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RESEARCH NOTES

A New Method for Isolating and Purifying Natural Drug Taxol*

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Abstract A pharmacological interaction target (PIT) method for solving the difficult problem in the separation of taxol from cephalonmanine was proposed. A two-phase extraction technique was used to carry out the PIT separation process. The effects of buffer, temperature and protein on the separation were investigated. Feasible disassembly conditions were also discussed. The final purity of taxol can reach 95% or higher.
Keywords taxol, purification, pharmacological interaction target method

1 INTRODUCTION

As a natural anticancer drug, taxol has been widely investigated since it was first isolated from *taxus* by Wani *et al.*[1]. Because of the coexistence of many taxane anal-ogues of taxol in the crude material, the isolation and purification of taxol are difficult and costly. The particularly difficult step in the purification of taxol is its isolation from cephalonmannine, whose chemical structure and many properties are very similar to those of taxol.

Pharmacological interaction target (PIT) refers to the physiological tissues which drugs attack at or combine with when they work. So far, several PITs have been discovered, and microtubule protein (MTP) is one of the targets of several anticancer drugs including taxol. The results of in vitro research[2] suggest that the interaction between taxol and MTP is quite different from other antimitotic drugs such as colchicines and vinblastine. Taxol molecule does not inhibit MTP assembly into microtubule (MT) as other antimitotic drugs do. On the contrary, it promotes MTP assembly and associates with MT targetedly and reversibly. Pharmacological research also suggests that cephalomannine and other taxane analogues of taxol do not have such a property[2]. Inspired by above results, we develop a PIT method to purify taxol from the mixture of taxol and cephalomannine in this study.

2 EXPERIMENTAL MATERIALS AND METHOD

2.1 Materials

Calf brain and porcine brain for preparing MTP were kindly provided by Tianjin Packinghouse. 2-(*N*-morph-olino)ethanesulfonic acid (MES) was Sigma Co. manufactured with purity of 99.5%. Both ethylenediamine tetraacetic acid (EDTA) and adenosine triphosphate (ATP) are manufactured by BIB Co. with 99% and 98% purity respectively. Ethylene glycolbis (2-aminoethyl-ether)-*N*,*N*, *N*′, *N*′-tetraacetic acid (EGTA) with 99% purity was obtained from B.M. Co. Bio-grade sodium glutamate was manufactured by Huzhou Biochemistry Factory. AR-grade $MgCl_2$ and phosphate were provided by North China Special Reagent Co. All these reagents were used without further purification.

2.2 Experimental method

MTP is prepared from porcine and calf brain tissue by 3 circles of temperature reversible polymerization[3−5]. Because the MTP assembly-disassembly is reversible, an experimental method is designed as follows. First, the mixture of taxol and cephalomannine is dissolved in an organic solvent, and MTP is dissolved in a biological buffer. Second, under certain conditions, the organic and water phases are mixed gently and MTP begins to assemble. At the same time taxol associates with MT. When the association of taxol and MT reaches equilibrium, MT deposit is disparted with the liquid phase. Third, the deposit obtained in Step 2 is released to a new mixture that comprises new organic solvent and buffer. Then, under appropriate disassembly conditions, taxol is released into organic solvent.

2.3 Analysis method

The composition of organic phase is analyzed by high performance liquid chromotography (HPLC) system (Tsp Co., P4000 pump, UV3000 detector and ChromQuest^{TM}), C18 column, TURNER, UV detector at 227nm with 0.001mAU sensitivity. The solvents of fluid phase are methanol (65%) and water (35%) and the flow rate of the fluid phase is 1ml· min^{-1}.

3 RESULTS AND DISCUSSION

According to the HPLC performance under the same analysis conditions, dichloromethane was selected as the organic solvent in our experiments.

3.1 Effect of buffer

The effect of buffer on the MTP assembly process has

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been studied extensively[6], but the results were obtained without taxol in homogeneous phase. Therefore, it is necessary to investigate the buffer effect on the association process of taxol and MTP when two phases coexist. In this work, three different buffers were used:

BF1 20mmol· L^{-1} phosphate + 0.1mol· L^{-1} sodium glutamate, pH=6.75;

MES buffer 0.1mol· L^{-1} MES + 1mmol· L^{-1} EGTA + 1mmol· L^{-1} ATP + 0.5mmol· L^{-1} $MgCl_2$, pH=6.75;

PEDTA buffer 1mmol· L^{-1} EDTA +phosphate buffer, pH=6.6.

Three curves are obtained at 15℃ with the three buffers, as shown in Fig.1, which indicates the effect of different buffers on taxol promoted MTP assembly process. The solid line stands for a continuous decreasing progress, which suggests that the reaction between taxol and MTP takes place as expected and the taxol concentration in organic phase is lowered by this process. For MES buffer, taxol concentration decreases quickly at the beginning of the reaction, and after about 4.5 h, the taxol concentration nearly reaches a constant value, which means that the reaction reaches equilibrium. The dash-dotted and the dotted lines show different trend. It is not clear so far why the three curves do not have a similar profile. Generally speaking, taxol concentration has a trend to decrease when using any one of these three buffers.

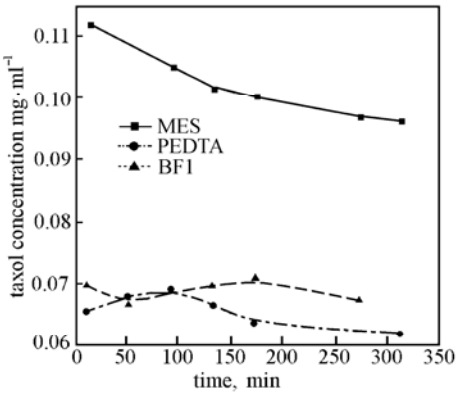

Figure 1 The effect of different buffers to the separation results

3.2 Effect of temperature

The MTP assembly process is an endothermic process, so high temperature is favorable for both MTP activity and its assembly rate. In order to examine the effect of temperature on the taxol-catalyzed MTP assembly process, experiments are conducted at three temperature levels. The results are shown in Fig.2.

From Fig.2, it can be seen that at 15℃, the assembly process reaches equilibrium in 3h. The taxol concentration in organic phase decreases steadily with time, which indicates that taxol has associated with MTP and left organic phase. The situations at 20 and 30℃ are different.

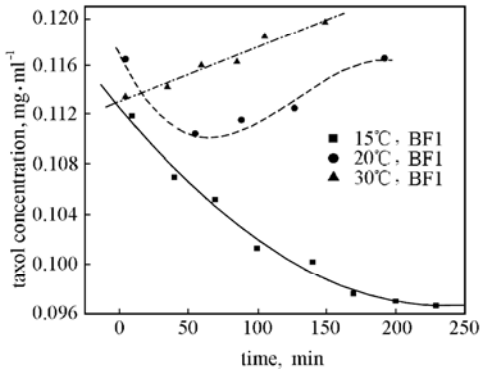

Figure 2 The effect of temperature

At 20℃, the taxol concentration decreases in the first hour, then increases gradually. While at 30℃, the taxol concentration rises gradually with time. These phenomena may be caused by the following reasons: at high temperature, dichloromethane volatilizes much more violently than at low temperature. The volatilization of dichloromethane results in taxol concentration rising. If this effect is great enough to counteract the decrease of taxol concentration in organic phase, taxol concentration in organic phase may increase gradually.

3.3 MTP stability

The effect of MTP on the separation process of PIT method is chiefly expressed by its activity. The fresh MTP has higher activity and this can lead to quicker assembly and shorter time needed to reach equilibrium. Experimental result suggests that taxol catalyzed MTP assembly equilibrium can be reached nearly in the same period even though the MTP preserved time (ranged from 3 days to 25 days) in nitrogen is different, as shown in Fig.3. This result suggests that MTP could be preserved in liquid nitrogen for a long period without obvious activity loss.

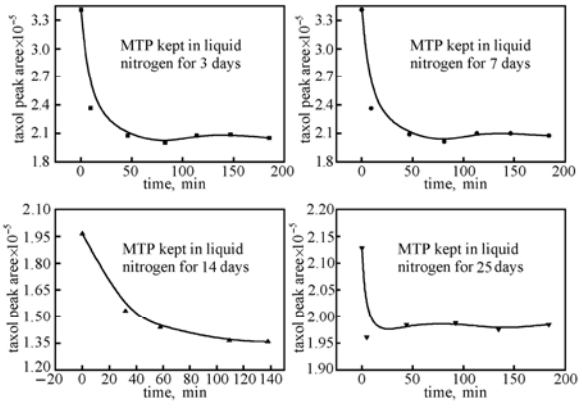

Figure 3 The activity of MTP

3.4 Disassembly condition of MT with taxol existing

The MT disassembly results are shown in Fig.4 and the disassembly conditions are indicated in the figure legends. Fig.4 suggests that the best disassembly result can be obtained at 0℃ with the addition of Ca^{2+}, adding

EDTA takes the second place, while lowering temperature alone (either 0℃ or liquid nitrogen) can not achieve satisfactory results. Such results are different from those conclusions obtained by other researchers because their experiments were carried out under a homogeneous phase condition.[7,8]

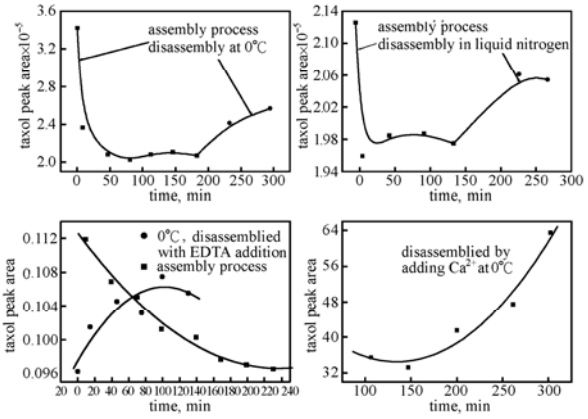

Figure 4 Experimental results of disassembly

From Fig.4 we can also see that taxol can not be released from the MT-taxol complex completely. This is also an evidence that there is an assembly-disassembly equilibrium although the reverse process of this equilibrium (disassembly) is negligible in homogeneous phase system in the presence of taxol.

3.5 Kinetic study

Based on experimental results, an apparent kinetic correlation of the interaction between taxol and MTP may be obtained as $\lg c_{\text{taxol}} = -2.87685t - 3.88854$, where c is concentration. This correlation suggests that the interaction between taxol and MTP is a first order reaction.

3.6 Purification results by the PIT method

The purification results are shown in Table1.The selectivity is defined as the ratio of purification time of taxol to cephalomannine. After purification by the PIT method, taxol purity in sample I increases from 58.2% to 85.48% and in sample II it increases from 13.65% to 95.81%. The selectivity of sample I is 4.08 and that of sample II is 6.27.

Table 1 Purification results of taxol

Sample	I composition (by mass), %		II composition (by mass), %	
	Taxol	Cephalo-mannine	Taxol	Cephalo-mannine
before purified	58.20	39.89	13.65	3.74
after purified by PIT method	85.48	14.52	95.81	4.19
purification time	1.47	0.36	7.02	1.12
selectivity	4.08		6.27	

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