Functional changes in the central nervous system: An early but unrecognized complication of type 1 diabetes mellitus

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Abstract

Purpose: Diabetic neuropathy is a chronic and disabling condition that affects a significant number of individuals with diabetes mellitus (DM). Long considered a disease of the peripheral nervous system, there is now increasing evidence of central nervous system (CNS) involvement. Recent advances in neuroimaging methods have improved our understanding of how diabetic neuropathy affects the CNS. The aim of this study was to explore the relationship between cerebrospinal fluid (CSF) concentration of polyols, plasma glucose, and 18-fluordeoxyglucose (18-FDG) uptake correlation in the CNS of Type 1 diabetes mice models.

Materials and Methods: We conducted a study using two different Type 1 DM mice models: Streptozotocin induced and non-obese diabetic (NOD) mice models. Positron emission tomography with 18-FDG was used to determine glucose uptake in the frontal cortex and hippocampus. CSF samples were taken from the cisterna magna, while polyol species in the CSF were determined using gas chromatographic/mass spectrometric assays by Sigma Aldrich.

Results: The concentration of CSF myoinositol was significantly higher in NOD mice compared to controls (P = 0.001). The 18-FDG uptake was significantly attenuated in NOD mice compared to controls. CSF-myoinositol negatively correlated with 18-FDG uptake in the hippocampal area (ρ = −0.571, P = 0.003) and frontal cortex (ρ = −0.521, P = 0.044).

Conclusion: We demonstrated that in the early course of Type 1 DM, there are functional changes in the CNS, precisely in the frontal cortex and hippocampal region, evidenced by elevated myoinositol, which might reflect the initiation of cerebral tissue damage. Whether this suggests that hyperglycemia is toxic for the brain needs further investigation.

Key words: Type 1 diabetes mellitus; central neuropathy; polyols; positron emission tomography
1. Introduction

Diabetes mellitus (DM) represents an intermediary metabolism disorder primarily characterized by hyperglycemia. It is associated with the development of microvascular complications: Retinopathy and nephropathy. However, small-vessel abnormalities represent a part of the cardiovascular and peripheral nerve pathology as well [1]. High glucose levels are associated with non-vascular damage as well, and it is related to peripheral nerve, myelin sheath, and non-myelinated autonomic nerves. These abnormalities cause loss of sensation, and proprioception, as well as dysregulation of autonomic function and have been extensively investigated [2]. Although cognitive dysfunction has been recognized in patients with DM, there has been little concern about the influence of hyperglycemia on the structure or function of the central nervous system (CNS), and the term “central neuropathy” has been unrecognized until recently [3]. Moreover, the exact nature, magnitude, and pattern, as well as its pathophysiological background have been a matter of debate. So far, age of disease-onset, its duration, glycemic control, severe hypoglycemia episodes, and microvascular complications as well as the presence of diabetes-related comorbidities (hypertension, dyslipidemia, cerebrovascular disease [CVD] and depression) have been suggested as potential factors which might impact cognitive function in these patients [4]. There is evidence that children with Type 1 DM, particularly those with early disease-onset (i.e. before the age of 7), are most adversely affected [5]. They perform more poorly on neuropsychological tests, mainly those measuring verbal and visual learning and memory, as well as attention/executive function skills (planning, inhibitory control, sustained attention, abstract problem-solving, and decision-making). This appears to be clinically significant and to have a negative impact on intelligence and academic achievement. Similarly, adult patients with Type 1 DM demonstrate a significant reduction of overall cognition and intelligence, psychomotor performance, the speed of information processing, cognitive flexibility, and visual and sustained attention [6]. Furthermore, neurophysiologic studies (electroencephalogram, evoked potential studies, and response latencies) provide clear evidence of CNS changes in association with Type 1 diabetes in particular [7], while structural neuroimaging has demonstrated changes in metabolites in both brain gray and white matter when compared with similar control individuals without diabetes [8]. These changes seem to be related to higher glycated hemoglobin A (HbA1c) levels, suggesting that hyperglycemia might contribute to these pathologic abnormalities.

When considering the mechanisms for tissue damage in diabetes, abnormal glucose levels are considered the primary agent; however, the associated abnormal insulin levels are commonly overlooked. In the brain, insulin regulates multiple cellular processes: It stimulates neuronal growth and differentiation, synaptic plasticity, cellular proliferation, and neurotransmission [9]. In Type 1 DM, insulin levels are maintained exogenously at arbitrary, without an exact relationship to physiologic need. Thus, the abundance of glucose in the brain is changing the metabolites in that tissue: Reduces brain taurine [9] and increases brain inositol [10].

Thus, the aim of this study was to explore the relationship between cerebrospinal fluid (CSF) concentrations of sorbitol, inositol, myoinositol, plasma glucose, and 18-fluordeoxyglucose uptake correlation in the CNS of Type 1 DM mice models.

2. Materials and Methods

We conducted a study using two different Type 1 DM mice models: Streptozotocin (STZ) induced and non-obese diabetic (NOD) mice. 18 C57 BL/6, 7-week-old male mice were treated intraperitoneally with STZ (Sigma-Aldrich) (80 mg/kg) for 3 consecutive days (days 8-10) [11]. Furthermore, 24 NOD mice were purchased from Charles River (Budapest, Hungary). Male mice were included in the experiment because they tend to be more susceptible to both STZ-induced and NOD diabetes then females [12]. Fasting blood glucose levels were measured once daily. Mice with fasting blood glucose levels >13.5 mmol/L or random blood glucose levels >20 mmol/L were considered to have developed diabetes. All mice were maintained on a 12-h light-dark cycle, with 22 ± 2°C room temperature and had access to food pellets and tap water ad libitum, and were treated with premixed as part insulin (70/30) in the dose of 1 U/kg. Glucose was measured once daily for
28 days. The experiment was performed in accordance with the current laws of the Republic of Croatia and with the guidelines of the European Community Council Directive of 22 September 2010 the EU adopted (2010/63/EU). All applicable institutional and/or national guidelines for the care and use of animals were followed.

Positron emission tomography (PET) represents one of the most advanced nuclear imaging methods used in clinical and preclinical studies. It can provide a quantitative measure of the three-dimensional distribution of radiopharmaceutical as a function of time, in a live subject, noninvasively [13]. Among all PET radiopharmaceuticals, 18-fluorodeoxyglucose (18-FDG) as a glucose analogue is the PET tracer most often used worldwide [12]. The use of 18-FDG makes PET imaging an excellent tool for monitoring glucose uptake in all tissues. In this study, a ClearPET high-performance small animal PET scanner was used with a special focus on 18-FDG uptake in the frontal cortex and hippocampus. Performance of the commercial ClearPET in terms of its spatial resolution, sensitivity, and quality of phantoms and preclinical images were described by Roldan [13].

CSF samples were taken from the cisterna magna, while polyol species in the CSF were determined as suggested by gas chromatographic/mass spectrometric assays obtained by Sigma Aldrich. Briefly, CSF (3 µL) was mixed with internal standard ([2H16myo-inositol), deproteinized and evaporated under vacuum. The residue was heated with acetic anhydride/pyridine/4-dimethylaminopyridine, then dissolved in hexane/ethyl acetate and washed with sodium bicarbonate solution. The organic layer was evaporated to dryness, and the residue was reconstituted in ethyl acetate. An aliquot of this solution was injected into the gas chromatograph/mass spectrometer. Polyols were resolved on a capillary column (bonded 50% phenyl-50% methyl polysiloxane), and individual species were detected and quantitated by a chemical ionization technique in an ion trap mass spectrometer. Each polyol yielded a highly abundant fragment ion corresponding to the loss of one CH3COOH residue from the protonated molecule. The ions, m/z 273 for 1,5-anhydroisorbitol, m/z 373 for myo-inositol, and m/z 375 for taurine was monitored. The constituent ion m/z 379 of the internal standard was also acquired simultaneously. The concentration of each polyol species in CSF or plasma was read from the standard curve generated. The relative standard deviation for quantitation was no more than 9%.

The two groups of animals were observed separately and each of them was subdivided into diabetic and healthy control mice.

2.1. Statistical analysis
The data are presented with median and range due to small sample size and abnormal data distribution. The differences between observed parameters were assessed by the non-parametric Mann–Whitney test while the correlations were assessed with Spearman’s coefficient of correlation. The calculation was performed using SPSS (Chicago, IL, USA) 18.0 version for Windows. Statistically significant results were considered P < 0.05.

3. Results
A total of 14 animals developed diabetes in the NOD mice group while 12 C57BL/6J mice were treated with STZ and developed diabetes. All the animals were approximately 10 weeks old. The average 28-day glucose concentration in the NOD group was 12.7 mmol/L and 11.9 mmol/L in the STZ group. The concentration of CSF myoinositol was significantly higher in NOD mice with diabetes compared to controls while no other differences in the metabolites of interest were found (Table 1). Similarly, 18-FDG uptake was significantly attenuated in the NOD mice group compared to controls (Table 1 and Figure 1). Furthermore, CSF-myoinositol correlated negatively with 18-FDG uptake in the hippocampal area (ρ = −0.571, P = 0.003) and frontal cortex (ρ = −0.521, P = 0.044). No other significant correlations were observed.

4. Discussion
Although the peripheral nervous system in DM has been extensively investigated, the term “central neuropathy” has been unknown until recently. Neuroimaging investigations are sensitive in determining both peripheral and central neuropathy in diabetic patients, while CSF disturbances in polyol concentrations have been observed in individuals with cognitive impairment without diabetes without detectable structural changes. In this study, we demonstrated that early in the course of Type 1...
DM development there are functional changes in the CNS, precisely in the frontal cortex and hippocampal region, that correlate with glycemia and myo-inositol concentration, which might reflect the initiation of cerebral tissue damage. This suggests that hyperglycemia, a biomarker of insulin openia, is toxic for the brain at any stage. It was already described that hyperglycemia in the brain reduces taurine [14] and increases brain inositol [10], which is in accordance with our study results. Elevated inositol reflects gliosis [15], an indicator of brain injury. By reflecting abnormal insulin levels and reducing intracellular taurine, hyperglycemia may impair neuron growth and maturation.

Glucose is transported across the blood–brain barrier into extracellular fluid by facilitated diffusion, mediated through glucose transporter protein 1 and 3 [16,17]. Once in neurons and astrocytes, glucose is phosphorylated by hexokinase as the initial step of glucose metabolism. The glucose-6-phosphate thus produced can enter several metabolic pathways in the brain [18]. As a consequence of those mechanisms, there is a linear relationship between plasma glucose concentrations and brain glucose content over a range of plasma glucose values up to ~30 mmol/L [19]. Long-term high glucose levels in the brain probably result in glucotoxicity and thus lead to neuronal damage which can be observed with functional imaging such as PET before structural changes occur.

So far, the meta-analytic reviews have documented subtle neurocognitive deficits in pediatric [20] and adult [21] populations with Type 1 diabetes: Basic intelligence, psychomotor processing speed, mental flexibility, and attention are noted to be permanently reduced [22]. Neurophysiologic studies (electroencephalogram, evoked potential studies, and response latencies) provide further evidence of CNS changes in association with Type 1 DM. It is also known that children with diabetes onset

<table>
<thead>
<tr>
<th>Variable</th>
<th>NOD without manifest DM (N=10)</th>
<th>NOD with DM (N=14)</th>
<th>P</th>
<th>Controls (N=6)</th>
<th>STZ-induced diabetes (N=12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>28-day average glucose (mmol/L)</td>
<td>6.2 (5.2-9.3)</td>
<td>12.7 (6.4-28.6)</td>
<td>0.001</td>
<td>6.5 (5.1-6.7)</td>
<td>11.9 (7.2-27.10)</td>
<td>0.001</td>
</tr>
<tr>
<td>CSF sorbitol (ng/µL)</td>
<td>1.91 (1.28-3.61)</td>
<td>2.20 (1.60-4.51)</td>
<td>0.101</td>
<td>3.17 (1.12-5.42)</td>
<td>3.16 (2.3-6.5)</td>
<td>0.778</td>
</tr>
<tr>
<td>CSF myo-inositol (ng/µL)</td>
<td>20.31 (14.11-26.39)</td>
<td>26.65 (16.30-37.70)</td>
<td>0.012</td>
<td>12.71 (9.17-17.15)</td>
<td>15.74 (8.9-20.58)</td>
<td>0.261</td>
</tr>
<tr>
<td>18-FDG uptake (% of total) in hippocampus</td>
<td>0.66 (0.51-0.75)</td>
<td>0.50 (0.38-0.60)</td>
<td>0.001</td>
<td>0.79 (0.71-0.85)</td>
<td>0.73 (0.58-0.86)</td>
<td>0.133</td>
</tr>
<tr>
<td>18-FDG uptake (% of total) in frontal cortex</td>
<td>0.72 (0.56-0.83)</td>
<td>0.63 (0.48-0.74)</td>
<td>0.057</td>
<td>0.79 (0.73-0.90)</td>
<td>0.73 (0.61-0.86)</td>
<td>0.091</td>
</tr>
</tbody>
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Table 1: Differences in average glucose concentration, CNS metabolites, and 18-FDG uptake in NOD and STZ-induced diabetic mice

18-FDG-18-fluordeoxyglucose, CSF-Cerebrospinal fluid, NOD-Non-obese diabetic, DM-Diabetes mellitus, CNS-Central nervous system, STZ-Streptozotocin

Figure 1. Differences in 18-fluordeoxyglucose hippocampal uptake between thenon-obese diabetic and streptozotocin induced mice models (healthy refers to mice without manifest diabetes)
before 5 years of age have permanent neurocognitive impairment more commonly than age-matched peers and siblings [23]. Structural neuroimaging studies have demonstrated that patients with diabetes have changes in metabolites in both brain gray and white matter when compared with similar controls without diabetes [7]. These changes seem to be related to higher HbA1c levels, suggesting that high glucose levels may contribute to these pathologic abnormalities.

Furthermore, the results of the Epidemiology of Diabetes Interventions Complications study [24] showed that patients with poor metabolic control (HbA1c >8.8%) compared to those with better control (HbA1c <7.4%) show declines in motor speed and psychomotor efficiency. This data, however, are not in accordance with our study results where we demonstrated functional CNS changes early in the course of the disease. However, we consider that comorbid conditions such as hypertension, dyslipidemia, CVD, and depression could have a synergistic effect or potentially mask the effect of Type 1 DM on cognitive abilities. The notion and the similarities of the effects of these factors on cognition may be appealing but does not seem to be significant in the majority of this population because most studies, which included patients with Type 1 DM screened for and excluded patients with CVD and depression.

Unlike Type 1, Type 2 DM is diagnosed at an older age and is commonly associated with obesity, insulin resistance, hypertension, dyslipidemia, and CVD, all of which have a negative impact on the brain and therefore on cognitive function. There is an increasing number of reports that Type 2 DM is associated with a significantly increased risk of dementia due to both Alzheimer’s and vascular disease [15]. Large prospective studies have shown that Type 2 DM is not only associated with a significant cognitive decline over 20 years but also with a doubled risk for developing dementia. The mechanisms and risk factors associated with such severe cognitive impairments are still not well understood and it is still unclear whether elimination or reduction of certain risk factors (smoking, obesity, insulin resistance, hypertension, dyslipidemia, and atrial fibrillation) would have a favorable effect on cognitive function in Type 2 DM. However, a recent clinical report states that high blood glucose levels estimated by HbA1c were associated with poorer cognitive function after 12 years of follow-up [8].

Although we cannot derive any general conclusion based on our study results, it seems that central neuropathies in DM might be related to the level of glycemia and metabolic control early in disease onset, and thus, result in severe cognitive dysfunction later in the course of the disease. Moreover, we hypothesize that “metabolic memory” might have a great impact in this pathophysiological issue, which definitely needs to be clarified in further study investigations.

Author Contributions

KB gave the idea for the study, performed statistical analysis and wrote the manuscript. MĆ, LSK and LAT performed data acquisition, critically reviewed the manuscript and gave the final approval. NB, IK, and GM gave advice regarding statistical analyses and data acquisition, critically reviewed the manuscript and gave the final approval.

References


