

Preparation, Characterization and Pharmacokinetics in Vivo of Oxymatrine-Phospholipid Complex

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Abstract

The aim of the present study was to prepare oxymatrine-phospholipid complex to enhance oral bioavailability of oxymatrine and to study its physicochemical properties and to compare the pharmacokinetic characteristics and bioavailability after oral administration of oxymatrine-phospholipid complex in rats. Using tetrahydrofuran as a reaction medium, oxymatrine and phospholipids were resolved into the medium, after the organic solvent was removed under vacuum condition, oxymatrine-phospholipid complex was formed. The new complex's physicochemical properties including differential scanning calorimetry (DSC), X-ray diffraction (XRD), N-octanol/water Partition Coefficient were tested. The concentrations of oxymatrine after oral administration of oxymatrine-phospholipid complex and oxymatrine at different time in rats were determined by HPCE. The pharmacokinetic parameters were computed by software program 3p87. The data showed that oxymatrine and phospholipids in the oxymatrine-phospholipid complex were combined by non-covalent bond, not forming a new compound and the solubility of oxymatrine-phospholipid complex in n-octanol was effectively enhanced. The better hepatocytes permeability was obtained by the phospholipid complex. We found that mean plasma concentration-time curve of oxymatrine after oral administration of oxymatrine-phospholipid complex and oxymatrine in rats was both in accordance with open two-compartment model with first-order absorption. Pharmacokinetic parameters of oxymatrine, physical mixture and the complex in rats were T_{max} 1.71, 1.91 and 2.17 h, C_{max} 0.164, 0.247 and 0.437 $\mu\text{g}\cdot\text{ml}^{-1}$, $AUC_{0-\infty}$ 2.87, 3.23 and 9.43 $\mu\text{g}\cdot\text{h}\cdot\text{ml}^{-1}$, respectively. The bioavailability of oxymatrine in rats was increased remarkably after oral administration of oxymatrine-phospholipid complex comparing to oxymatrine and the physical mixture. This was mainly due to an impressive improvement of the lipophilic property of oxymatrine-phospholipid complex.

Keywords: Oxymatrine; Phospholipid complex; Physicochemical properties; Pharmacokinetics

Introduction

Oxymatrine (OMT) is a kind of alkaloid extracted from a Chinese herb (*Sophora alopecuroides* L.) (Lai et al., 2003). Basic and clinical researches suggested that oxymatrine had the fol-

lowing pharmacological effects such as anti-virus, protecting hepatocytes, anti-hepatic fibrosis, immune regulation, etc (Liu et al., 2003; Dong et al., 2003; Xiang et al., 2002; Yang et al., 2002; Chen et al., 2001; Li et al., 1998). In particular, wide attention was paid to its inhibitory effect on hepatitis B virus (HBV) in recent years. Oxymatrine has been proved to have distinct anti-virus effect in the treatment of chronic hepatitis B (CHB). The slight liposolubility of oxymatrine result in the poor permeation across the intestinal epithelial cells and minor the gastrointestinal (GI) tract absorption in rats (Chen et al., 2002; Yu et al., 2002; Chen et al., 2001; Wang et al., 2000). So the wide clinical application of oxymatrine remains a question because of the poor clinical therapeutic effect after oral administration. There are usually several factors responsible for this, but a particularly widespread problem is poor absorption due to slow in the lumen of the gastro-intestinal tract. There are numerous advantages of phospholipids in addition to solubilizing property while considering them for a carrier system. Phospholipids are an important component of cell membrane, having the actions of keeping cell membrane fluidity and treating hepatic disorder. In this paper, OMT-phospholipid complex (OMT-PLC) was studied in order to improve oral bioavailability of OMT.

The objective of this study is: (1) to improve oral bioavailability of OMT, OMT-phospholipid complex was prepared. It is expected that OMT combined with phospholipids might increase the oral bioavailability; (2) the physicochemical characters of OMT-PLC were evaluated, such as DSC, XRD. The n-octanol/water partition coefficient (P) study of OMT-PLC was performed in order to evaluate the improved solubility properties of OMT-PLC in comparison with OMT material and the physical mixture; (3) After oral administration of three formulations: OMT, the physical mixture and OMT-PLC, the pharmacokinetics and bioavailability of OMT in rats were studied. The formulation OMT-PLC, which exhibits comparable to the commercial products or even higher bioavailability, may be clinical candidate for future clinical study.

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Materials and Methods

Materials

Oxymatrine was purchased from Ningxia-bo-er-tai-li Ltd, purity 99.13%, and phospholipid was purchased from Hua-qing-mei-hen Ltd., and the phosphatidyl content was approximately 82% (w/w). The other chemical reagents were of analytical grade or better.

Preparation of Oxymatrine-phospholipid Complexes

The required amounts of oxymatrine and phospholipids were placed in a 100 ml round-bottom flask and dissolved in tetrahydrofuran. After tetrahydrofuran was evaporated off under vacuum at 40°C, the dried residues were gathered and placed in desiccators overnight, then crushed in the mortar and sieved with a 100 mesh. The resultant oxymatrine-phospholipid complex was transferred into a glass bottle, flushed with nitrogen and stored in the room temperature.

Determination of the Content of Oxymatrine in Phospholipids Complex

The content of oxymatrine in phospholipids complex was determined as follows. Approximately 5mg of phospholipids complex were dissolved in 50 ml of solvent A (acetonitrile: dehydrated alcohol = 80:20, v/v), and a 20 µl aliquot of the resulting solution was injected into a HPLC system. The stationary phase, NH₂ column (150mm×4.6 mm, 5µm), was kept at 25°C. The mobile phase was a mixture of acetonitrile : dehydrated alcohol: 3% H₃PO₄ (80:10:10, v/v). The flow rate was 1.0 ml/min. Effluent was monitored at 220 nm.

Differential Scanning Calorimetry (DSC)

The samples sealed in the aluminum crimp cell were heated at the speed of 5 µ·ml⁻¹ from 0 to 300ml in the atmosphere of nitrogen (Dupont 1090B, Dupont, USA). Peak transition onset temperature was determined by means of an analyzer. The peak transition onset temperatures of phospholipids, pure oxymatrine, the mixture of phospholipids and oxymatrine and the oxymatrine-phospholipids complex were compared.

X-ray Diffractometry (XRD)

The X-ray diffractogram (D/max-r A, Rigaku Denki, Japan) was scanned with the diffraction angle increasing from 5° to 50°, 2θ angle, with a step angle of 0.04° and a count time of 1s.

N-octanol/water Partition Coefficient(P) of OMT-PLC

P of OMT determination of OMT material, phospholipids complex or the physical mixture was carried out by adding 0.2g of OMT material, phospholipids complex or physical mixture to a series of 10 ml water solutions (pH 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, 7.5) in sealed glass containers at 25°C, respectively. Each experiment was performed in triplicate. All the sixty-three liquids were agitated for 24 h and centrifuged to remove excessive residues (15 min, 4000 rpm), respectively. Each liquid was added 10 ml n-octanol and agitated for 24 h. Then they were centrifuged at 4000 rpm for 15 min, respectively. The water phase and n-octanol phase were separated. The water phase and n-octanol phase were filtrated through a 0.45µm membrane, respectively. The 1ml filtrate was mixed with 9ml of methanol

and a 20µl aliquot of the resulting solution was injected into a HPLC and detected as the previous description, the concentrations of OMT were measured, respectively.

P of OMT of OMT material, physical mixture and phospholipids complex were calculated as follows:

$$P = C_o / C_w$$

where C_o was the concentration of OMT material, OMT-PLC or the physical mixture in n-octanol; C_w was the concentration of OMT material, OMT-PLC or its physical mixture in water.

Rat Bioavailability Experiments

Capillary Electrophoresis

Capillary electrophoresis was performed on a HPCE instrument (Trisep-2010) equipped with a UV detector set at 205 nm (Luo et al., 1999). Separation and analysis were carried out on an uncoated fused-silica capillary tube (50µm I.D., 56 cm total length and 36 cm from the injection point to the detector) at 25°C. Before each run, the capillary tube was washed with 0.1 M NaOH for 5 min, bidistilled water for 5 min, and then with the operating buffer tris hydroxymethyl aminomethane (Tris, 40mM)-sodium dihydrogen phosphate (10 mM)-4% avantin buffered at pH 7.6 for 5 min. The operating buffer used was degassed by vacuum filtration through a 0.2 µm membrane filter, followed by agitation in an ultrasonic bath. The samples to be analyzed were injected automatically, using the pressure injection mode, in which the sample is pressurized for 3 s. The electrophoresis was performed at 20 kV (about 50 mA) using normal polarity.

Plasma Sample Preparation and Validity

The rats were anaesthetized with aether, and 500µl blood was taken from the eyeground veins. The plasma was obtained after centrifugation (15 min, 4000 rpm) was stored at -20°C until analyzed. When the plasma sample was thawed, 50µl of cimetidine solution (CMD, 1.4 mg· ml⁻¹, internal standard), 100µl of 1M Na₂CO₃ solution and 500µl of borate buffer saline (pH 8.0) were added, and agitated for 30 s. After 4ml aether was added to the solution above, this mixture was shaken for 3 min and then centrifuged (15 min, 4000 rpm). The organic phase was quantitatively decanted into a clear tapered centrifuging tube and the eluate was evaporated under nitrogen at 37°C. The residues were resuspended in 100µl of mobile phase and centrifuged (15 min, 4000 rpm). Aliquots (20µl) of the supernatant were injected for HPCE analysis.

Pharmacokinetic Studies of Oxymatrine-phospholipids Complex and Oxymatrine in Rats

Eighteen male rats (body weight 180~220g) divided randomly into three groups were fasted for 12 h, but allowed to take water freely. A sample equivalent to 100mg/kg of oxymatrine phospholipids complex suspended in 2ml of water was orally administered to one group of rats. The solutions of oxymatrine and physical mixture equivalent to 100mg/kg of oxymatrine were orally administered to the other group of rats, respectively. Peak concentrations (C_{max}) and peak times (T_{max}) were derived directly from the experimental points. The other pharmacokinetic parameters were computed by software program 3p87.

Results and Discussion

Preparation of Oxymatrine –phospholipid Complex

We prepared oxymatrine –phospholipid complex according to different quantity ratio of phospholipids and drugs, such as 1, 2, 3 and 4. The results showed that when the ratio was more than 3, the appearance of resultant materials appeared viscous and it was not easy that resultant materials were prepared to other preparations, but when the ratio was lower than 3, the stability of phospholipid complexes was worse and the complex ratio was poor. For the purpose to get the best quality and use the fewest quantity ratio of phospholipids, at last we prepared oxymatrine–phospholipid complex in term of the quantity ratio 3. The content of oxymatrine in the phospholipids complex was 24.86% (w/w).

Differential Scanning Calorimetry

Figure 1 showed (included as a supplementary information) the DSC curves of phospholipids, oxymatrine physical mixture and phospholipids complex. DSC of phospholipids complex showed the endothermal peaks of oxymatrine and phospholipid are disappeared and the phase transition temperature is lower than the phase transition temperature of phospholipids, it was considered that oxymatrine and phospholipids should have some interaction, such as the combination of hydrogen bonds or van der Waals force (Venema et al., 1988; Lasonder et al., 1990). After the combination of oxymatrine and the phospholipids molecule polarity parts, the carbon–hydrogen chain in phospholipids could turn freely and enwrap the phospholipids molecule polarity parts, which made the sequence decrease between phospholipids aliphatic hydrocarbon chains, made the second endothermal peak of phospholipids disappear and depressed the phase transition temperature.

X-ray Diffractometry

Figure 2 shows (included as a supplementary information) the powder X-ray diffraction patterns of oxymatrine, phospholipids, their physical mixture and the complex. The oxymatrine powder diffraction pattern shown in Figure 2(c) displayed partial sharp crystalline peaks, which is the characteristic of a molecule with some crystallinity. In contrast, phospholipids shown in Figure 2(b) were amorphous lacking crystalline peaks. Compared with that of the physical mixture, the crystalline peaks had disappeared in the complex shown in Figure 2(a). This suggested that oxymatrine in the phospholipids lipid matrix was either molecularly dispersed or amorphous form. However, as seen in Figure 2(d), some crystalline drug signal was still detectable in the physical mixtures of oxymatrine and phospholipids.

N-octanol/water Partition Coefficient(P) of OMT-PLC

Table 1 showed the n-octanol/water partition coefficient (P) of OMT, the physical mixture and OMT-PLC at different pH. The data showed that OMT-PLC markedly increased the lipophilicity of OMT, and P of OMT-PLC in n-octanol and water was about 10 multiples more than that of OMT material, these were due to the strong dispersibility or/and amorphous form of the phospholipids complexes and polar group of OMT were masked by phospholipids. However, P of OMT in physical mixture was about 4 multiples more than that of OMT mate-

samples	pH	Cw/ g.ml ⁻¹	Co/ g.ml ⁻¹	P (Co / Cw)
OMT	1.5	0.183±0.0098	0.022±0.0038	0.120
	2.5	0.175±0.0086	0.024±0.0046	0.137
	3.5	0.173±0.0034	0.023±0.0073	0.133
	4.5	0.165±0.0007	0.034±0.0057	0.206
	5.5	0.168±0.0097	0.037±0.0068	0.220
	6.5	0.164±0.0086	0.031±0.0038	0.189
	7.5	0.167±0.0067	0.033±0.0043	0.197
Physical mixture	1.5	0.136±0.0076	0.066±0.0075	0.485
	2.5	0.137±0.0084	0.069±0.0037	0.503
	3.5	0.139±0.0039	0.068±0.0028	0.489
	4.5	0.133±0.0059	0.066±0.0063	0.496
	5.5	0.142±0.0065	0.057±0.0052	0.401
	6.5	0.148±0.0098	0.055±0.0084	0.372
	7.5	0.147±0.0056	0.047±0.0057	0.320
OMT-PLC	1.5	0.074±0.0050	0.125±0.0063	1.69
	2.5	0.063±0.0185	0.133±0.0057	2.11
	3.5	0.064±0.0044	0.127±0.0052	1.98
	4.5	0.066±0.0050	0.138±0.0057	2.091
	5.5	0.065±0.0015	0.129±0.0036	1.984
	6.5	0.064±0.0089	0.128±0.0073	2.0
	7.5	0.066±0.0088	0.127±0.0067	1.924

Table 1: N-octanol/water partition coefficient (P) of oxymatrine, physical mixture and OMT-PLC at different pH. Values are mean±S.D. (n=3).

rial, because the phospholipids slightly improved the hydrophilicity and lipophilicity of OMT in physical mixture by means of its solubilization.

Rats Bioavailability

Oxymatrine in plasma was completely separated under analytical conditions (Figure 3) (included as a supplementary information). The calibration curve was found to be linear 13.501-0.046 ($r=0.9992$, where x is the concentration ratio of OTM to CA and y is the corresponding peak-area ratio UDCA/CA) in the 0.0179 to 0.1790 mg·ml⁻¹ range. The results attained from the method recoveries of high, middle and low concentrations were 85.15, 87.11 and 88.37%, respectively. The R.S.D. in days were 3.18, 3.52 and 3.29%, respectively, the R.S.D. intra-days were 3.41, 3.74 and 3.45%, respectively, which showed recoveries and RSD in days or intra-days were satisfying, and the

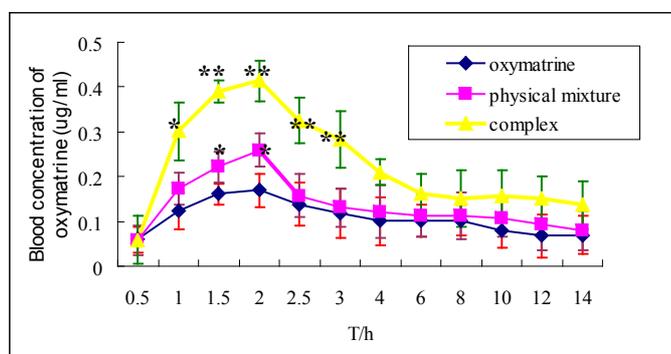


Figure 4: Mean plasma concentration-time curve of oxymatrine in rats after oral administration of oxymatrine-phospholipid complex, oxymatrine and physical mixture equivalent to 100 mg/kg of oxymatrine ($n=6$), respectively. Values are mean±SD ($n=6$ /group/time point). * $P < 0.05$ and ** $P < 0.01$ are statistical significances with the OMT-PLC versus OMT or physical mixture.

lowest detection limit was 35 ng·ml⁻¹.

Figure 4 showed the sample equivalent to 100 mg/kg of oxymatrine of phospholipids complex and oxymatrine was respectively orally administered to rats ($n = 6$). From the above profile and Table 2, it was known that the average value of C_{\max} is 0.437 $\mu\text{g}\cdot\text{ml}^{-1}$ after oral administration of phospholipids complex with a T_{\max} of about 2.17 h. However, the average value of C_{\max} was 0.164 $\mu\text{g}\cdot\text{ml}^{-1}$ after oral administration of oxymatrine solution with a T_{\max} of about 1.71 h, the average value of C_{\max} was 0.247 $\mu\text{g}\cdot\text{ml}^{-1}$ after oral administration of oxymatrine solution with a T_{\max} of about 1.91 h. The average value of $\text{AUC}_{0-\infty}$ of oxymatrine, the physical mixture and the complex in rats were 2.87, 3.23 and 9.43 $\mu\text{g}\cdot\text{h}\cdot\text{ml}^{-1}$, respectively. And the bioavailability of oxymatrine was 3.29 multiples than oxymatrine. The pharmacokinetic data were simulated by non-linear least squares. The results showed that open two-compartment model and 1st-order absorption were fitted to both phospholipids complex and oxymatrine plasma concentration-time course in vivo of rats.

Parameters	Oxymatrine	Physical mixture	Complex
AUC_{0-24} ($\mu\text{g}\cdot\text{h}\cdot\text{ml}^{-1}$)	1.97±0.218	2.42±0.371	6.21±0.859 * *
$\text{AUC}_{0-\infty}$ ($\mu\text{g}\cdot\text{h}\cdot\text{ml}^{-1}$)	2.87±0.417	3.23±0.317	9.43±0.384 * *
CL (mLh^{-1})	4.72±0.45	4.83±0.76	4.97±0.85
T_{\max} (h)	1.71±0.54	1.91±0.64	2.17±0.46
C_{\max} ($\mu\text{g}\cdot\text{ml}^{-1}$)	0.164±0.045	0.247±0.075	0.437±0.083 * *

Table 2: The main pharmacokinetic parameters of phospholipids complex, physical mixture and oxymatrine in rats ($n=6$). *P <0.05 are statistical significances with the OMT-PLC versus OMT or physical mixture.

Conclusion

In this study, OMT-PLC was successfully prepared by a simple method. DSC and XRD curves of phospholipids complex showed that OMT and phospholipids combined and formed some kind bond, such as hydrogen bonds or van der Waals force. The N-octanol/water partition coefficient (P) of OMT-PLC studies showed OMT-PLC surprisingly increased the hydrophilicity and lipophilicity of OMT, and P of OMT-PLC in n-octanol and water was about 10 multiples more than that of UCDA material. The blood concentration of OMT was precisely assayed by HPCE Compared with OMT material and the physical mixture, the phospholipid complex can markedly improve the bioavailability of OMT in vivo of rats. It would be further studied about the absorbed mechanism of OMT-PLC through small intestine and therapeutic evaluation in vivo. The OMT-PLC would be more prospective preparation in future.

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