Insulin-like growth factor system in human central nervous system, multiple sclerosis and amyotrophic lateral sclerosis

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Chapter 4

Insulin-like growth factor-I receptors in normal appearing white matter and chronic plaques in multiple sclerosis

Nadine Wilczak and Jacques De Keyser

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Abstract

Preclinical studies suggest that insulin-like growth factor-I (IGF-I) plays an important role in oligodendrocyte survival and myelination. We used human recombinant $^{125}$IGF-I to study IGF-I receptors in post-mortem brain tissue from patients with multiple sclerosis (MS). In normal appearing white matter, we found that IGF-I receptor densities and binding characteristics were not different between MS patients and controls. In chronic plaques, histologically characterised by astrogliosis, we found densities of IGF-I receptors, which were in the same range as those measured in the normal appearing white matter. *In vitro* studies have shown that IGF-I also acts as a mitogenic factor for astrocytes. Since MS lesions are rapidly invaded by reactive astrocytes, IGF-I may not only protect oligodendrocytes and stimulate remyelination but also enhance the astrogliosis that limits repair.
Introduction

Insulin-like growth factor I (IGF-I) promotes the survival and regeneration of oligodendrocytes in culture systems and stimulates the synthesis of myelin by regulating the expression of myelin genes in oligodendrocytes \(^{2,11,12}\). Transgenic mice, which overexpress IGF-I, have an increased myelin content in their central nervous system \(^4\), whereas oligodendrocytes and myelin are deficient in mice homozygous for disrupted IGF-I gene \(^3\). We have previously shown that the central white matter in neonates, undergoing active myelination, contains a 3-fold higher density of IGF-I receptors than in adults, suggesting that also in human brain IGF-I and IGF-I receptors play an important role in the myelination process \(^6\). In the monophasic model of experimental allergic encephalomyelitis (EAE), treatment with IGF-I reduces clinical deficits and lesion severity \(^9,16\). Therefore, it has been suggested that IGF-I may be useful as a treatment for multiple sclerosis (MS). However, information concerning IGF-I receptors in the central nervous system in MS is lacking. We compared IGF-I receptors in post-mortem obtained brain specimens from patients with MS and controls.

Material and Methods

**Brain tissues**

The experiments on post-mortem brains were approved by an Ethical Committee. Slices (± 0.5 cm thick) of frontal lobe were obtained from 4 patients with neuropathologically confirmed MS (3 females and 1 male; mean age 69 ± 7 years; mean post-mortem delay 8 ± 1h) and 5 neurologically intact controls (3 females and 2 males; mean age 68 ± 9 years; mean post-mortem delay 10 ± 2 h). The slices were quickly frozen in liquid nitrogen or with dry ice and stored at –80°C.

**Radioligand binding assays**

Cryostat sections of 20-µm thickness were thaw-mounted on gelatine-coated microscopic glass slides and dried overnight. The optimal incubation conditions were determined in previous experiments \(^7\). In brief, the slide-mounted sections were pre-incubated for 15 min at
25°C in 25 mM Tris-HCl pH 7.5 containing 10 mM MgCl₂ and 0.1% bovine serum albumin. Consecutive sections were incubated in duplicate for 60 min at 25°C in the same buffer with 0.1 nM human recombinant [125I]IGF-I (Amersham, Buckinhhamshire, UK). After incubation, the sections were washed 3 times for 1 min each in the same buffer to remove unbound ligand and then quickly dipped in distilled water.

For competition binding experiments, the cerebral cortex was removed from the white matter. The washed sections were wiped from the slides using glass fiber filters and radioactivity was determined in a gamma-counter. Binding isotherms were analysed by non-linear least-square curve fitting. The dissociation constants (Ki values) of the competitors IGF-I, DES(1–3)IGF-I and R³IGF-I (Gropep, Adelaide, Australia) were calculated from the corresponding IC₅₀ values by the method of Cheng and Prusoff⁵. DES(1–3)IGF-I and R³IGF-I are analogues of IGF-I which display high affinity for IGF-I receptors but not for IGF-binding proteins¹⁰. In these experiments, non-specific binding of [125I]IGF-I was determined in the presence of 0.5 µM IGF-I.

For quantitative autoradiography, the sections were dried, placed in X-ray cassettes together with commercially available [125I]standards (Amersham) and apposed to [³H] Ultrofilm (Amersham) for 4 days. Non-specific binding of [125I]IGF-I was determined in consecutive sections in the presence of 0.5 µM DES(1–3)IGF-I. The autoradiographic images obtained on the films were scanned and analysed by computer-assisted densitometry using NIH Image Analysis software Macintosh. Mean optical densities were determined and converted into disintegration/min/mg protein and fmol/mg protein, based on the experimentally determined relation between polymer and brain paste standards. Specific binding values were obtained by the subtraction of non-specific binding from total binding.

Consecutive sections were also stained with luxol fast blue (myelin staining) and examined by light microscopy to identify normal appearing white matter and demyelinated plaques. Glial fibrillary acidic protein (GFAP) immunoperoxidase staining was used to identify astrocytes.
Results

**Binding studies and receptor concentrations**

Representative competition binding experiments in central white matter are shown in Figure 1. Both DES(1–3)IGF-I and R³IGF-I displaced the same amount of [¹²⁵I]IGF-I, indicating that in the conditions used in our assays, [¹²⁵I]IGF-I binds to IGF-I receptors and not to IGF-binding proteins. Computer analysis of the binding data revealed that the competition curves were best described by a one-component binding model.

The calculated $K_i$ values for IGF-I, DES(1–3)IGF-I and R³IGF-I were similar between MS patients and controls (Table 1). IGF-I receptor densities in normal appearing white matter and cerebral cortex from MS patients were not different from those in controls (Table 2).

Seven demyelinated plaques were identified by luxol fast blue staining of the frozen sections. Histologically all demyelinated lesions were identified as inactive chronic plaques, devoid of an inflammatory response (absence of monocytic infiltrations) and characterised by extensive astrogliosis. The astrocytes were identified by their morphology and GFAP immunoreactivity (Figure 2). In all these plaques, we measured only slightly reduced densities of IGF-I receptors (Table 2); the difference in receptor densities between normal appearing white matter and plaques was not detectable on visual inspection of the autoradiographs (Figure 3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>MS patients (n = 3)</th>
<th>Controls (n = 3)</th>
</tr>
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<tbody>
<tr>
<td>IGF-I</td>
<td>1.04 ± 0.18</td>
<td>0.90 ± 0.20</td>
</tr>
<tr>
<td>DES(1-3)IGF-I</td>
<td>0.95 ± 0.23</td>
<td>0.75 ± 0.07</td>
</tr>
<tr>
<td>R³IGF-I</td>
<td>0.77 ± 0.12</td>
<td>0.95 ± 0.22</td>
</tr>
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$K_i$ values (nM) of IGF-I, DES(1-3)IGF-I, and R³IGF-I for competition with [¹²⁵I]IGF-I binding in microtome sections of central white matter (from the frontal lobe) are given as the mean ± S.E.M.
Table 2  
IGF-I receptor densities in human frontal cortex and central white matter from patients with MS and controls.

<table>
<thead>
<tr>
<th></th>
<th>Cerebral cortex</th>
<th>normal appearing white matter</th>
<th>plaques (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 5)</td>
<td>23.83 ± 1.54</td>
<td>11.81 ± 2.05</td>
<td>-</td>
</tr>
<tr>
<td>MS patients (n = 4)</td>
<td>23.07 ± 2.52</td>
<td>11.43 ± 2.03</td>
<td>8.66 ± 1.31</td>
</tr>
</tbody>
</table>

Receptor densities were obtained from [\(^{125}\)I]IGF-I receptor autoradiographs and are expressed in fmol/mg protein. Non-specific binding of [\(^{125}\)I]IGF-I was determined in consecutive sections in the presence of 0.5 µM DES(1-3)IGF-I. Values are means ± S.D.

Figure 1

Representative competition binding curves for IGF-I (O), DES(1-3)IGF-I (●) and R\(^3\)IGF-I (□) inhibition of 0.1 nM [\(^{125}\)I]IGF-I binding on microtome sections of central white matter from a patient with MS. 

\(Ki\) values are given in Table 1.
Figure 2

Immunoperoxidase staining for glial fibrillary acidic protein (X250) demonstrating the dense astrogliosis in one of the chronic plaques.

Figure 3

Consecutive microtome sections (20-µm thickness) from the frontal lobe of a patient with MS. The left section shows the myelin staining with luxol fast blue discriminating a plaque (the pale area) from normal appearing white matter (surrounding the pale area). The right section shows an autoradiograph of the specific binding (= total minus nonspecific binding) of $[^{125}\text{I}]$IGF-I receptors in white matter and cerebral cortex. Both the normal appearing white matter and plaque contain a substantial amount of IGF-I receptors. The dark rim on the right side of the section is the cerebral cortex, which contains a much higher IGF-I receptor density.
Discussion

MS is a chronic demyelinating disorder and is the most common cause of disability in the young adult. The aetiology remains unknown. One hypothesis suggests that MS patients may be relatively deficient in intrinsic mechanisms for limiting CNS tissue injury following common insults, especially in the capacity for myelin repair. IGF-I and IGF-I receptors appear to play a key role in the myelination process.

Our findings indicate that there is no deficiency in IGF-I receptors in MS brain and that interventions for the promotion of remyelination in MS through IGF-I receptor activation might be feasible. IGF-I can cross the blood–brain barrier in normal adult rats. In active inflammatory demyelinating lesions, higher levels of IGF-I may be expected because there is a disruption of the blood–brain barrier. Studies in the EAE model also suggest that the systemic administration of IGF-I may have potential utility in the treatment of MS. However, we found that IGF-I receptor densities in chronic MS plaques were only slightly reduced and, on visual inspection of the autoradiographs, we were unable to distinguish plaques from normal appearing white matter. Chronic plaques are devoid of oligodendrocytes and contain a dense network of astrocytes responsible for the characteristic astrogliosis. In vitro studies have shown that IGF-I is also a mitogenic compound for astrocytes. This may have implications for the clinical use of IGF-I in MS. Acute MS lesions are rapidly invaded by reactive astrocytes. Therefore, the administration of IGF-I to MS patients might not only protect oligodendrocytes and stimulate remyelination but also enhance the astrogliosis that limits repair. This may suggest that there exists a narrow therapeutic time window for IGF-I to be beneficial in MS when administered in patients with a relapse, or it should be given prophylactically in the early stages of the disease.

Additional research is needed before administering IGF-I to patients with MS. Valuable information can be obtained by studying the effects of IGF-I in the chronic relapsing form of EAE where substantial astrogliosis also occurs, just as it does in MS.

Acknowledgements

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References


