion.
Frofloxacin 9.14×10^6[23] $5.4 \times 10^{-4} \sim 1.1 \times 10^{-4}$	99.6%~99.9%/0.4%~0.1% The effect on the digestive function of the stomach is extremely significant.	0.0064%~0.0032%/99.9936%~99.9968% Does not affect the efficacy.	Nausea, vomiting, abdominal discomfort, diarrhea, loss of appetite, abdominal pain, indigestion.
Cefixime 1.02×10^4[24] $2.2 \times 10^{-4} \sim 4.4 \times 10^{-4}$	95.7%~97.8%/4.3%~2.2% The effect on the digestive function of the stomach is extremely significant.	0.015%~0.008%/99.985%~99.992% Does not affect the efficacy.	Soft stools, diarrhea, upset stomach, loss of appetite, heating.

Cefetamet pivoxil $1.17 \times 104[25]$ $9.8 \times 10^{-4} \sim 2.0 \times 10^{-3}$	92.0%~95.9%/8.0%~4.1% The effect on the digestive function of the stomach is extremely significant.	0.0032%~0.0016%/99.9968%~99.9984% Does not affect the efficacy.	Diarrhea, nausea, vomiting.

Table 1: Protein binding rate and drug binding rate of oral drugs and PEP and its effects after taking the drug.

The normal basal gastric juice volume was 10~100 ml, and the PEP concentration was calculated according to the basal gastric juice volume of 50 ml: $2.6 \times 10^{-8} \sim 4.3 \times 10^{-8}$ [26].

The most important reference in the study of drug-digestive protease binding rate is protein binding rate and drug binding rate. The higher the protein binding rate, the less the free digestive enzymes, the digestive function of the digestive system will also decrease; the greater the drug binding rate, the less the free drugs, the efficacy will also be reduced. After oral drugs enter the human body, their effective components will be absorbed in the human digestive system, most of the effective components of drugs through the gastrointestinal mucosa into the human blood circulation system to act on the affected area. As shown in Figure 1, taking the binding reaction of levofloxacin with PEP as an example, the binding model of levofloxacin and pepsin was obtained by nonlinear fitting: $W(B) = -1.894 \times 10^4 Q^2 + 2.374 \times 10^2 Q + 0.9029$, $r = 0.9985$; $W(Q) = 5.286 \times 10^2 Q^2 - 0.6412 + 2.553 \times 10^{-4}$, $r = 0.9978$. In the range of the actual dosage of levofloxacin, the binding rate data of the drug and PEP, the free rate of the drug and the free rate of PEP can be obtained by combining the model. The effect of the interaction between levofloxacin and PEP on the efficacy and digestibility can be discussed by the data of binding rate and free rate. After oral administration of the tablet into the digestive system, the starch substance in the tablet is digested, and the effective component of the drug enters the gastric juice in contact with PEP and then binds within the actual dosage range of levofloxacin, The protein binding rate between the drug and PEP was 95.3%~97.6%, and the free PEP was only 4.7%~2.4%, indicating that the digestive capacity of the stomach was greatly reduced due to the binding of levofloxacin. The drug binding rate of levofloxacin was 0.012%~0.006%, and the free rate of levofloxacin was 99.988%~99.994%, which indicated that the efficacy of oral levofloxacin was almost not affected by PEP binding.
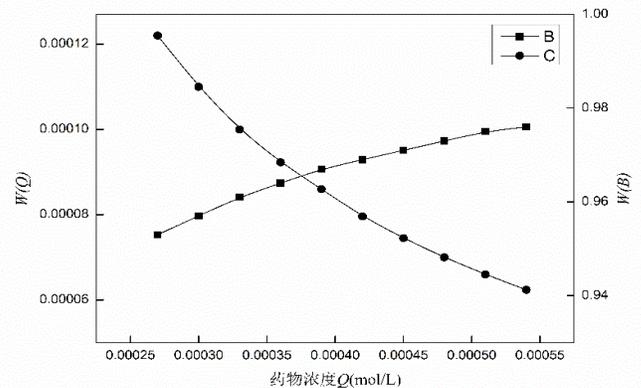

Figure 1: Binding model of levofloxacin and PEP.

Table 1 lists data on the binding rate of some of the oral drugs often exposed to PEP in life. After the combination of oral drugs with gastric protein, the protein binding rate of most oral drugs, such as levofloxacin, gatifloxacin and so on, is relatively high. Based on the results of the data, it is speculated that taking these drugs has a greater impact on gastric digestive function. According to the adverse drug reactions recorded in the drug instructions, most of these drugs cause gastrointestinal dysfunction, the common manifestations are abdominal discomfort or pain, diarrhea, nausea or vomiting. However, there are also some oral drugs with low binding rate to PEP, such as baclofen, torvastatin and metformin. The free amount of PEP is less affected by the combination of drugs and PEP, so these drugs have little effect on gastric digestive function after oral administration. According to the adverse reactions recorded in the drug instructions, stomach digestive discomfort occasionally occurs after taking these drugs, but the symptoms are relatively mild.

Drug; Ka (L/mol)；Common amount (mol/L)	W_B/100%-W_B Prediction of intestinal digestive function	W_Q/100%-W_Q Prediction of drug efficacy	Drug instruction
Ciprofloxacin hydrochloride 7.74×10^4[27] $6.5 \times 10^{-4} \sim 1.3 \times 10^{-3}$	98.0%~99.0%/2.0%~1.0% Significant effect on intestinal digestive function	2.32%~1.19%/97.68%~98.81% Does not affect the efficacy	Abdominal discomfort or pain, diarrhea, nausea or vomiting
Ofloxacin 4.52×10^6[28] $5.5 \times 10^{-4} \sim 1.1 \times 10^{-3}$	99.95%~99.96%/20.05%~0.04% Significant effect on intestinal digestive function	2.83%~1.41%/97.17%~98.59% Does not affect the efficacy	Abdominal discomfort or pain, diarrhea, nausea or vomiting
Enoxacin 3.93×10^4[28] $6.2 \times 10^{-4} \sim 1.3 \times 10^{-3}$	95.9%~98.0%/4.1%~2.0% Significant effect on intestinal digestive function	2.42%~1.18%/97.58%~98.82% Does not affect the efficacy	Common abdominal discomfort or pain, diarrhea, nausea or vomiting
Norfloxacin 1.08×10^5[29] $1.3 \times 10^{-3} \sim 1.8 \times 10^{-3}$	99.3%~99.5%/0.7%~0.5% Significant effect on intestinal digestive function	1.24%~0.86%/98.76%~99.14% Does not affect the efficacy	Abdominal discomfort or pain, diarrhea, nausea or vomiting
β-carotene 2.28×10^5 [19] $6.7 \times 10^{-6} \sim 8.4 \times 10^{-6}$	34.2%~40.3%/65.8%~59.7% Have a certain influence on the digestive function of the intestine	62.5%~60.23%/37.5%~39.77% It has a greater impact on the efficacy of the drug.	Mild abdominal discomfort, diarrhea
Astaxanthin 2.39×10^6 [19] $6.7 \times 10^{-6} \sim 2.0 \times 10^{-5}$	53.2%~82.2%/46.8%~17.8% Have a certain influence on the digestive function of the intestine	86.31%~61.82%/13.69%~38.18% It has a greater impact on the efficacy of the drug.	Digestive dysfunction
Vitamin B_1 4.83×10^5[30] $3.3 \times 10^{-6} \sim 5.0 \times 10^{-6}$	23.6%~33.4%/76.4%~67.6% Have a certain influence on the digestive function of the intestine	78.8%~75.3%/21.2%~24.7% It has a greater impact on the efficacy of the drug.	Headache, loss of appetite
Vitamin B_9 1.30×10^5[30] $1.1 \times 10^{-5} \sim 2.3 \times 10^{-5}$	64.3%~82.7%/35.7%~17.3% Have a certain influence on the digestive function of the intestine	67.5~56.3%/32.5%~43.7% It has a greater impact on the efficacy of the drug.	Loss of appetite
α-tocopherol 3.02×10^3[31] $6.9 \times 10^{-6} \sim 4.6 \times 10^{-5}$	1.45%~11.7%/98.55%~88.3% It has a slight effect on the digestive function of the intestines.	4.56%~3.95%/95.44%~96.05% The effect is almost unaffected	Occasionally, loss of appetite, vomiting, diarrhea
Tolvaptan 2.22×10^4[20] $3.3 \times 10^{-5} \sim 6.7 \times 10^{-5}$	38.0%~56.3%/62.0%~43.7% Have a certain influence on the digestive function of the intestine	17.1%~12.8%/82.9%~87.2% The effect on the efficacy is small.	Nausea, vomiting, loss of appetite
Colchicine tablets 1.06×10^4[32] $2.5 \times 10^{-6} \sim 5.0 \times 10^{-6}$	2.25%~4.39%/97.75%~95.61% It has a slight effect on the digestive function of the intestines.	13.3%~13.1%/86.7%~86.9% The effect on the efficacy is small.	Occasionally nausea, loss of appetite
Rutin 1.58×10^4[33] $3.3 \times 10^{-5} \sim 6.6 \times 10^{-5}$	30.8%~49.6%/69.2%~50.4% Have a certain influence on the digestive function of the intestine	14.1%~11.1%/85.9%~88.9% The effect on the efficacy is small.	Occasionally gastrointestinal reactions, manifested as nausea and constipation

Table 2: Protein binding rate and drug binding rate of oral drugs and TRP and its effects after taking the drug.

Determination of TRP concentration in duodenal fluid by enzyme rate method: $6.3 \times 10^{-6} \sim 2.5 \times 10^{-5}$ mol/L in fasting state, the volume of intestinal fluid was 45~319 mL, the average value was 105 mL, and the concentration of trypsin in duodenal fluid was determined by enzyme rate method [34].

After oral drugs enter the human body, the main part of absorption is the small intestine. As shown in Figure 2, taking the binding reaction of enoxacin with TRP as an example, the binding model can be obtained by nonlinear fitting according to the obtained data: $W(B) = -3.338 \times 10^4 Q^2 + 0.9450 \times 10^2 Q + 0.9135$, r = 0.9988; $W(Q) = 1.827 \times 10^4 Q^2 - 0.5295 \times 10^2 Q + 0.04982$, r = 0.9982. In the range of the actual dosage of enoxacin, the binding rate data of the drug to TRP, the free rate of the drug and the free rate of TRP can be obtained by the binding model. When enoxacin interacts with TRP, the effects of efficacy and digestibility can be discussed by the binding rate and free rate data.

In the range of the actual dosage of the oral drug, after the drug molecule enters the small intestinal fluid with the digestive system, the TRP in the small intestinal fluid is in contact with the drug molecule. The free rate of TRP was reduced from 100% to 4.1%~2.0% due to the binding of enoxacin to TRP, which indicated that the number of free TRP was very small due to the binding of enoxacin to TRP. That is to say, enoxacin has a great effect on the digestive function of the small intestine. The content of free enoxacin was 97.58%~98.82%. The results showed that the efficacy of enoxacin was almost not affected by the binding effect.
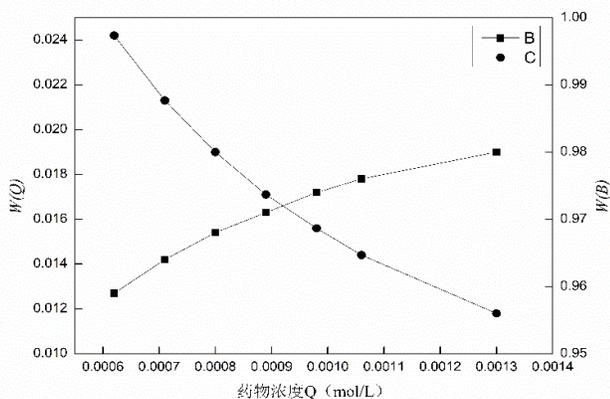

Figure 2: Binding model of enrofloxacin and TRP.

From the data in Table 2, it can be seen that the protein binding rate of ciprofloxacin hydrochloride, ofloxacin, enoxacin, norfloxacin and vitamin B_9 is high, so that the amount of free TRP is very small, The results showed that the combination of drugs and TRP had a significant effect on the digestive function of the small intestine, so the common adverse symptoms after taking these drugs were abdominal discomfort or pain, diarrhea, nausea or vomiting, which were consistent with the drug instructions. Other oral drugs, such as vitamin B_1, α-tocopherol, etc., have relatively low protein binding rates to TRP, indicating that the binding of drugs to TRP has little effect on the digestive function of the small intestine. This is consistent with the fact that the occasional adverse symptoms after taking these drugs are anorexia and constipation, and the discomfort symptoms are relatively mild.

From the results of Table 1 and Table 2, it can be seen that the effects of common oral drugs on digestive function predicted by the protein binding rate of digestive proteases to drugs are consistent with the adverse drug reaction symptoms recorded in the drug instructions. Therefore, it is feasible to infer the effect of drugs on digestive function according to the protein binding rate between drugs and digestive proteases. In addition, the effect of digestive protease on the efficacy of common oral drugs can also be simply predicted. most of the effects of pepsin on drug efficacy can be ignored, and TRP has a greater impact on the efficacy of some drugs, such as astaxanthin, vitamin B_1, vitamin B_9 and so on.

In fact, it is a very complex process for drugs to be absorbed into the human body. Many drugs are actually absorbed in the digestive system in the stomach and small intestine. The spectral experiment is only a simple estimation of the binding rate of the system according to the drug dose, the binding constant and the number of binding sites of the drug and protein under simulated physiological conditions. This method is compared with equilibrium dialysis [35], high performance liquid chromatography [36], ultrafiltration [37], microdialysis [38] and liquid chromatography-tandem mass spectrometry [39], which are commonly used to study the binding rate of drugs and proteins. There will be some errors in the binding constant and the number of binding sites, resulting in a small number of adverse reactions predicted by this method are not completely consistent with the adverse reactions reported by the drugs. However, the experimental equipment of these commonly used binding rate research methods is expensive, the test period is long and the concentration range of the test drug is narrow. The method of using spectroscopy to study the protein binding rate is simple to operate and has a wide range of application, so this method is desirable. As far as the efficacy is concerned, there are many physiological factors that affect the efficacy, such as digestive system factors, circulatory system factors and disease factors. The effect of enzymes in gastric juice and intestinal juice on the efficacy belongs to the influence of digestive system factors on the efficacy, accounting for only a small part of the impact. Therefore, simply using the combination of digestive enzymes and drugs to study the drug efficacy and the effect of drugs on human digestive function, the conclusion will be different from the actual situation.

Conclusion

Under simulated physiological conditions, the binding rate of drugs to digestive proteins can be easily and quickly obtained by spectroscopy and the effects of some new oral drugs on the digestive function of human stomach and small intestine can be simply predicted by this method. Because the action process of drugs in human body is a very complex mechanism, there is a certain error between the predicted results of a small number of oral drugs and the actual reported results. The method provided in this paper provides a simple reference for the rapid judgment of the clinical safety of drugs, and has a certain reference value for the rapid judgment of the effect of new drugs on gastrointestinal function.

Acknowledgements

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