·论　著·

Comparison on the Pharmacokinetic of Metformin Hydrochloride between Normal and Diabetic Rats

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ABSTRACT：OBJECTIVE To compare the pharmacokinetic differences of metformin hydrochloride between normal and diabetic rats. **METHODS** Metformin hydrochloride was orally administrated to the normal and diabetic rats. The blood samples were collected at different time. The concentration of metformin hydrochloride in plasma was determined by HPLC method. The software of DAS1.0 was used to estimate the pharmacokinetic parameters. **RESULTS** The main pharmacokinetic parameters of metformin hydrochloride in normal and diabetic rats were C_{max}=9.41 μg·mL^{-1}, $t_{1/2}$=161.8 min, Cl/F=0.079 L·min^{-1}·kg^{-1} and C_{max}=20.09 μg·mL^{-1}, $t_{1/2}$ =1 718.93 min, Cl/F=0.012 L·min^{-1}·kg^{-1}, respectively. **CONCLUSION** The main pharmacokinetic parameters of metformin hydrochloride in normal and diabetic rats have significant differences (P<0.05).
KEY WORDS: metformin hydrochloride; rats; diabetic; pharmacokinetics

盐酸二甲双胍在正常大鼠与糖尿病大鼠体内的药动学比较

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摘要：目的 比较盐酸二甲双胍在正常大鼠与糖尿病大鼠体内的药动学差异。**方法** 正常大鼠与糖尿病模型大鼠，分别灌胃给予盐酸二甲双胍后，于不同时间点采集血样，用 HPLC 测定血药浓度并绘制药-时曲线，用 DAS1.0 统计软件计算药动学参数。**结果** 盐酸二甲双胍在正常大鼠与糖尿病大鼠体内的主要药动学参数分别为：C_{max}=9.41 μg·mL^{-1}、$t_{1/2}$=161.8 min、Cl/F=0.079 L·min^{-1}·kg^{-1}；C_{max}=20.09 μg·mL^{-1}、$t_{1/2}$ =1 718.93 min、Cl/F=0.012 L·min^{-1}·kg^{-1}。**结论** 盐酸二甲双胍在正常大鼠与糖尿病模型大鼠体内的药动学参数有明显差异(P<0.05)。
关键词：盐酸二甲双胍；大鼠；糖尿病；药动学
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1 Introduction

Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar levels, which result from defects in insulin secretion, or action, or both. There are two major types of diabetes, called type 1 and type 2. Type 1 diabetes was also called insulin dependent diabetes mellitus (IDDM) and the patient with type 1 diabetes must rely on insulin medication for survival. Type 2 diabetes was also referred to as non-insulin dependent diabetes mellitus (NIDDM), the patients with type 2 diabetes must be controlled by diet and oral hypoglycaemic agents such as metformin hydrochloride (MT).

MT is one of the oral antidiabetic drug, the main effects involve inhibiting absorption of glucose in intestinal tract, increasing uptake and utilization of glucose in skeletal muscles and peripheral tissues. Because of the less adverse effect, MT was widely used in clinic to treat the patients that were diagnosed NIDDM.

It has been reported that chromatography could be used to determine the concentration of MT in biological specimen [1-3]. Differences maybe exist in pharmacokinetic characteristics of MT between normal and pathologic states, but few studies were involved. In the present study, diabetes was induced in rats using streptozotocin (STZ), the pharmacokinetic difference of MT between normal and diabetic rats was compared by detecting the concentration of MT in rats after MT administration.

2 Apparatus and materials

2.1 Apparatus

The HPLC system was carried out using a Shimadzu LC-20A HPLC system (Kyoto, Japan)

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which consisted of a binary gradient pump (model LC-20A), a SPD-20MA diode-array detector, a SiL-20A auto sampler, a DGU-20A3 degasser and CTO-20A column oven. The apparatus was interfaced to a DELL PC compatible computer using LC solution software. UV spectrometer (model 753WBI, Shanghai),

2.2 Chemicals and reagents

MT used in analysis and animal experiment was purchased from the Chinese medicine control institute (Beijing, China) and the Yilin pharmaceutical factory (Beijing, China), respectively. STZ was purchased from Sigma company. Glucose oxidase test kits were obtained from Maker company (Beijing, China). Acetonitrile was HPLC grade, obtained from Shandong Yuwang Co. Ltd. (Shandong, China). Anorethisterone (ANTA) was purchased from the Chinese medicine control institute (Beijing, China).

2.3 Animals

Male Wistar rats weighing 210-250 g purchased from the laboratory animal center of Lanzhou university (Lanzhou, China)[SYXK(甘)2005-0007] and all the rats were allowed to equilibrate in standard and conditioned animal houses at the first hospital of Lanzhou university for one week before use. All the rats were fasted for 12 h with free access to water, prior to the experiments.

2.4 Statistical analysis

The results of determination were conducted by the software of DAS1.0, all data were expressed as mean ± SD. Statistical comparisons were performed by analysis of Student's *t*-test using SPSS version 12.0. All the tests were done at the 5 % level of significance.

3 Methods and results

3.1 Chromatographic condition

The HPLC assay was carried out using a VP-ODS C_{18} column(4.6 mm×150 mm，5 μm) at 40 ℃. The mobile phase consisted of acetonitrile and 2 mmol·L^{-1} sodium dodecyl sulfate in a volume ratio of 35:65. The flow rate was 1.0 mL·min^{-1}. The detection was performed at a wavelength of 235 nm and the sample injection volume was 20 μL. ANTA was selected as internal standard. Under this chromatographic condition, the HPLC chromatograms of blank plasma, plasma spiked with MT and ANTA , the plasma obtained 1 h after oral administration of 350 mg·kg^{-1} MT in nomal or diabetic rats, were shown in Fig 1. The retention times for MT and ANTA were approximately 8.76 and 14.38 min, respectively. It can be seen that plasma proteins do not interfere with the elution of MT and ANTA.

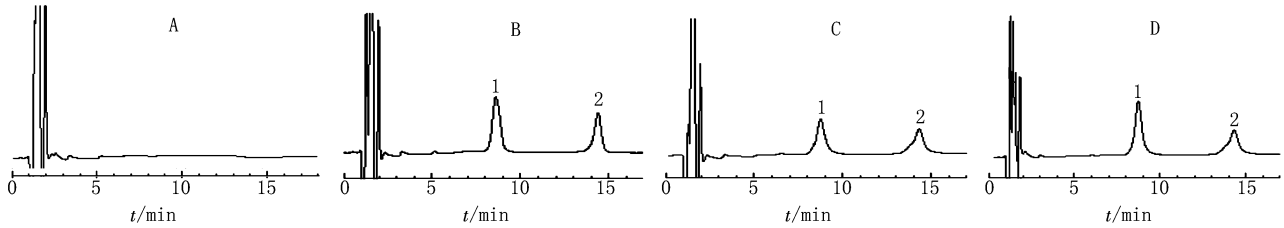

Fig 1 HPLC chromatograms

A-blank plasma; B-blank plasma spiked with metformin hydrochloride and internal standard; C-nomal rats plasma one hour after administration of 350 mg·kg^{-1} metformin hydrochloride; D-diabetic rats plasma one hour after administration of 350 mg·kg^{-1} metformin hydrochloride; 1-metformin hydrochloride; 2-internal standard

图 1 高效液相色谱图

A-空白血浆；B-空白血浆+二甲双胍对照品+内标；C-正常大鼠灌胃给药后 1 h 血浆样品；D-糖尿病大鼠灌胃给药后 1 h 血浆样品；1-盐酸二甲双胍；2-内标

3.2 Preparation of reference substance solution

To prepare the reference substance stock solution, ANTA 6.6 mg was scaled precisely and dissolved in methanol at a volume of 10 mL. The solution of internal standard was prepared by diluting ANTA stock solution with methanol to reach the concentration of 6.6 μg·mL^{-1}. The reference substance solution of MT was prepared by dissolving MT 6 mg with internal standard solution 10 mL, and the prepared reference substance solution was stored at 4 ℃.

3.3 Preparation of plasma samples

Each collected blood sample was immediately transferred to a heparinized microcentrifuge tube and centrifuged at 10 000 r·min^{-1} for 5 min. The plasma (100 μL) was then vortex-mixed with 400 μL of internal standard solution for 40 s. The mixture was centrifuged for 5 min to separate preciptitated proteins. The supernatant was filtered with membrane and 20 μL solution was directly injected

into the chromatography. The same sample processing was applied to the recovery and to the precision in plasma.

3.4 Calibration curve and limit of quantitation (LOQ)

Calibration curves in the concentration range of 0.33−22.8 μg·mL^{-1} for MT were constructed by plotting the peak–area ratio of each analyte/ANTA versus MT concentration in rat plasma. The linear regression analysis of the standard calibration plot for rat plasma was Y=0.168X−0.000 7(r=0.999 8), where Y and X represented the peak-area ratio and MT concentration, respectively. Ten independent blank plasma samples were measured singly. The LOQ was expressed as the analyte concentration corresponding to the sample blank value plus 5 standard deviations. The LOQ for MT was calculated to be 0.17 μg·mL^{-1} and the calibration line were accepted to determine the plasma MT concentration.

3.5 Precision and recovery

Plasma samples were spiked with MT at concentration of 0.71, 5.68 and 22.8 μg·mL^{-1}. Samples were processed in replicates (n=5) and subjected to HPLC analysis. The precision was calculated as the relative standard deviation of measurements. The recovery was determined as the ratio of peak-area of MT in plasma sample to that in methanol solution. The results indicated that the recoveries of MT in plasma from low to high concentration were more than 93%. The intra-day and inter-day variance were less than 3.1% and 5.8%, respectively. Assay accuracy was better than 96%.

3.6 Pharmacokinetic studies

Diabetes was induced in the rats using STZ. A dose equivalent to 60 mg·kg^{-1} body weight was dissolved in 0.5 mL citrate buffer (pH 4.5) and administered intraperitoneally to the rats [4]. After 3 days, the extent of diabetic induction was monitored based on blood-sugar level and weight decrease. Blood sugar levels of fasting rats exceeding 16.5 mmoL·mL^{-1} were accepted as the based level for diabetes [5]. A polyethylene tube (0.28 mm, I.D., 0.61 mm, O.D.) was inserted into the right femoral artery of the normal or diabetic rats while the animal was under anesthesia with ether. MT solution was then orally administered to the rats at a dose of 350 mg·kg^{-1}. Blood samples (0.25 mL) were collected at 0, 15, 30, 60, 90, 120, 150, 180, 240, 360, 480, 600 and 720 min after oral administration. The methods of sample processing and chromatographic assay applied for were described above. Fig 2 shows the mean±SD plasma concentration-time profile of MT after oral dosing. At the same time after MT administration, the plasma concentration of MT in diabetic rats was markedly higher than that of the normal rats. The pharmacokinetic parameters were analyzed using Drug and Statistics 1.0 program (DAS 1.0). All data are expressed as the mean ± SD and showed in Tab 1.

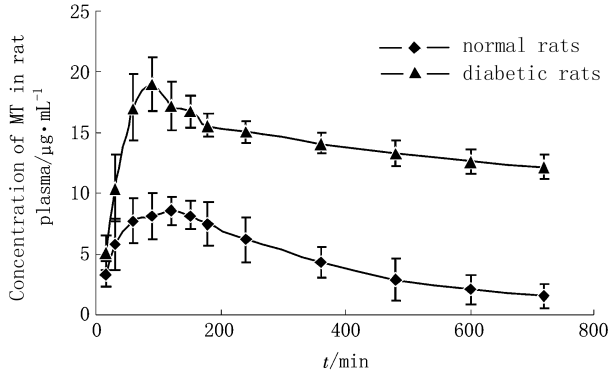

Fig 2 Mean plasma concentration-time curve of metformin hydrochloride after oral administration in normal rats and diabetic rats(n=5)

图 2 正常大鼠及糖尿病大鼠灌胃给予盐酸二甲双胍后平均血药浓度-时间曲线图(n=5)

Tab 1 Pharmacokinetic parameters of metformin hydrochlo-ride in normal and diabetic rats (n=5)

表 1 盐酸二甲双胍在正常大鼠及的糖尿病大鼠体内的药动学参数(n = 5)

Parameters	Normal rats	Diabetic rats
T_{max}/min	112.50±37.75	82.50±15.00
C_{max}/μg·mL^{-1}	9.41±1.67	20.09±4.42[1)]
$t_{1/2}$/min	161.80±56.37	1 718.93±415.22[1)]
Cl/F/L·min^{-1}·kg^{-1}	0.079±0.01	0.012±0.00[1)]
AUC/mg·min·L^{-1}	3 063.41±478.76	10 717.13±269.77[1)]

Note: Compared with normal rats, [1)]P<0.01

注：与正常组相比，[1)]P<0.01

4 Discussion

There were significant differences in the pharmacokinetic process of MT between normal and diabetic rats, though their pharmacokinetic processes were all consistent with one-compartment model. Compared with the normal rats, elimination of MT in diabetic rats was slower. The $t_{1/2}$ in diabetic rats was about ten-fold to that of normal one, while CL/F in

diabetic rats was decreased six-fold to that of normal rats.

MT was excreted mainly by kidney. Damages to kidney were induced when STZ was administered intraperitoneally, which were also coincident with diabetic nephropathy, one of the common diabetic complications. Maybe the damage to kidney was the key reason why elimination of MT in diabetic rats was slowed down. Moreover, in pathologic states, the transport ability of membrane was decreased generally, which may prolong the residence time and reduce the elimination of drug. Therefore, in order to inspect the pharmacokinetic characteristics of MT, diabetic models should be used and the accumulation of MT in pathologic states also should be considered when it was used.

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