Ion Pairing LC-MS/MS Method for Analysis of Intracellular Phosphorylated Metabolites

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Nucleoside analogues have been extensively used in medication. The nucleoside analogue cordycepin is the principal bioactive compound found in the caterpillar fungi (Cordyceps and Ophiocordyceps). It has been shown to have biological activity, including anti-inflammatory, immunomodulatory and anti-proliferative activity in many kinds of malignant cells. Intracellular drug interactions at the nucleotide level can be explained by understanding the intracellular metabolism of nucleoside analogues as well as their plasma metabolism since their efficacy of therapy or toxicity does not associate with the plasma level of nucleoside. Therefore, investigation of the metabolism of nucleoside analogues is required for a full understanding of their pharmacological activity and toxicity. For that reason, here an ion pairing LC-MS/MS method has been developed for quantitative analysis of the nucleoside analogue cordycepin and the metabolites and its application to cell culture, using in vitro and in vivo studies.

Several HPLC parameters and extraction techniques have been optimised, followed by optimisation of the mass spectrometry method by examining the fragmentation of nucleotides. The method was then validated and applied to study the metabolism of cordycepin in vitro and in vivo, to investigate the effect of the cordycepin treatment with or without pentostatin on the intracellular level of endogenous nucleotides, and to examine the intracellular metabolism of nucleoside analogue 4-thiouridine and the effect of its metabolite on the metabolic balance of adenine and uridine nucleotides.

The study on the intracellular metabolism of cordycepin in MCF7 and HeLa cells shows that cordycepin was rapidly metabolized into the deaminated form by adenosine deaminase (ADA) in culture medium as well as in cancer cells; therefore combination with pentostatin, an ADA inhibitor, resulting in the highly accumulated phosphorylated metabolite intracellularly. In contrast, cordycepin in C. militaris extracts showed much lower

degradation in non-heat-treated serum compared with pure cordycepin that indicates a strong evidence of the presence of a deaminase inhibitor in the extract of Cordyceps. Moreover, the determination of concentrations of cordycepin and the metabolites in the plasma and liver of rats dosed with cordycepin proves that the half-life of cordycepin and its metabolites are very short in the plasma; nevertheless they are accumulated in the liver with repeated administration. Treatment using cordycepin initially caused an increase in the intracellular concentrations of nucleoside triphosphate, but in the long term, the active metabolite of cordycepin likely induced a long term change in the cell resulting in a drop in nucleotide levels. Pentostatin on its own reduced nucleoside triphosphates levels in the long term and combination with cordycepin increased the effect of cordycepin on nucleotide concentrations. High levels of the accumulated cordycepin triphosphate led to a massive decline in nucleotide levels.

A study on the intracellular metabolism of nucleoside analogue 4-thiouridine has shown that generally the uptake of 4-thiouridine into NIH 3T3 cells was fast and the phosphorylated metabolite rapidly was developed only after two min labelling. However, it was also shown that its phosphorylation was not very efficient, but the level of the phosphorylated metabolite increased in serum-stimulated cells likely because the enzyme was upregulated in the presence of growth factor. Moreover, the present study provides additional evidence that 4-thiouridine and its metabolite have no adverse effect on the metabolic balance of adenine and uridine nucleotides.

This study confirms that pharmacological activity of nucleosides analogues and their cytotoxicity highly rely on the accumulation of their phosphorylated metabolites. Consequently, the activity and the level of the enzymes involved in their metabolism are highly influential on their pharmacological action as well as their toxicity.