Preformulation study and initial determination of biological Properties of isopropylidene shikimic acid

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Abstract: Isopropylidene shikimic acid (ISA), a new drug derviatived from Shikimic Acid, had been proved to be effective in the cerebral protection after cerebral ischemia and reperfusion. But there was little research on the physical pharmacy and biopharmaceutical properties about the drug. In order to provide some useful data for the pharmaceutical development of ISA, the solubility, stability and Oil/Water partition coefficient (LogP) were determined by the classic preformulation study method, and the transmembrane performance of ISA was studied by Franz -diffusion cell method *in vitro*. The results showed that ISA was water-soluble with a solubility 32.52mg/ml, which could be improved to 44.32 mg/ml by 1% (w/v) sodium dodecylsulfate; the LogP was -0.63; ISA was less stable in water but it was stable when pH greater than 6.0 and unstable when pH less than 6.0; the accumulated permeation rates at 1h were about 50% and more than 80% at 6h. Data obtained by the study indicated that the medium selection and pH control were important for liquid preparation of ISA, and avoiding dissolution and absorption in stomach was critical for the oral solid dosage forms. Mucosal drug delivery systems would be considered, according to the certain hydrophilic-lipophilic characters and good transmembrane capability.

Keywords: Preformulation study, isopropylidene shikimic acid, stability, partition coefficient, transmembrane.

INTRODUCTION

Shikimic Acid (SA), the active ingredient in traditional Chinese medicine Magnoliaceae plant Star Anise, has significant anti-thrombotic effect and anti-inflammatory effect in pharmacological studies (Wang *et al.*, 2002; Ma *et al.*, 2003; Ma *et al.*, 2004). 3,4-oxo- Isopropylidene Shikimic Acid (ISA) is a derivant of Shikimic Acid, and has been depurated upto the purity of 99.70% Currently a detailed research about the pharmacology, pharmacokinetic, pharmacodynamics and the mechanism of effect has been finished, including the cerebral protective effects such as anti-thrombotic, anti-inflammatory, anti-edema, as well as pharmacokinetics and tissue distribution after intravenous administration of ISA solution. The results suggested that ISA has significant pharmacological effects, even over the role of SA in some aspects. However, preformulation study for the pharmaceutical preparation design of this new drug was insufficient, only a simple study of the tablet and powder for injection had been performed so far, and little has been reported about the chemical or biopharmaceutical properties of ISA itself. In our study, the preformulation (Proniuk 2002) and pharmaceutics parameters, such as solubility, stability and oil-water partition coefficient and so on, had been determined (Sheikh, 2005), and the biological mucosal affinity and transmembrane permeability *in vitro* had also been inspected. So that it would provide a more systematic theoretical basis for the further development of the new drug ISA preparations.

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MATERIALS AND METHODS

Agilent 1260 high performance liquid chromatograph (Agilent Company), BT-125D Electronic Analytical Balance (Sartorius Company, Germany), TK-6A Transdermal Diffusion Tester (Shanghai Kai Kai Technology Trade Co., Ltd.) 3,4-oxo-Isopropylidene Shikimic Acid was provided by Beijing University of Chinese Medicine (Beijing, China), with batch number 101206 and a purity of 99.70%; Acetonitrile was HPLC grade from Merck (Germany), rthophosphoric acid, potassium dihydrogen phosphate and dipotassium hydrogen phosphate was Analytical Reagent (A.R.) from Beijing Chemical Works (China), Distilled water was used for all solutions.

Chromatographic conditions

The concentrations of ISA in samples were measured by HPLC method, with the chromatographic column was Agilent Eclipse Plus C18 (100mm×4.6mm, 3.5μm), the mobile phase was acetonitrile-0.05% phosphonic acid solution (volume ratio 10:90), flow rate was 1 $ml \cdot min^{-1}$, wavelength was 220 nm, column temperature was 30°C, and injected sample volume was 20μL.

Solubility

Equilibrium Solubility in distilled water and phosphate

buffer was determined by shaking flask method. Quantum sufficit powder of ISA was added into 50mL distilled water, and after shaking for about 24h in water bath at the temperature of 37°C, samples were collected and diluted for the HPLC determination. The equilibrium solubility could be calculated.

Stability in solution

According to some research papers and our early researches, ISA was found to be stable in solid state under the conditions of high temperature (60 °C), high humidity (90%) or strong light (4500Lx±500Lx). Therefore, we focused on the stability of ISA in solution and the influence of pH in this study, so that a suitable buffer would be found to be the medium of ISA solution (Lu, 2012). Phosphate buffers with the pH about 2, 3, 4, 5, 6, 7, 9 were prepared. About 150mg ISA was weighed and then added into 10ml volumetric flask dissolved and filled up to the scale with the aforementioned several buffers and distilled water respectively. And then these ISA solutions were placed at the temperature of 37°C, sampled and determined at 0, 1, 2, 3 and 6h respectively to inspect the stability according to the concentration of ISA at a series of time.

Apparent oil/water partition coefficient

HPLC determination of ISA concentration in water or buffer solution (pH7.0) was used for the assessment of partition coefficient (P) or logP. The ISA solutions with the concentration of about 4mg/ml were prepared by distilled water or buffer solution, which had been saturated by n-octyl alcohol in advance. The concentration of this solution was noted as C_A. Take the ISA solutions 10ml and n-octyl alcohol 10ml, which had been saturated by water or buffer solution in advance, into a conical flask and keep stirring for about 8h in water bath at the temperature of 37°C. After standing and demixing, the substratum, which was the aqueous phase, was sampled and diluted to be determined. The concentration was calculated and noted as C_w, so the concentration in the oil phase could be noted as. And then the P or logP would be calculated by the following equation. (Lecordier, 2008, Yao, 2009).

$$C_O = (C_A - C_W)V_W/(V_O C_W)$$

$$P = \frac{C_O}{C_W} = (C_A - C_W)V_W/(V_O C_W)$$

The Partition Coefficient of ISA in water and buffer solution was determined respectively.

Transmembrane permeability in vitro

An initial study of the biological characters of ISA was performed, which was about the transmembrane permeability *in vitro*. The Franz-diffusion cell method was used to simulate the transmembrane proceeding *in vitro*, Caprine mittele muschel mucous membranes were selected as the membrane model, and the drug concentrations of the reception solution were determined by the HPLC method. The sketch of the device was showed in the fig. 1, with the upper donor room, the lower receptor room and the membrane model in the middle (Bonferoni 1999, Ng 2010).

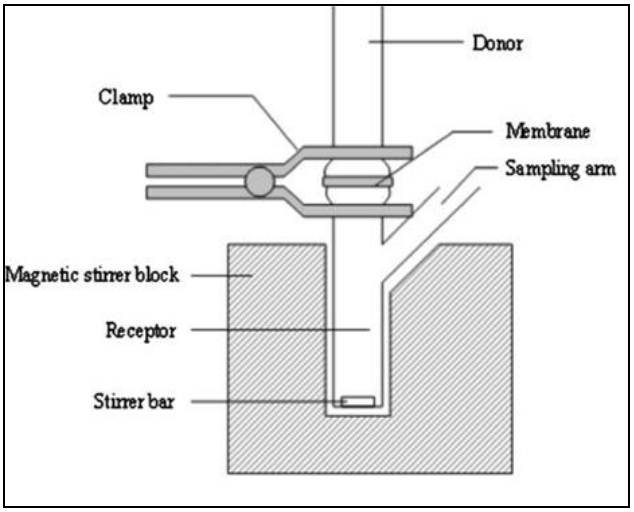

Fig 1: A Sketch of typical Franz Diffusion Cell for the experiment (cited from literature).

The transmembrane capability of ISA water solution and pH 7.0 buffer solution was compared. Sodium Chloride was selected as the receptor, and filling up the receptor room by about 7ml. ISA solutions with concentration about 10mg/ml were taken exactly 1ml and added into the donor room. The experiments were performed in water bath at the temperature of 37°C and samples were taken at 0, 10, 20, 30, 45, 60 min and 2, 4, 6h respectively. Supplementary sodium chloride would fill up the receptor room and keep the contact with the membrane. Samples were diluted and then determined by HPLC method. And the accumulated transit doses or the permeation rates would be calculated to evaluate the transmembrane permeability of ISA.

RESULTS

Solubility

Solubility of ISA in distilled water was 31.23mg/ml and in phosphate buffer was 32.52mg/ml. In our further solubilizing experiments, it was found that the solubility in phosphate buffer could be raised to 44.32 mg/ml by 1% (w/v) sodium dodecylsulfate, and relatively only raised to 33.39 mg/ml by 1% (v/v) polysorbate 80. This result could be valuable for the pharmaceutical formulation design.

Stability in solution

The stability of ISA in water and several phosphate buffers were listed in table 1. It is showed that ISA was

instable in water and buffers with the pH value less than 6.0. Drug stability showed significant pH-dependent, and stability become worse with decreasing of the pH. But drug was stable when pH greater than or equal to 6.0 in contrast. The crico-acetal or crico-ketal groups in the chemical structure of ISA may be the main reason, which were stable in alkaline condition and unstable in acid condition and then broken down to the SA (signed in fig. 2).

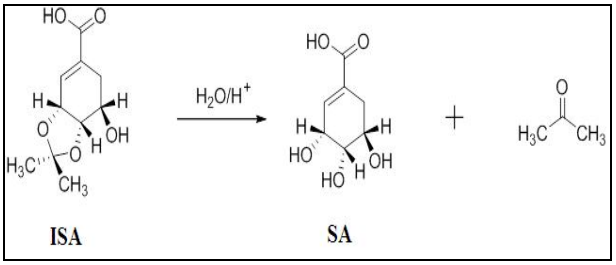

Fig. 2: Chemical structure of the ISA, SA and possible degradation pathway.

Partition Coefficient

The results of apparent Oil/Water partition coefficient were listed in table 2, together with the concentration of water phase and oil phase after partition equilibrium. The partition coefficient in water and buffer solution (pH 7) were close, LogP was -0.63 and -0.36 respectively.

Transmembrane Permeability in vitro

Transmembrane experiment *in vitro* was carried out for the ISA water solution and buffer solution, using the caprine mittele muschel mucous membranes as the membrane model. Accumulated Permeation Rates – Time curve was drawn in fig. 3. The results showed that the permeation rates were similar in different medium under the situation *in vitro*. The accumulated permeation rates at 1h were about 50%, therefore mucosal drug delivery and absorption may be considered in the preparation design.

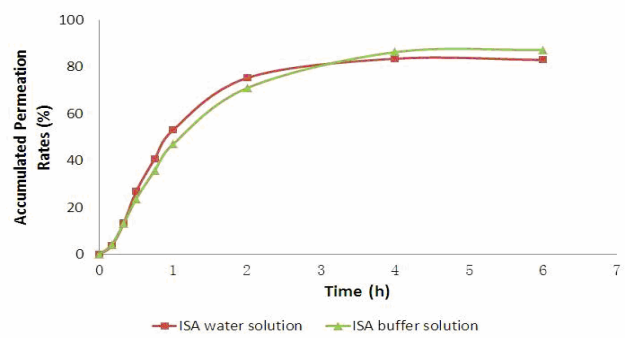

Fig. 3: Accumulated permeation rates – Time curve of the transmembrane permeability in vitro (n=6).

DISCUSSION

Isopropylidene Shikimic Acid, a derivant of Shikimic Acid, has good solubility but poor stability in water and buffers with pH value less than 6.0. In addition, it is more stable in phosphate buffers with pH greater than or equal to 6.0. Therefore phosphate buffers with pH 7.0 was selected as the medium of ISA solution in the pharmaceutical and analytical process. And in the product development, dissolution and absorption in stomach must be avoided, enteric-coated tablets could be a choice, and colonic targeting tablets may be suitable for the protective effects on experimental colitis newly discovered (Xing 2012).

ISA has a good transmembrane capability, but in our other experiment about the drug distribution *in vivo*, we found that it was difficult to pass the blood-brain barrier for ISA. According to the pharmacologic action of ISA, it would be more effective when delivered targeting into brain. The apparent Oil/Water partition coefficient of ISA, which

Table 1: The stability of ISA in water and several phosphate buffers

Time (h)	Stability（%）							
	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 9.0	Water
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1	86.91	98.63	99.07	99.22	99.96	100.03	99.96	98.55
2	74.58	97.14	98.02	98.50	99.84	99.93	99.91	96.93
3	63.45	95.67	97.00	97.78	99.83	99.91	99.94	93.89
4	53.62	94.13	96.08	97.17	99.79	99.99	99.91	93.01
6	45.10	92.47	95.04	96.52	99.70	100.00	99.81	91.98
24	0.05	47.08	83.11	87.94	99.85	99.99	100.37	80.90

Table 2: The concentration after partition and partition coefficient (P and LogP)

Medium of ISA solution	Volume (ml)		Concentration mg/ml)		Partition coefficient (P)	LogP
	Water phase	Oil phase	Water phase	Oil phase		
Distilled water	10	10	3.254	0.76	0.233559	-0.63
Buffer (pH 7.0)	10	10	2.557	1.106	0.432538	-0.36

expressed as LogP, is -0.63 in water and -0.36 in buffer, which were suitable for the entrapment of liposome or nanoparticle preparation. These could be helpful for the formulation design.

CONCLUSION

On the basis of the main structure of Shikimic Acid and the chemical or biological characters of ISA determined in our researches, more reconstitution or modification could be performed to improve the lipophilicity or stability, and so on.

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