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SHORT COMMUNICATION

Matrix-metalloproteinases and proinflammatory cytokines in children with febrile convulsions and epilepsy—Cause or consequence?

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Summary This is the first investigation of MMPs in children with febrile seizures. In a prospective, cross sectional study, serum levels of matrix metalloproteinases (MMP8/9), tissue inhibitor of metalloproteinases (TIMP1/2), of children with FS ($n = 13$), children with febrile infection (FI, $n = 13$) and children with unprovoked generalized seizures (US, $n = 11$) were compared. Neither provoked nor unprovoked seizures in FS and US seem to elevate levels of MMPs or TIMPs, whereas in case of febrile infection blood level of MMP8 was significant elevated. Seizures in general might have no influence on this distinctive inflammatory process or even might have suppressive impact.

Introduction

Approximately 2—5% of children suffer from febrile seizures (FS). Adults with temporal lobe epilepsy and hippocampal sclerosis (HS) have a history of repetitive or prolonged febrile seizures in up to 40% suggesting inflammatory processes during FS might contribute to epilepsy development (Cendes et al., 1993; French et al., 1993; Vezzani and Granata, 2005).

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases, regulating cell—matrix composition with substrate affinity for different extracellular matrix components (Bellayr et al., 2009). As proteolytic enzymes, they play an important role in acute and chronic inflammatory processes by activating other cytokines (CK) and cleaving CK receptors. They are regulated by natural

Abbreviations: CNS, central nervous system; CK, cytokine; CRP, C-reactive protein; CSF, cerebral spinal fluid; EEG, electroencephalogram; ESM, ethosuximide; FI, febrile infection; FS, febrile seizures; HS, hippocampus sclerosis; IL-6, interleukin-6; LEV, levetiracetam; LTG, lamotrigine; MMP, matrix metalloproteinase; OXC, oxcarbazepine; PB, Phenobarbital; TIMP, tissue inhibitor of metalloproteinase; TNF-α, tumour necrosis factor-alpha; US, unprovoked epileptic seizure; VPA, valproic acid; ZNS, zonisamide.

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antagonists, the so-called tissue inhibitors of metalloproteinases (TIMPs) (Elkington et al., 2005). Elevated CSF MMP-8 and MMP-9 in children with bacterial meningitis was associated with neurological developmental delay and secondary epilepsy syndromes (Leppert et al., 2000). MMP-9 levels were elevated after stimulation with kainate in adult rat hippocampi (Szklarczyk et al., 2000).

CK are water soluble messengers acting via specific receptors playing an important role in human infections. They are a strong stimulus for brain MMP release (Elkington et al., 2005). TNF-alpha (TNF-α) and interleukin (IL)-6 with diverse function such as induction of apoptosis or stimulation of other immune competent cells, are elevated in various central nervous system (CNS) diseases (Vezzani and Granata, 2005).

We explored whether children with FS have a specific serum profile of MMPs, TIMPs and CK in comparison to children with febrile infection (FI) or unprovoked generalized seizures (US).

Methods

Participants and study design

We performed a prospective, cross sectional study from March 2005 until August 2008 data of 37 children (age 6 months to 6.5 years) — in an Austrian centre. We compared CK and MMP/TIMP blood levels within 24 h of admission of three groups: Children with *febrile seizures* (FS), *febrile infection* (FI) or *unprovoked generalized seizures* (US). Inclusion criteria for FS were simple FS with fever of at least 38 °C, for FI infectious diseases with fever of at least 38 °C no longer than 48 h and for unprovoked generalized seizure (US) with and without diagnosis of idiopathic generalized epilepsy (distance to last seizure >4 weeks). Children with meningitis, underlying autoimmune diseases (asthma, rheumatologic disease), oncologic disease and anaemia were excluded.

We recorded patient age, gender, fever onset and maximum degree, drugs, seizure semiology and duration, postictal neurological deficits, history of seizures before actual event. Clinical history including pregnancy, birth and psychomotor development was assessed using a standardized questionnaire. The study was approved by the ethics committee from the Medical University Innsbruck (AM2281). Informed consent was obtained from the parents.

Biochemical analysis

Routine blood analysis in each child included blood count, CRP, liver enzymes, electrolytes, blood glucose and the following inflammatory parameters: TNF-α, IL-6, MMP8/9 and TIMP1/2. Laboratory testing for IL-6 and TNF-α was done by enzyme-linked immuno-sorbent assay (ELISA) (Kits by Biosource Europe Belgien Nivelles). Testing for MMP8/9 and TIMP1/2 was done by enzyme-linked immuno-sorbent assay (ELISA) (Kits by Biomedica Group).

Statistics

The results of the three groups (FS, FI, US) were analyzed as follows: Data are presented as median and range. Normal distribution was tested with Kolmogorow—Smirnov test. Logarithmic transformation was applied for TNF-α, IL-6 and CRP to achieve normal distribution. Two-way analysis for variance (ANOVA) was performed with group and sex as categorical variables, using post hoc pairwise comparison (Scheffé's test). All statistical significance tests were two-tailed, with alpha <0.05 indicating statistical significance. Statistical analysis was completed using the Statistical Package for Social Science for Windows version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographic data

37 (18 females) children were included. 13 suffered from FS (5 females 2.8 [1.0—3.8]), 13 were admitted to hospital for febrile infections (FI) (10 females, 2.1 [0.6—6.0]) and 11 had US (3 females, 3.2 [1.8—6.2]) (Table 1). In case of admission with FS 7/13 children had a viral upper respiratory tract infection, 4/13 had gastroenteritis, and 2/13 otitis media. 6/13 children with FI had viral upper respiratory tract infection, 3/13 with gastroenteritis, 3/13 with otitis media and 1/13 with pneumonia. There was no significant difference regarding age, weight, body mass index (BMI), maximum degree of fever and seizure duration (Table 1). In children with US ($n = 10$) 5/10 had no antiepileptic drug medication, and 6/10 had a genetic (former idiopathic) epilepsy with generalized tonic—clonic seizures, which was treated in 2/5 with valproic acid (VPA), in 2/5 with phenobarbital (PB), in 1/5 with levetiracetam (LEV) and in 1/5 with VPA + lamotrigine (LTG).

Laboratory findings

There was a significant difference ($p < 0.002$) concerning serum concentrations of MMP8 between FS (18.0 [5.0—153.0]) versus FI (112.2 [4.2—200.0]), as well as for ($p < 0.001$) FI (112.2 [4.2—200.0]) versus US (7.4 [4.1—108.0]) (Table 2). Significant differences were also present in MMP8/TIMP1 ratio between FS—FI ($p < 0.001$) and FI—US ($p < 0.001$) and furthermore MMP8/TIMP2 ratio between FS—FI ($p < 0.05$) and FI—US ($p < 0.001$). There was no significant difference between the groups concerning IL-6, TNF-alpha, CRP, MMP9, TIMP1, TIMP2, MMP9/MMP1 ratio and MMP9/TIMP2 ratio (Table 2).

Discussion

This is the first study focusing on the role of MMPs and TIMPs in the pathogenesis of FS in children. The most important finding is that patients with FI showed significantly higher concentrations of MMP8, MMP8/TIMP1 ratio as well as MMP8/TIMP2 ratio, whereas there was no elevation of MMP8 in both groups with seizures (FS/US).