LA1

The activity of selected proteolytic enzymes and glycosidases in whole saliva in cystic fibrosis patients

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Introduction: CFTR protein is a component of cytoplasmatic membranes separating intracellular compartments. Impaired CFTR function influences the sialization and sulphhydrylations of proteins and thus changing their enzymatic properties. Observed abnormalities of lysosomal enzymes function may contribute to clinical image of cystic fibrosis and especially a susceptibility to chronic Pseudomonas aeruginosa infection. The aim of the study was the assessment of activity of selected lysosomal enzymes and its correlation with clinical features in a group of cystic fibrosis patients.

Materials and methods: The study was performed in a group of 66 cystic fibrosis (CF) patients and 66 healthy controls. 5 ml of whole saliva was collected from each participant before and after stimulation of saliva excretion. Total protein and glycoprotein concentration, along with activity of selected lysosomal enzymes (CTSD, ICPA, NeuA, GluA) and concentration of total sialic acid was assessed. The results were correlated with clinical features of CF patients: Schwachman score, FEV1 and the presence of chronic bacterial infection of lower respiratory tract. The smears of saliva of CF patients were also performed (H+E, May-Grunwald-Giemza staining).

Results: The activity of LCPA in CF group before stimulation was 205.13 ± 103.45 nmol/mL, after stimulation 124.26 ± 56.18 nmol/mL (p<0.001). Activity of CTSD in CF group before stimulation was 132.00 ± 57.714 nmol/mg, after stimulation 124.09 ± 46.682 nmol/mg (p=0.99). Activity of GluA in CF group was 4.85 ± 0.824 nmol/mg before stimulation, 6.44 ± 1.829 nmol/mg (p<0.001) after stimulation. Activity of NeuA in CF group was 35.71 ± 22.631 mU/mg before, and 39.32 ± 15.673 mU/mg (p=0.28) after stimulation. Concentration of sialic acid in CF group before stimulation was 3.16 ± 1.314 µg/mL, after stimulation 2.78 ± 1.248 µg/mL (p=0.042). There was no significant correlation between enzymatic activity (LCPA, CTSD, Neu i GluA) and the presence of chronic bacterial infection (P. aeruginosa, S. aureus, S. aureus MRSA).

Conclusions: The activity of studied proteolytic enzymes in saliva of CF patients differs from healthy subjects. LCPA activity correlated negatively with chronic P. aeruginosa infection in CF patients. Concentration of sialic acid in saliva of CF patients is higher comparing to healthy subjects.

LA2

Analyzing brain tumours for O6-methylguanine-DNA methyltransferase hypermethylation in the routine clinical setting

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Malignant gliomas, including glioblastoma multiforme and oligodendroglioma, are the most common primary tumours of the central nervous system, but the current options for their treatment are limited and the prognosis remains poor. Alkylating drugs, such as temozolomide, are most commonly chosen for the therapy of gliomas. Recent reports suggest that a DNA-repair protein called O6-methylguanine-DNA methyltransferase (MGMT), can be involved in creating tumour resistance. Epigenetic silencing of the MGMT promoter is associated with loss of MGMT expression and diminished DNA-repair activity and is regarded as a good predictive factor. That is why, good, reliable methods for the analysis of MGMT methylation status are needed in order to be applied in the routine clinical setting.

The aim of this study was to evaluate and compare methods for MGMT methylation status analysis and also to verify if these methods could be implemented in the routine clinical setting. MGMT hypermethylation was detected by Methylation-Specific Multiplex Ligation Dependent Probe Amplification (MS-MLPA), pyrosequencing and Methylation-Specific PCR (MSP).

As the result of the performed investigation the working protocols for MS-MLPA, pyrosequencing and MSP were established. It was demonstrated that these techniques are suitable for the analysis of clinically available tissues. This study also revealed that different commercially available kits for MGMT methylation status analysis, detect different CpG sites in MGMT promoter, raising the question of the importance of these particular sites in MGMT silencing. Despite these differences, the average hypermethylated population was found to be of similar rate to that found in the literature, namely 57% according to MS-MLPA, 40.7% according to pyrosequencing and 59% according to MSP. An unexpected tendency for shorter survival rate among patients with MGMT hypermethylation was observed. One possible explanation of this observation might be the lack of treatment and the age of those patients. The study on a larger and more homogenous population of patients is needed in order to fully assess the application of MS-MLPA, pyrosequencing and MSP in a routine clinical setting. Nevertheless this study shows that these methods are promising tools in brain cancer diagnosis and can be helpful in establishing tailor-made treatment.