Advanced Glycoxidation End Products in Patients with Multiple Sclerosis

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Abstract: Advanced glycoxidation end products (AGEs) play an important role in the pathogenesis of neurodegenerations and we studied if AGEs could represent a useful marker in patients with multiple sclerosis (MS). AGE-products were assessed in cerebrospinal fluid (CSF) and serum of 31 patients with MS and 8 controls. We did not find any statistically significant differences in patients with MS and controls either in CSF or in serum. We have observed a significant association between pentosidine and total AGEs as well as a relationship of both to the protein content in CSF in MS patients. Despite of the involvement of both oxidative stress and RAGE (receptor for AGEs) in the pathogenesis of MS and its experimental model, neither pentosidine nor total AGE were shown as useful markers in this indication. Other compounds and ligands of RAGE are probably of higher significance in MS.

Key words: Advanced glycoxidation end products – AGEs – Pentosidine – RAGE – multiple sclerosis – Oxidative stress.

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Abbreviations: AGEs – advanced glycation (glycoxidation) end-products, AU – arbitrary units, CSF – cerebrospinal fluid, EAE - experimental autoimmune encephalomyelitis, HPLC– high performance liquid chromatography, IFCC – International Federation of Clinical Chemistry, IQR – interquartil range, MBP – myelin basic protein, MS – multiple sclerosis, NF-κB – nuclear factor kappa B, RAGE – receptor for advanced glycation end products

Introduction

It is generally accepted, that advanced glycation (glycoxidation) end products (AGEs), a group of compounds with characteristic pigmentation and fluorescence, take part in the pathogenesis of many chronic diseases and their complications. Only some of these products have already been characterized (e.g. pentosidine, carboxymethyllysine, carboxyethyllysine or imidazolone), but it is well known, that they can modify proteins and thereby cause their damage and act via specific receptors, e.g. RAGE. AGE-RAGE interaction activates nuclear factor NF-κB, which is followed by stimulation of transcription of genes for cytokines, growth factors and adhesive molecules and a large variety of further toxic effects [1, 2, 3]. Formation of AGEs as well as their action is connected with oxidative stress.

Apart from diabetes mellitus, atherosclerosis and chronic renal failure, the attention is paid to neurodegenerative diseases. In Alzheimer's disease, AGE-modified proteins can be found both intracellulary in neurofibrils and extracellulary in plaques [4, 5]. Additionally, the expression of RAGE in damaged neurons is increased [5]. Unlike diabetics and patients with renal diseases, serum level of AGEs remains normal [6]. Accumulated AGEs were discovered also in neurofilaments in Pick's disease [7], amyotrophic lateral sclerosis [8], in Lewy bodies in Parkinson's disease [9] and in Rosenthal fibres in Alexander disease [10].

Since oxidative stress [11] and RAGE expression [12] play a role in the pathogenesis of multiple sclerosis (MS) and its experimental model – experimental autoimmune encephalomyelitis (EAE), we were interested whether advanced glycoxidation end products could represent a useful marker in patients with multiple sclerosis. To test this hypothesis, we determined pentosidine and total AGEs in cerebrospinal fluid and serum in patients with multiple sclerosis and compared them with their levels in non-multiple sclerosis subjects. Simultaneously, we measured myelin basic protein, total protein and glucose and studied the relationship of advanced glycoxidation end products to these parameters.

Material and Methods

Studied groups

The studied group consisted of 31 patients with multiple sclerosis (13 men and 18 women, mean age 39±11 years) indicated to diagnostic lumbal puncture. Duration of the disease was 2–32 years (median 9 years), 17 patients had relapse-remitting disease and 14 patients secondary progressive disease. 14 of these patients (7 men and 7 women, mean age 36±10 years) were in stable clinical status with minimal activity of the disease and 17 patients (6 men and 11 women, mean age 41±11 years) experienced acute worsening by the time of the study (10 patients with secondary progression and 7 patients with relapse-remitting

disease). Patients with diabetes mellitus, renal insufficiency and malignant tumors were excluded from the study.

8 patients without multiple sclerosis indicated to lumbal puncture (1 man and 7 women, mean age 42±14 years) were used as controls. Findings in both cerebrospinal fluid and serum in these patients were non-specific. Patients with diabetes mellitus, renal insufficiency and malignant tumors were excluded from the study. None of the studied patients had signs of acute neuroinfection.

All patients gave their informed consent with the diagnostic lumbal puncture and simultaneous blood collection (puncture of cubital vein) for diagnostic purposes and additional determination of advanced glycation end products as new potential marker of the activity of disease.

Methods

Pentosidine assay

Pentosidine was determined in acidic hydrolysates of sera and cerebrospinal fluids by reversed phase high performance liquid chromatography (HPLC, Schimadzu, C18) according to Špaček [13]. We monitored the emission signal at 385 nm upon excitation at 335 nm. The concentration of pentosidine is expressed in nmol/l and nmol/g protein.

AGE-assay

AGEs were estimated using a spectrofluorimetric method (excitation 350 nm, emission 435 nm) in sera diluted 1:50 with phosphate buffer according to Henle [14] and Munch [15] (spectrofluorimeter Fluoromax-3, Jobin Yvon Horiba, USA) and in undiluted cerebrospinal fluids in a special microcuvette. Results are expressed in arbitrary units (AU) and in AU/g protein.

Other parameters

Myelin basic protein (MBP) was determined immunochemically by radioimmunoassay (standard kit DSL, USA). Total protein and glucose were determined by standard clinical chemistry methods recommended by International Federation of Clinical Chemistry (IFCC): total protein in serum with biuret reaction (Beckmann, USA), total protein in cerebrospinal fluid with pyrrogallol red (Beckmann, USA) and glucose both in serum and cerebrospinal fluid with glucose-oxidase reaction followed by electrode detection (Beckmann, USA).

Statistical analysis

Results are expressed as medians and interquartil ranges. Mann-Whitney U test was used for evaluation of differences between groups and Spearman's correlation coefficient for description of association between parameters. All results were considered as statistically significant at $p<0.05$.

Results

Concentrations of advanced glycation end products and other biochemical parameters in cerebrospinal fluid and serum are shown in Table 1 and Table 2. We did not find any statistically significant differences in total AGEs and pentosidine in patients with multiple sclerosis compared to controls and in patients with multiple sclerosis in the phase of worsening compared to patients with multiple sclerosis in stable clinical status either in cerebrospinal fluid or in serum. Concentration of pentosidine corrected for the protein content is 2 fold higher in cerebrospinal fluid than in serum. AGEs are elevated in the cerebrospinal fluid as well (in the present study 4–5 fold), probably also due to different composition of both fluids.

Examining correlations between AGEs, pentosidine and total protein in cerebrospinal fluid and serum, we have found a significant correlation between pentosidine and AGEs in cerebrospinal fluid in all multiple sclerosis patients, worsening multiple sclerosis patients and controls. Additionally, we describe a slight, but significant correlation between serum a cerebrospinal fluid AGEs in the whole group of multiple sclerosis patients. There was also a significant correlation between cerebrospinal fluid pentosidine and total protein, and AGEs and total protein in all multiple sclerosis patients and worsening multiple sclerosis patients (Table 3). On the other hand, correlations between serum pentosidine

Table 1

Parameters (units)	Patients with multiple sclerosis (N=31)		Controls (N=8)
	Stable clinical status (N=14)	Worsening (N=17)	
CSF-pentosidine (nmol/l)	0.81 (0.58–1.09)		0.62 (0.55–0.77)
	0.64 (0.47–1.07)	0.84 (0.61–1.08)	
CSF-pentosidine/g prot. (nmol/g prot.)	2.07 (1.71–2.98)		2.25 (2.22–2.46)
	2.22 (1.01–3.12)	2.07 (1.88–2.81)	
S-pentosidine (nmol/l)	74.23 (60.46–87.25)		70.62 (56.31–93.40)
	75.94 (57.26–85.98)	74.23 (66.04–87.59)	
S-pentosidine/g prot. (nmol/g prot.)	1.05 (0.81–1.17)		0.93 (0.79–1.23)
	1.04 (0.77–1.15)	1.05 (0.85–1.24)	
CSF-AGEs (AU)	6.83 (5.03–8.58) $\times 10^3$		5.78 (4.57–7.05 $\times 10^3$
	7.59 (5.07–8.89) $\times 10^3$	6.50 (4.99–8.02) $\times 10^3$	
CSF-AGEs/g prot. (AU/g prot.)	1.75 (1.40–2.25) $\times 10^4$		2.05 (1.72–2.54) $\times 10^4$
	2.08 (1.41–2.52) $\times 10^4$	1.61 (1.41–2.08) $\times 10^4$	
S-AGEs (AU)	2.89 (2.67–3.47) $\times 10^5$		2.92 (2.65–3.15) $\times 10^5$
	2.86 (2.51–3.47) $\times 10^5$	2.89 (2.80–3.47) $\times 10^5$	
S-AGEs/g prot. (AU/g prot.)	4.07 (3.48–4.74) $\times 10^3$		4.01 (3.45–4.37) $\times 10^3$
	4.19 (3.15–4.74) $\times 10^3$	4.01 (3.65–4.65) $\times 10^3$	

and serum AGEs, serum pentosidine and serum total protein, serum AGEs and serum total protein as well as serum pentosidine and cerebrospinal fluid pentosidine were not statistically significant. Additionally, other correlations between parameters (pentosidine and AGEs with MBP and glucose) were not statistically significant in any of the studied groups.

In summary, we did not show any significant difference between total AGEs and pentosidine in cerebrospinal fluid and serum in patients with multiple sclerosis and in healthy subjects. We have observed a significant association between pentosidine and AGEs as well as the relationship of both to the protein content in cerebrospinal fluid mainly in patients with multiple sclerosis.

Table 2

Parameters (units)	Patients with multiple sclerosis (N=31)		Controls (N=8)
	Stable clinical status (N=14)	Worsening (N=17)	
CSF-MBP (ng/l)	0.96 (0.40–1.35) × 0.93 (0.40–1.47)	0.98 (0.57–1.19)	0.73 (0.58–0.90)
S-MBP (ng/l)	3.83 (3.04–4.78) 3.93 (3.05–4.37)	3.80 (3.19–4.80)	4.54 (4.11–5.26)
CSF-total protein (g/l)	0.39 (0.28–0.43) × 0.34 (0.24–0.42)	0.39 (0.31–0.43)	0.30 (0.23–0.32)
S-total protein (g/l)	74.3 (70.0–78.9) 75.3 (69.5–79.2)	72.1 (70.7–77.7)	73.0 (71.0–76.7)
CSF-glucose (mmol/l)	3.5 (3.3–3.7) × 3.7 (3.3–3.9)	3.5 (3.3–3.7)	4.1 (3.8–4.4)
S-glucose (mmol/l)	5.2 (4.8–5.5) 5.3 (4.8–5.5)	5.2 (4.5–5.5)	5.2 (4.9–5.8)

Table 3

Correlations	Patients with MS whole group	Patients with MS worsening	Controls
CSF-pentosidine vs CSF-AGEs	r=0.54, p=0.0016	r=0.66, p=0.0038	r=0.74, p=0.037
CSF-AGEs vs S-AGEs	r=0.36, p=0.044		
CSF-pentosidine vs CSF-total protein	r=0.50, p=0.0037	r=0.77, p=0.0003	
CSF-AGEs vs CSF-total protein	r=0.54, p=0.002	r=0.55, p=0.022	

Discussion

Advanced glycation end products are involved in the pathogenesis of several neurodegenerative diseases. However, in multiple sclerosis, the significance of AGE-determination seems to be limited, as shown in the present study.

Concerning AGEs in serum, our results in multiple sclerosis patients are in line with previous findings that in neurodegenerative diseases, serum AGE-levels remain normal [6] or are even slightly decreased [16]. In lesions affecting central nervous system, concentrations of several substances in blood may not reflect changes in the brain and for this reason, analysis of cerebrospinal fluid or tissue samples (post-mortem) are preferable [16]. Determination of AGEs in cerebrospinal fluid was performed only in a small number of studies and was aimed at Alzheimer's disease and vascular dementia, where significant elevation was shown [16, 17]. After correction of AGEs for protein content, higher AGE-levels are observed in cerebrospinal fluid than in serum both in controls and multiple sclerosis patients as shown in our study, as well as in Alzheimer's disease and vascular dementia [16], probably due to minimalization of metabolic influences and interferences present in serum, however, other reasons cannot be excluded. The majority of AGEs is protein-bound, and a tight correlation between AGEs and protein content was observed in cerebrospinal fluid but and not in serum, given probably by the composition of serum, as already mentioned above.

It was shown that oxidative stress plays an outstanding role in the pathogenesis of multiple sclerosis – e.g. Liu et al. have shown that bilirubin as a potent antioxidant suppressed experimental autoimmune encephalomyelitis, an experimental model of multiple sclerosis [18] and Besler and Comoglu [19] described an elevation of oxidized lipids and a decrease of total antioxidant capacity in serum. As oxidative stress enhances formation of AGEs and pentosidine and other AGEs are also called glycoxidation products, we have expected their elevation in patients with multiple sclerosis (at least in those in the phase of worsening), which was not proved.

Additionally, RAGE (receptor for advanced glycation end products), a multiligand receptor, is present on T cells, which react with the major components of myelin sheats, mononuclear phygocytes and endothelium. Its proinflammatory ligands, S100-calgranulins, are upregulated in multiple sclerosis and its model, experimental autoimmune encephalomyelitis. Blockade of RAGE suppresses experimental autoimmune encephalomyelitis, when the disease was caused e.g. by myelin basic protein. Inhibition of RAGE markedly decreased infiltration of the central nervous system by immune and inflammatory cells [12]. As AGEs also interact with this receptor and are related to inflammation (via RAGE influence transcription of genes for cytokines), we hypothetised that they could be of importance in multiple sclerosis as well. We did not find even any association of AGEs with myelin basic protein, which might

be also involved in the pathogenesis of this disease (however, despite being elevated in cerebrospinal fluid of multiple sclerosis patients, in our hands, it is not a reliable marker, either).

Conclusion

We can conclude that despite of the involvement of both the oxidative stress and RAGE (receptor for AGEs) expression in the pathogenesis of multiple sclerosis and in its experimental model, neither pentosidine nor total AGEs were shown as useful markers in this indication. Other compounds as well as other ligands of RAGE are probably of higher significance in this disease.

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