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A thermodynamic study of β-N-acetylhexosaminidase enzyme heterogeneity in cerebrospinal fluid from patients with multiple sclerosis

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Abstract

Background. ß-N-acetylhexosaminidase (Hex) is a lysosomal hydrolase, whose determination in the cerebrospinal fluid (CSF) of patients with multiple sclerosis (MS) has provided discordant results.
Methods. Total Hex and its isoenzymes Hex A and Hex B were determined using a thermodynamic procedure in the CSF of 27 patients with definitive MS, 8 with possible MS, 9 with meningitis, 14 with other neurological diseases, and in 10 controls without any neurological disease.
Results. In the group of patients with definitive MS, the total Hex and Hex A were significantly higher than in the control group ($p<0.001$), with a possible association of greater enzymatic activities with the presence of oligoclonal bands and recent relapse; however, an overlap was detected for the activities of total Hex and its isoenzymes between the groups of patients with different neurological diseases. A significant correlation was obtained for neuron-specific enolase (NSE) with total Hex and Hex A and Hex B isoenzymes ($p<0.001$); however, in the partial correlation statistical significance was only obtained between NSE and Hex A ($p<0.001$) which is the most abundant Hex isoenzyme in the brain.
Conclusions. Although the inflammatory process in MS mainly takes place in the perivascular zone, with little activity in the cerebral parenchyma, the significant increase of NSE and Hex A isoenzyme in CSF reveals a neuronal damage. The disease status may have effect on the CSF Hex activity.

Key words: ß-N-Acetylhexosamninidase isoenzymes; neuron-specific enolase; multiple sclerosis; cerebrospinal fluid.

Introduction

Multiple Sclerosis (MS) is one of the most common demyelinating diseases that affect the central nervous system, and the most frequent cause of non-traumatic neurological incapacity in young and middle-aged adults in Europe and USA (1). The autoimmune nature of this illness explains why most research, either in relation to its pathogenesis, diagnosis or treatment, is focused on its immunological aspects (1,2). However, in MS an accumulation of lysosomal enzymes occurs in the plaques that may play an important role in the destruction of myelin (3–5), although the clinical value of determining its activity in cerebrospinal fluid is a controversial issue (5–8).

β-N-acetylhexosaminidase (Hex, EC 3.2.1.52) is a lysosomal hydrolase present in human brain homogenates, and apart from the principal acidic Hex A and Hex

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B isoenzymes, an additional minor fraction (Hex C) with optimum neutral pH has been characterised (9). Goi et al (7) and Hultberg and Olsson (8) found an enzymatic activity for total Hex in CSF of patients with MS that was significantly lower than in control subjects. However, this finding was not confirmed by Datti et al (10), detecting at the same time in the isoenzyme separation by column chromatography in DEAE-cellulose an increase of the relative proportion of Hex A, and an additional fraction that is more acidic than the normal Hex A isoenzyme. In accordance with these authors, the explanation would be an anomalous glycosylation of the enzyme protein and the presence of a Hex A fraction of tissue origin, both in CSF and in serum from patients with MS (11).

Using the chromogenic substrate 3-3´-dichlorophenolsulphoftaleinyl-N-acetyl-β-D-glucosaminide, the apparent enzyme activation energy of Hex is directly related to the relative proportion of the Hex A (αβ heterodimer) and Hex B (ββ homodimer) isoenzymes (12, 13). The presence of the α subunit in Hex A means that its activation energy is significantly lower ($p<0.001$) than that of the Hex B isoenzyme (13), independently of their sialylation degrees (12). The aim of our research was to make a thermodynamic study of the Hex enzyme heterogeneity in CSF from patients with MS.

Patients and methods

CSF samples collected by lumbar puncture were obtained from 27 patients with definitive MS diagnosis and 8 patients with possible MS according to the criteria of Poser et al (14). A further 9 patients with meningitis were studied (5 with polymorphonuclear pleocytosis and 4 with mononuclear pleocytosis), and 14 patients with other neurological diseases. As a control group 10 patients without any neurological illness, who were given a lumbar puncture for diagnostic purposes in the Emergency Unit, were included. The study was approved by the ethical committee of the University of Santiago de Compostela Hospital Clinic, and all participants provided their consent to participate.

The determination of total Hex activities in CSF and serum at 25 ºC, 30 ºC, 35 ºC and 37 ºC using 3-3´-dichlorophenolsulphoftaleinyl-N-acetyl-β-D-glucosaminide as substrate, and the calculation of the slopes of Arrhenius plots and the apparent activation energies for the evaluation of the Hex isoenzyme composition, were carried out as previously described (12). Isoelectric focusing of concentrated CSF and undiluted serum for the detection of oligoclonal bands was performed on polyacrylamide gel. The neuron-specific enolase (NSE, EC 4.2.1.11) was determined by enzyme immunoanalysis in a Cobas Core analyzer, using reagents marketed by Roche Diagnostics. The samples of CSF and serum were kept at a temperature of –80ºC for a period of no more than 7 days prior to their analysis.

Statistical analysis of the data was made using the SPSS package (SPSS Inc., Chicago, USA), and the Kolmogorov-Smirnov test was applied to check for normality. Parametric tests (Student t-test and Pearson´s correlation coefficient) were

used when the data had a Gaussian distribution; otherwise, non-parametric tests (Mann-Witney U-test and Spearman´s correlation coefficient) were used. The results were expressed as mean±SEM (median).

Results

Table 1 shows the results obtained for total Hex and its isoenzymes in the different groups of controls and patients. A significant correlation was found for all of the cases studied between the Hex A and Hex B isoenzymes ($r=0.660$, $p<0.001$), but also for the total Hex with the relative proportion of Hex A ($r=0.474$, $p<0.01$), which would indicate that the increases in CSF for total Hex activity is preferably produced at the expense of the Hex A isoenzyme.

The distribution of the activities of total Hex and the relative proportion of Hex A isoenzyme in the different groups of patients studied are shown in Figure 1. In the MS patients, the levels of total Hex and Hex A, as well as the relative proportion of this isoenzyme, were significantly higher than in the control group ($p<0.001$); however, there is an overlap between these biochemical variables when considering the groups of patients with different neurological diseases. Twentythree (85%) of the 27 patients with definitive MS diagnosis had oligoclonal bands in their CSF, while in the 8 patients with possible MS this prevalence was only 50%. In the patients with definitive MS, the presence of oligoclonal bands appears to be associated with elevated Hex activity in CSF, as well as the relative proportion of Hex A (Figure 1), and the higher Hex activities were found in two patients with a recent relapse.

NSE was determined in 36 cases (11 with definitive MS, 2 with possible MS, 9 with meningitis, 7 with other neurological diseases and 7 controls), and a significant correlation was found for this variable with the total Hex (Figure 2), Hex A ($r=0.757$, $p<0.001$) and Hex B ($r= 0.622$, $p<0.001$). In the partial correlation between NSE and Hex A, keeping Hex B constant, the statistical significance was maintained ($r=0.589$, $p<0.001$); however, in the partial correlation of NSE with Hex B, keeping Hex A constant, statistical significance was not achieved ($r=0.249$, $p=0.148$).

The total serum Hex activity in the group of patients with definitive MS was significantly higher than in the control group, however a significant difference was not found for the Hex isoenzyme composition, as indicated in Table 2.

Discussion

In the group of patients with definitive MS, the activities in CSF for total Hex and Hex A, as well as the proportion of this isoenzyme, were significantly higher than in the control group (Table 1, Figure 1); however, in relation to the other groups of neurological patients studied, there is an overlap between the values found for these biochemical variables. A significant correlation between the total Hex and the

Table 1. Levels of total Hex and its isoenzyme composition in CSF. Significance with respect to the control group: *** p<0.001, * p<0.05, NS Not significant.

	n	Total Hex (U/L) Mean±SEM (Median)	Hex A (U/L) Mean±SEM (Median)	Hex A (%) Mean±SEM (Median)
Controls	10	1.18 ± 0.14 (1.04)	0.66 ± 0.09 (0.49)	55.0 ± 1.59 (55.5)
Def. MS	27	2.26 ± 0.12 (2.05)***	1.64 ± 0.10 (1.46)***	69.5 ± 1.15 (71.0)***
Poss. MS	8	1.84 ± 0.20 (1.68)*	1.14 ± 0.14 (1.03)*	61.6 ± 2.78 (61.0)*
OND	14	1.99 ± 0.16 (2.00)***	1.17 ± 0.10 (1.09)***	58.2 ± 1.89 (60.0) NS
MN	9	1.57 ± 0.26 (1.23)NS	0.99 ± 1.18 (0.70) NS	61.7 ± 1.18 (62.5)***

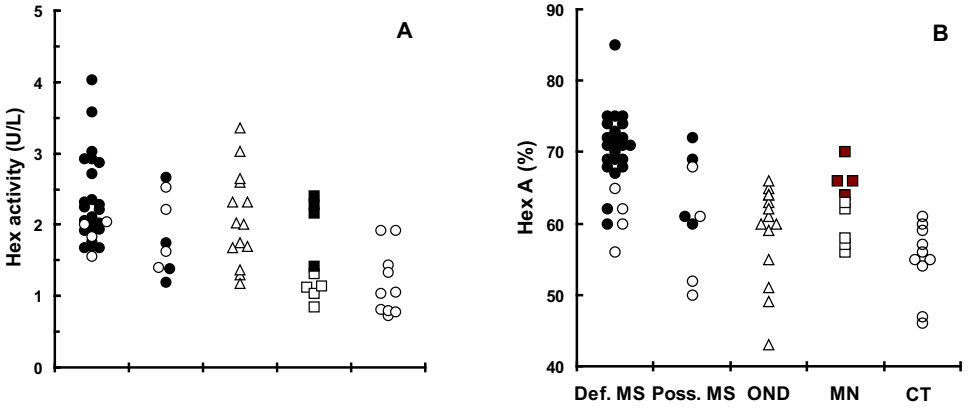

Figure 1
Distribution of total Hex activity (A) and relative proportion of Hex A isoenzyme (B) in cerebrospinal fluid. Patients with multiple sclerosis (MS) with (●) or without (○) oligoclonal bands, meningitis (MN) with mononuclear (■) or polymorphonuclear (□) pleocytosis, other neurological diseases (OND) and controls (CT).

relative proportion as a percentage of Hex A was found in all of the cases studied, which suggest that the increases of the enzymatic activity in CSF would preferably be due to the increase of this isoenzyme.

The prevalence of oligoclonal bands in the CSF of patients with definitive and possible MS is in agreement with the results previously obtained in Spain by Falip et al (15). The presence of CSF oligoclonal bands in MS is related to the inflammatory process, and is the result of the synthesis of immunoglobulins (mainly IgG) by lymphocytes and plasma cells, which surround the demyelinisation plaques and perivascular zones (16,17), and appears to be associated with a greater activity of total Hex and Hex A (Figure 1). Likewise, the disease status may have a significant effect on the CSF enzyme activity, because the higher Hex activities were found in two patients with a recent relapse.

NSE (γγ homodimer) in CSF is considered as a suitable marker for neuron damage (18,19). The results obtained in our MS patients are analogous to those ob-

tained by Royds et al (19), with higher levels of NSE in relation to the control group (10.1±0.99 ng/mL vs 5.0± 0.93 ng/mL, $p<0.001$), which would be the result of a damage caused to the axons passing through demyelinised areas. The most important increase described for the enolase of glial origin (αα homodimer), is compatible with the proliferation of astrocytes in MS (19).

A significant correlation was found between the levels of NSE, total Hex, Hex A and Hex B in CSF, although the partial correlation study revealed the statistical significance for the correlation of NSE with Hex A, but not with Hex B isoenzyme. Although the main inflammatory process in MS occurs in perivascular regions, with little activity in the cerebral parenchyma (20), and the extent of axonal damage is controversial (1), the significant increases in CSF of NSE and Hex A, which is the predominant Hex isoenzyme in the brain (21), reveal a neuronal damage in this disease.

The thermodynamic results obtained for the enzyme heterogeneity of Hex in the CSF of patients with MS reveal an increase of the Hex A isoenzyme activity and of its relative proportion (Table 1), and are in agreement with the chromatographic data obtained by Datti et al (10). An anomalous glycosylation, as has been described in several neurological diseases for different proteins (22), and the presence of mature (tissue) forms as well as precursor forms which escape from incorporation into lysosomes and are secreted to the extracellular medium, may explain the chromatographic heterogeneity described for Hex in CSF and serum from MS patients (10,11). However, as the desialylated and tissue forms of Hex are rapidly cleared from circulation (23, 24), it is necessary to carry out a more extensive confirmatory study in order to discover the specificity and sensitivity of this serum Hex A heterogeneity in MS patients.

No significant differences were found in our study, for the serum Hex isoenzyme composition, between the groups of MS patients and controls (table 2), not confirming the increase in Hex B isoenzyme proportion previously described by other authors (11). The fact that serum was used instead of plasma in both studies may introduce, as a disturbing factor, the presence in the samples of Hex activity released from the platelets during blood coagulation; however, it does not appear that the relative proportion of the Hex A and Hex B isoenzymes may be modified in a significant degree (25).

In conclusion, although the inflammatory process in MS mainly occurs in the

Table 2. Levels of total Hex and its isoenzyme composition in serum. Significance with respect to the control group: *** $p<0.001$, NS Not significant.

	n	Total Hex (U/L) Mean±SEM (Median)	Hex A (U/L) Mean±SEM (Median)	Hex A (%) Mean±SEM (Median)
Controls	104	6.88 ± 0.14 (6.50)	4.66 ± 0.08 (4.47)	68.2 ± 0.40 (68.1)
Def. MS	26	8.52 ± 0.36 (8.64)***	5.84 ± 0.26 (5.65)***	68.9 ± 0.96 (68.0) ^{NS}
Poss. MS	8	7.27 ± 0.57 (7.00)^{NS}	5.26 ± 0.50 (4.56) ^{NS}	66.0 ± 2.45 (67.4) ^{NS}

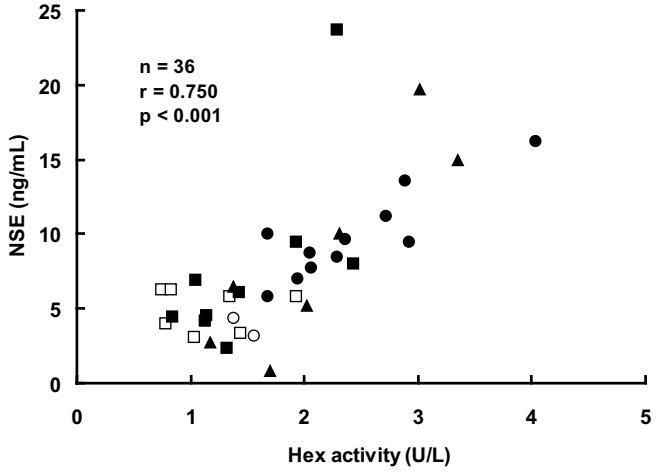

Figure 2
Correlation between NSE and total Hex activity in cerebrospinal fluid from patients with definitive (●) and possible (○) multiple sclerosis, meningitis (■), other neurological diseases (▲) and controls (□).

perivascular zone, with little activity in the cerebral parenchyma, neuronal damage leads to a significant increase of Hex A isoenzyme and NSE in the CSF of these patients.

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