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Aspects of inflammation and nitric oxide in Cluster Headache

AKADEMISK AVHANDLING
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Summary

Cluster Headache (CH) is an uncommon headache disorder, with severe implications for the individual patient. The headache is excruciating, unilateral and appearing in attacks. It is common that CH patients show ipsilateral associated symptoms, like for example conjunctival injection, lacrimation and nasal congestion. The pathophysiology of CH is still not completely understood. The overall objective of this thesis was to explore if inflammation and nitric oxide participate in the pathophysiology of CH.

Study I

The aim of study I was to identify differentially expressed genes during clinical phases of CH, assuming that changes of pathophysiological importance would also be observed in peripheral venous blood. Blood samples were drawn at 3 consecutive occasions from 3 episodic CH patients: during attacks, between attacks and in remission, and at 1 occasion from 3 matched controls. Global gene expression was analyzed with microarray tehnology using the Affymetrix Human Genome U133 2.0 Plus GeneChip® Set. In addition, quantitative RT-PCR on S100P gene expression was analyzed in 6 patients and 14 controls. Small differences were seen intraindividually and large differences interindividually. Intraindividual comparisons showed upregulation of severaS100 calcium binding proteins; S100A8 (calgranulin A), S100A12 (calgranulin C), and S100P during active phase of the disease compared to remission. The S100A8 and S100A12 proteins are considered markers of non-infectious inflammatory disease, while increased levels of S100P have been associated to different forms of cancer. RT-PCR analysis of S100P confirmed the Affymetrix´ results.

Study II

We investigated the cytokine interleukin-2 (IL-2) as a possible marker of immune system involvement in the pathophysiology of CH. Eight episodic CH patients and 16 healthy headache-free control subjects matched for age and gender were studied. Venous blood samples were drawn from the CH patients at three occasions; during active period between headache attacks, during attack and in remission. Venous blood samples were drawn once from each control subject. We analyzed IL-2 gene expression, using quantitative real-time polymerase chain reaction (RT-PCR). Patients with CH had significantly increased relative IL-2 gene expression levels during active period between headache attacks compared to during attacks, remission and controls.

Study III

In this study we have investigated white blood cell accumulation into potential inflammatory areas intracranially in 14 CH patients, both in active period and in remission, and 5 control subjects, with single photon emission computer tomography (WBC-SPECT). To enable precise definition of regions of interest (ROI:s) in the brain, all CH patients and control subjects also underwent magnetic resonance imaging (MRI) of the brain. We found no statistically significant difference in ^{99m}Tc-labeled WBC uptake between CH patients in active period and controls. Furthermore CH patients in active period were not significantly different in uptake compared to CH patients in remission.

Study IV

We investigated the role of nitric oxide (NO) in CH, by measuring its oxidation products, nitrite and nitrate, in the cerebrospinal fluid (CSF). We collected CSF from 14 episodic CH patients. Lumbar puncture was performed at two occasions: in active period between headache attacks, and in remission, not earlier than three weeks after their last headache attack. Eleven healthy volunteers served as controls. To estimate NO production, we determined the levels of NO-oxidation end products (NOx), that is, the sum of nitrite and nitrate, by using capillary electrophoresis (CE). CH patients in active period had significantly increased NOx levels compared with those in remission and control subjects. CH patients had also significantly enhanced NOx levels in remission compared to control subjects.

Keywords: Cluster Headache, gene expression, inflammation, WBC-SPECT, nitric oxide.

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