The feasibility of the nucleosides assay in PBMCs by analysing with LC-MS/MS

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Introduction

PBMC or peripheral blood mononuclear cell is any blood cell having a round nucleus. Lymphocytes and monocytes are PBMCs. These blood cells are a critical component in the immune system to fight infection. Nucleoside and deoxyribonucleoside were quantified in PBMCs because a lot of anticancer drugs are nucleoside analogs. I had to know the quantity of endogenous nucleoside. Indeed our nucleoside analogs do not flood among the endogenous nucleoside in cellular matrix and do not pull a resistance in drugs. The best technique of separation and extraction of the PBMCs for the whole blood then to quantify their nucleotides was looked for. Two techniques were compared: tube Ficoll and tube CPTTM (Cell Preparation Tube).

Experimental Conditions

To isolate PBMCs, tube Ficoll with a centrifugation at 1800 tr/min during 20min at room temperature and tube CPTTM with a centrifugation at 2900tr/min during 20min at room temperature were used. The purity of PBMCs was controlled with a cytocentrifugation and counted PBMCs with a counting chamber and phase contrast microscopy. High performance liquid chromatography coupled with mass spectrometry was used for the extraction and quantification of nucleosides PBMCs. The system is made of a Surveyor AS autosampler injector, an oven, a Surveyor MS quaternary pump, and a Thermo Finnigan Ultra-Quantum triple quadrupole mass spectrometer equipped with an electrospray ionisation source. The extraction of nucleoside was performed on a OASIS® WAX column. Liquid chromatography was performed in the gradient. Two eluents, buffer with pH adjusted to 10 and 50% acetonitrile in water were constituted the gradient which was used for LC. The acquisition mode used was Single Reaction Monitoring.

		Ficoll		CTP	
		EDTA	sodium heparine	sodium heparine	sodium citrate
T_0	PBMCs/mL of blood	1,292,500	1,004,000	2,300,000	2,296,000
	Purity (%)	57	64	98.5	98.5
T_{4h}	PBMCs/mL of blood	1,343,000	1,069,000	2,590,000	2,275,000
	Purity (%)			98.5	99
T_{24h}	PBMCs/mL of blood	1,695,000	1,075,900	2,525,000	2,795,000
	Purity (%)			99	97.5

Table 1 : Results of two techniques which isolate PBMCs of whole blood

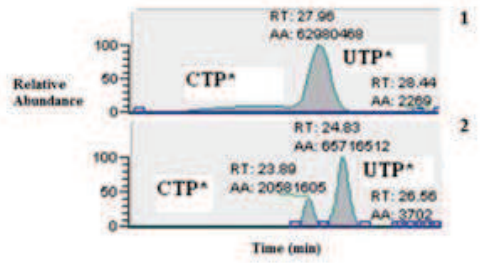

Figure 1 : Chromatogram of CTP* and UTP* with column of nucleoside extraction by LC-MS/MS

Figure 2 : Chromatogram of CTP* and UTP* without column nucleoside extraction by LC-MS/MS

Results and discussion

T_0, T_{4h} and T_{24h} are different times when samples of the taken blood were treated for the isolation of PBMCs with tube Ficoll and tube CPT™. Different anticoagulants were used such as sodium heparine. Table 1 presents the results of PBMCs isolation. For the quantification of nucleosides, we had big problems to separate CTP* and UTP*. These problems could come from the column of extraction (Figure 1 and 2).

Conclusion

The technique with tubes CPTTM seems better than tubes Ficoll. We obtained better results, with a purity of the PBMCs and a number of PBMCs high. By using these we have gained time but it was expensive. On the other hand, nucleotides assay in vivo has yet to be perfected.

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