# The study on intestinal absorption kinetics of harmine hydrochloride in rats *in situ*

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**ABSTRACT: OBJECTIVE** To provide biological pharmaceutical basis for dosage forms design of harmine hydrochloride by studying its absorption kinetics in various intestinal segments of rat. **METHOD** The absorption kinetics of harmine hydrochloride from duodenum, jejunum, ileum, and colon were investigated using in situ perfusion method in the rats. The drug concentrations in cycling system were determined using RP-HPLC. **RESULTS** The absorption rate constants (Ka) of harmine hydrochloride were (0.3092 ±0.059), (0.2064 ±0.044), (0.2858 ±0.081), (0.2009 ±0.080) $h^{-1}$ at duodenum, jejunum, ileum, and colon, respectively. **CONCLUSION** Harmine hydrochloride was absorbed perfectly in the duodenum, jejunum, ileum, and colon.

**KEY WORDS:** harmine hydrochloride; absorption kinetics; in situ perfusion method

# 盐酸去氢骆驼蓬碱大鼠肠吸收动力学的研究

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摘要:目的　研究盐酸去氢骆驼蓬碱在大鼠胃肠道各段的吸收动力学特征,为其剂型设计提供生物药剂学依据。方法　采用大鼠在体肠循环法分别研究盐酸去氢骆驼蓬碱在大鼠十二指肠、空肠、回肠及结肠中的吸收动力学特征。采用反相高效液相色谱法测定循环液中的药物浓度。结果　盐酸去氢骆驼蓬碱在十二指肠、空肠、回肠及结肠中的吸收速度常数 Ka 分别为:(0.3092 ±0.059), (0.2064 ±0.044), (0.2858 ±0.081), (0.2009 ±0.080) $h^{-1}$。结论　盐酸去氢骆驼蓬碱在十二指肠、空肠、回肠及结肠中均能较好地吸收。

关键词:盐酸去氢骆驼蓬碱;吸收动力学;在体肠循环法

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*Peganum harmala* L., a plant of zygopyllaceae, used by Uygur and Mongolian. It was reported by the Recorder of Ethnopharmacology of China that its seeds usually was used as folk medicine[1]. It was reported early in 1841 that the ingredients in *Peganum harmala* L. were alkaloids, including harmine, harmaline, harmalol, harman, and etc.[2,3]. Since 1980, the total alkaloid preparation was used to treat malignant tumor of digestive tract in clinic and laboratory in China with an effective rate of treating twenty-one cases about 85.7%[4]. The antineoplastic activity of *Peganum harmala* L. has attracted the wide attention of medical world in China[5~10].

Harmine is a main active alkaloid in seeds of *Peganum harmala* L[5]. It is very meaningful for dosage form design that the characteristics of absorption of harmine in individual intestinal segments were examined clearly. There were several useful *in vitro* and in vivo systems already in existence for studying drug absorption[11~15]. *In situ* perfusion of intestinal segments of the rat was frequently used to study the absorption kinetics of drugs because the physiological condition of rat is similar to humans'. Thus, the in situ perfusion of intestinal segment of the rat was employed to study the intestinal absorption kinetics of harmine hydrochloride.

## 1　Equipment and materials

### 1.1　Equipment

A peristaltic pump (model RDB-6B, Instrument Plant of Shazhou County, Jiangsu Province), an UV-VIS spectrophotometer (model DMS-100, Varian Co. USA.), a constant temperature water-bath (Medical Apparatus and Instrument Factory of Peking), a multifunctional magnetic stirrer (Electric Appliance Plant of Tianchang City, Anhui Province). an HPLC

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system (model SSI-2001 with series Ⅳ pump, chromatography data system, model 525 UV absorbance detector, ODS $C_{18}$ column, 7725I injector, and model SSI-OV100 column oven, Science Systems Inc. USA.) was used.

**1.2** Materials

Reference standard of harmine hydrochloride was purchased from Sigma Co. USA. Raw material of harmine hydrochloride was supplied by Department of Pharmaceutical, College of Pharmacy, Xinjiang Medical University (content was of 99.8%). Tinidazole was used as internal standard and was of pharmaceutical. All other chemicals were of analytical or chromatographic grade.

**1.3** Test animal

Wistar albino rats weighing (225 ± 25) g were purchased from Medical Experimental Animal Center of Xinjiang. The grade was first grade and the certificate number was 16-001.

## 2 Experiment method and results

**2.1** Establishment of analysis

**2.1.1** Preparation of standard solutions The reference standard of harmine hydrochloride, which was dried to constant weight at 80 ℃, weight of 0.0234g was exactly weighed and dissolved with water to 25 mL. The standard solution of harmine hydrochloride of concentration of 0.936 mg/mL was prepared for reservation. Phenolsulfonphthalein standard solution was prepared by weighing exactly 0.0451g and being dissolved with water to 200 mL. And it's concentration was 0.2255 mg/mL. Tinidazole weight of 0.10042g, which was dried to constant weight at 105 ℃, was exactly weighed and was dissolved with water to 100 mL and a internal standard solution of 1.0042 mg/mL was prepared for reservation.

**2.1.2** Selection of detector wavelength The determining solution of harmine hydrochloride (4.68 mg/mL), tinidazole(10.042 mg/mL), and phenolsulfonphthalein (11.275 mg/mL) were prepared with flow phase, respectively. Solutions were scanned from 200 to 400nm by DMS-100 UV-VIS spectrophotometer, respectively. The results showed that harmine hydrochloride had maxim absorbance at 321nm and tinidazole and phenolsulfonphthalein also had ultraviolet absorption. Thus the 321nm was determined as detector wavelength.

**2.1.3** Chromatographic condition To extract the standard solutions of harmine hydrochloride, phenolsulfonphthalein, and tinidazole just the right amount, respectively and dilute to concentration of 7.448 mg/mL, 9.02 mg/mL, and 6.0252 mg/mL with flow phase. 20μL of above dilutions was injected into HPLC. The mobile phase was methanol-0.01 mol/L ammonia sulfate (60∶40, using phosphoric acid adjust pH to 3.91). The flow rate was about 1.0 mL/min and the column temperature was (30 ± 1) ℃. The detector wavelength was 321nm. The chromatogram has shown that the resolution among the three components was satisfied and the retention time of harmine, tinidazole, and phenolsulfonphthalein were 4.8, 3.9, and 3.2 min, respectively (seeing Fig 1). The number of theoretical plates of harmine hydrochloride was not less than 2000.

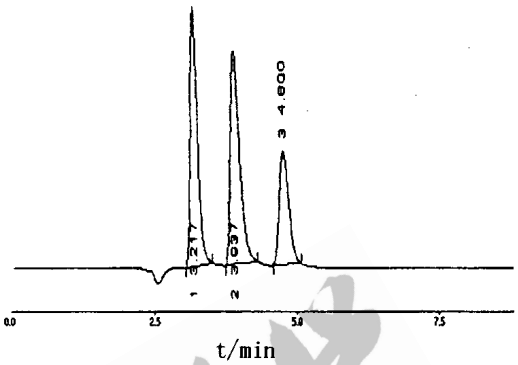


**Fig 1** HPLC Chromatogram of tinidazole (1), phenolsulfonphthalein(2), and harmine(3)

图 1

**2.1.4** Preparation of standard curve To extract the standard solutions of harmine and phenolsulfonphthalein just the right amount, respectively, into same 25 mL flasks and added equal amount of internal solution 0.75 mL (extracted the standard solution of tinidazole 5.0 mL and diluted to 25 mL with flow phase). Then get a series of concentration of harmine hydrochloride of 1.871, 3.744, 7.488, 14.976, 22.464, 29.952 mg/mL and of phenolsulfonphthalein of 2.255, 4.510, 9.020, 15.785, 22.550, 31.570 mg/mL respectively. Amount of 20μL solution was injected into HPLC at the conditions introduced above and recorded the chromatogram. The peak area of harmine (Ah), tindizole (As), and phenolsulfonphthalein (Ap) were recorded, reppectively. A suitable calibration curve between harmine levels and peak area rate: C(mg/mL) = 2.6203 Ah/As-0.7314 ($r$ = 0.9999), and between phenolsulfonphthalein levels and peak area rate: C(mg/mL) = 16.7952 Ap/As-0.0753 ($r$ = 0.9999), were attained, respectively. The calibration graph was linear for 2 ~ 30 mg/mL of harmine hydrochloride and phenolsulfonphthalein, respectively.

**2.1.5** Determination of recovery To extract the standard solutions of harmine hydrochloride, phenolsulfonphthalein, and tinidazole just the right amount, respectively, into same 10 mL flask, in which contains 1.0 mL of blank intestinal perfusion solution, and added flow phase to scale circle. The solution was filtered with suitable microporous filtering film of 0.45μm and the filtrate of 20μL were injected into HPLC. Determined the peak area according to the method of preparation of standard curve. The recoveries were calculated by putting the peak area of harmine hydrochloride and phenolsulfonphthalein respectively into individual standard curve. The results of recovery were shown in Table 1.

**Tab 1** The results of recovery test ( $n = 5$)

**表 1 回收率试验结果**

| Harmine hydrochloride | | | | phenolsulfonphthalein | | | |
|---|---|---|---|---|---|---|---|
| Add. amount (μg/mL) | Det. amount (μg/mL) | recovery (%) | Average recovery (%) | Add. amount (μg/mL) | Det. amount (μg/mL) | recovery (%) | Average recovery (%) |
| 1.871 | 1.879 ±0.04 | 100.4 ±2.1 | | 2.255 | 2.251 ±0.01 | 99.8 ±0.6 | |
| 7.488 | 7.523 ±0.13 | 100.5 ±1.7 | 100.6 ±0.3 | 9.020 | 8.933 ±0.04 | 99.0 ±0.4 | 99.3 ±0.4 |
| 22.464 | 22.661 ±0.26 | 100.9 ±1.2 | | 22.550 | 22.351 ±0.02 | 99.1 ±0.1 | |

**2.1.6** Precision and repeatability The samples of different concentration were determined in one day and in different day. The precision and repeatability were calculated, as illustrated in Table 2.

**Tab 2** The results of precision and repeatability ( $n = 5$)

**表 2 精密度及重复性实验结果**

| Harmine hydrochloride | | | Phenolsulfonphthalein | | |
|---|---|---|---|---|---|
| Conc. (μg/mL) | *RSD*% of intraday | *RSD*% of interday | Conc. (μg/mL) | *RSD*% of intraday | *RSD*% of interday |
| 2.048 | 2.68 | 2.37 | 2.255 | 0.74 | 1.48 |
| 7.488 | 1.45 | 1.36 | 9.020 | 0.37 | 0.73 |
| 22.464 | 0.51 | 0.54 | 22.550 | 1.45 | 1.39 |

**2.2** Absorption experiments

**2.2.1** The Preparation of perfusion solution The perfuse solution consisted of harmine hydrochloride dissolved in Tyrode solution (pH7.4 ~ 8.1). The phenolsulfonphthalein was added to the perfusion solution to act as a nonabsorbable marker to help identify any transfer of water into or out of the intestinal lumen and to monitor the structural integrity of the intestinal segment. The concentration of harmine hydrochloride and phwnolsulfonphthalein in perfusion solution were 10.45 and 39.539 mg/mL, respectively. The perfusion solution was freely prepared shortly before starting the experiments.

**2.2.2** Test method Wistar albino rats (200 ~ 250g) were fasted with free access to drinking water 24 hour prior to surgery. The rats were given 20% urethane solution (1mL/100g, body weight) abdominal injection 1/2 hour prior to surgery. When the rats were anesthetized, it was fixed dorsally position on surgical board. The small intestine was exposed by a midline abdominal incision about 3 ~ 4cm long and a intestinal segment (10-cm) of duodenum, jejunum, ileum, and colon with intact blood supply was isolated from the abdominal cavity. Two sides of the segment were incised obliquely and two glass cannulas (3mm I.D., 5-mm O.D.) were inserted into the segment and were ligated with silk suture. Silicon perfusion tubes were connected with these cannulas and with peristaltic pump. Before starting the perfusion, the segments were cleaned by rinsing it with isotonic saline of 37℃ temperature. At starting perfusion, 50mL perfusion solution was pumped at rate of 5mL/min through a heat exchange device to bring the solution to body temperature (-37℃). After 10min of equilibration, 2.0mL of the perfusion fluid were withdrawn in order to determine the initial concentration of harmine hydrochloride and phenolsulfonphthalein. Then the perfusion rate was adjusted to 2.0mL/min. All perfusion were performed at rate of 2.0mL/min for 3 hours. A magnetic stirrer stirred the perfusion solution. During perfusion experiments, perfusion samples of 2.0mL were collected into 5mL flask at designed intervals for analysis of harmine hydrochloride and phenolsulfonphthalein. 2.0mL Tyrode solution containing phenolsulfonphthalein 39.539 mg/mL was supplemented to perfusion solution contemporaneously. At the completion of each perfusion experiment, the entire intestinal segment was excised from each animal. The length and radius of intestinal were determined for calculating the absorption area.

**2.2.3** Analytical methods The concentration of harmine hydrochloride and phenolsulfonphthalein in perfusion solution was determined by HPLC. Perfusion samples (2.0mL) were diluted with Tyrode solution to 5.0mL after the addition of interval standard (tinidazole). The solution was filtered using suitable microporous filtering film of 0.45mm and the filtrate of 20mL were injected into HPLC for simultaneous determining the concentration of harmine and phenolsulfonphthalein, respectively.

## 3 Results

The residual amount of drug in perfusion solution was calculated by the method of Ping[16]. From the semilog plot of the residual amount of drug in perfusion fluid versus time, the absorption constant (Ka) and the half-life of absorption ($t_{1/2}$) was

calculated. In addition, the total absorption rate (PA%) in 3 hours and the absorption rate per area per unit time (mg.cm$^{-1}$.min$^{-1}$) (R%) were calculated from data determined. All these results were summarized in Table 3 and were presented in Figure 3. The statistic results of absorption constant (Ka) at different intestinal segments of rat were analyzed using SAS (Statistical Analysis System, SAS Institution, USA) software program and were summarized in Table 4 and 5.

The absorption constant (Ka) of harmine hydrochloride at different intestinal segment was statistically different from each other, as illustrated in Table 4. There was statistically significant difference ($P<0.05$ or $P<0.01$) in the Ka between duodenum and jejunum, ileum, and colon. And there was no significant difference ($P>0.05$) in the Ka of jejunum, ileum, and colon, as illustrated in Table 5.

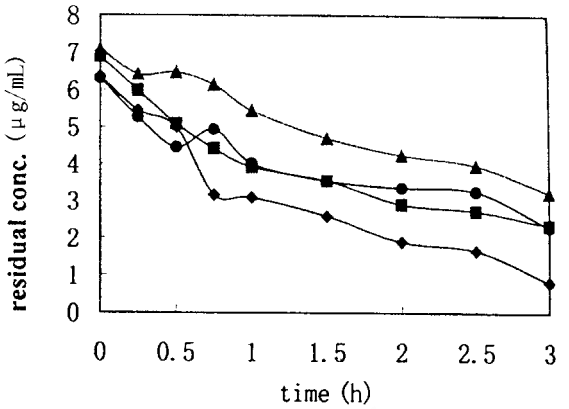


**Fig 2** The graph of residual concentration of harmine hydrochloride in perfusion fluid verse time (◆: duodenum; ■: ileum; ▲: jejunum; ●: colon;)

图 2 循环液中盐酸去氢骆驼蓬碱浓度与时间曲线(◆:十二指肠;■:空肠;▲:回肠;●:结肠;)

**Tab 3** Parameters of absorption of harmine hydrochloride at different intestinal segment in rat ($n=5$)

表 3 盐酸去氢骆驼蓬碱在大鼠小肠不同部位的吸收参数

| parameters | duodenum | jejunum | ileum | colon |
|---|---|---|---|---|
| dose(mg) | 0.5225 | 0.5225 | 0.5225 | 0.5225 |
| Ka($h^{-1}$) | 0.3092±0.059 | 0.2064±0.044 | 0.2858±0.081 | 0.2009±0.080 |
| $T_{1/2}$(h) | 1.809±0.304 | 3.479±0.695 | 2.549±0.530 | 3.993±1.767 |
| PA% | 69.99±10.06 | 48.30±11.89 | 57.80±6.60 | 47.14±12.45 |
| R%(mg/mL/min) | 22.30±9.19 | 22.88±11.63 | 14.75±2.06 | 10.49±2.17 |

**Tab 4** Analysis of variance of absorption constant (Ka)

表 4 吸收常数方差分析

| parameter | Source | Sum of square | DF | Mean square | F value | P value |
|---|---|---|---|---|---|---|
| Ka | In groups | 0.3207 | 4 | 0.0802 | 30.76 | 0.0001 |
| | Out groups | 0.0469 | 18 | 0.0026 | | |

**Tab 5** T test of absorption constant (Ka) at different intestinal segments in rat

表 5 大鼠小肠不同部位吸收常数的 $t$ 检验

| | duodenum | jejunum | ileum | colon |
|---|---|---|---|---|
| duodenum | | $P<0.0002$ | $P<0.0479$ | $P<0.0028$ |
| jejunum | | | $P>0.05$ | $P>0.05$ |
| ileum | | | | $P>0.05$ |

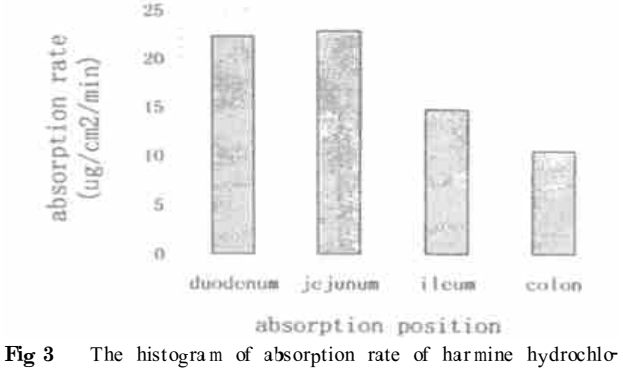


**Fig 3** The histogram of absorption rate of harmine hydrochlorideper unit area per unit time at different intestinal segments in rat

图 3 盐酸去氢骆驼蓬碱在大鼠小肠不同部位单位时间单位面积内吸收速率的柱状图

## 4 Discussion

**4.1** A variety of models have been proposed to study the absorption kinetics of drugs. Roughly three types of experiments are available: (1). In vivo, the drugs are administered into blood-perfusion gastrointestinal segments. From the disappearance of the drugs from the intestinal lumen the uptake into the organism is calculated. (2). *In vitro*, transfer across the intestinal wall and tissue content of drugs is measured with non-blood perfusion isolated segments of the small intestine. (3). Physicochemical models *in vitro*, permeability coefficients of $^{14}$C-clanobutin in vivo on blood perfusion tied-off intestinal across artificial membranes[12]. In this paper, the in situ method was employed to study the absorption kinetics of harmine hydrochloride. In in situ, the perfusion experiment was performed without amputating blood vessel and nerve. Drugs could be carried away by blood while the drug permeated through epithelial cell of gastrointestinal. Furthermore, in in situ models, the influence of the gastric content evacuation and the intrinsic movement of digestive canal for the experiment results could be avoid-

ed. The absorption rate of drug was calculated from determining the disappearance of drug in digestive canal. Thus, the experiment result could present the reality condition of drug absorption in digestive canal. However, the duration of experiments is limited by nonphysiological parameters like anesthesia and surgical trauma[13]. The movement status of perfusion fluid in intestine differed from intrinsic movement status of intestinal fluid is another drawback. The flow of perfusion fluid influenced intestinal flora, which differed from that of normal situation of drug in intestines. Besides, this set-up usually excludes crossover experimental schemes. So, this in situ model alone is not adequate to characterize the absorption process, especially when metabolism or accumulation of the drug in the intestinal wall occurs. But, it is frequently used to study the absorption kinetics of drug and to study the factors influencing absorption of drug.

**4.2** If a drug is absorbed well in entire intestine, especially in colon and rectum, the dosage forms design for single dose release system ought to have the drug released homogeneously in 24 hours. But, if a drug is absorbed in stomach or the upper of intestine, the dosage forms design for single dose release system ought to prolong the retention time of drug in upper of gastrointestinal. While, if a drug is absorbed well mainly at upper and middle of gastrointestinal, but little at lower or under gastrointestinal, the dosage forms design for single dose release system ought to slow down the propel of drug preparation in gastrointestinal and to promote drug absorption at lower part of gastrointestinal.

**4.3** In conclusion, harmine hydrochloride is absorbed well at entire gastrointestinal. If a dosage form for single dose release system of harmine hydrochloride is designed, the drug release rate will be controlled.

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