ABSTRACT

Analysis of oxidative and free radical induced DNA damage

Diploma thesis

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Free radicals and reactive oxygen species (ROS) are highly reactive molecules capable of modifications of biomolecules, including DNA. 5',8-cyclopurine-2'deoxynucleosides represent a group of DNA lesions characterized by concomitant damage to both sugar and base moieties of the same purine nucleoside that are together with 8-oxo-2'-deoxypurines among the major lesions formed by attack of free radicals (e.g. hydroxyl radical).

Quantification of oxidative and free radical induced DNA lesions as biomarkers of oxidative stress has a high importance in study of their role in human health and disease. For quantification of these DNA lesions in gamma irradiated samples, high performance liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) will be utilized. Before injection into the LC/MS/MS, irradiated samples, treated by enzymatic digestion in order to gain free nucleosides, have to be desalted and DNA lesions have to be separated from undamaged nucleosides.

A new HPLC/UV method was developed for separation of (5'R)-5',8-cyclo-2'-deoxyadenosine; (5'R)-5',8-cyclo-2'-deoxyguanosine; (5'S)-5',8-cyclo-2'-deoxyadenosine; (5'S)-5',8-cyclo-2'-deoxyguanosine; 8-oxo-2'-deoxyguanosine and 8-oxo-2'-deoxyadenosine from 2'-deoxyadenosine; 2'-deoxycytidine; 2'-deoxyguanosine and 2'-deoxythymidine in aqueous solutions (without salts). Analytes were separated on Phenomenex LUNA C18 (2) [150 x 4,6 mm, 5µm] analytical column protected by Phenomenex LUNA C18 (2) [30 x 4,6 mm, 5µm] pre-column using gradient elution with acetonitrile (ACN) and 2 mM ammonium formate buffer as mobile phase. Flow rate of mobile phase was 1 mL/min. Detection vas achieved using UV-detector with detection at 260 nm. Separated DNA lesions were collected by Gilson FC 203B Fraction Collector in given time ranges.

Recovery from HPLC was 100 ± 2%; recovery from the subsequent lyophilization step was in the range from 77% to 103%, however further and more precise data will come from LC/MS/MS analysis

This method will be further updated for samples containing salts in the same concentrations as in real samples.