Fluoxetine as disease modifying treatment in multiple sclerosis

Rationale, evaluation of the use of MRI to monitor treatment, and preliminary findings

Jop Mostert

The research presented in this thesis and the publication of this thesis was supported by the MS foundation ‘MS anders’, Amsterdam, The Netherlands.

Printed by: Gildeprint, Enschede

ISBN 9789490122164

RIJKSUNIVERSITEIT GRONINGEN

Fluoxetine as disease modifying treatment in multiple sclerosis.

Rationale, evaluation of the use of MRI to monitor treatment, and preliminary findings.

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
dinsdag 19 mei 2009
om 13.15 uur

door

Jop Pieter Mostert
geboren op 19 januari 1977
te Leiderdorp

Promotores:	Prof. dr. J.H.A. De Keyser Prof. dr. M.A. van Buchem
Copromotor:	Dr. D.J. Heersema
Beoordelingscommissie:	Prof. dr. F. Barkhof Prof. dr. J.B.M. Kuks Prof. dr. J.W. Snoek

Paranimfen: Marc Kuipers
Harold Weitenberg

Contents

Chapter 1

Introduction

Multiple sclerosis (MS) is a chronic disorder of the central nervous system (CNS). It usually starts in young adulthood and leads to cumulative disability. In Groningen the prevalence was estimated at 79 per 100000.[1] About 16000 people in the Netherlands have MS. Worldwide the number of patients is estimated at 2.5 million.[2] Women are affected more frequently than men (ratio male : female = 1 : 2).

The majority of MS patients presents with recurrent episodes of neurological deficit, which are followed by complete or partial recovery. These episodes are called relapses or exacerbations. This disease course is called relapsing remitting MS.[3] After 10 years 40% and after 20 years around 70% of these patients have gradual progression of symptoms independent of relapses.[4,5] The disease course is then called secondary progressive. Ten to 20 % of patients have a primary progressive disease course, in which patients experience gradual worsening of symptoms from the beginning of their disease. Around 25 % of the patients with MS have a 'benign' disease course. Ten years after onset these patients have suffered some exacerbations, but they have recovered well and they are not impaired in their daily activities of living.[6]

The cause of MS is unknown. Based upon epidemiological, migration and genetic studies, it is hypothesized that a genetic predisposition in conjunction with unknown infectious agents or other environmental factors, activates the production of auto reactive T cells.[7] Autoimmune responses would lead to focal inflammatory demyelination, which is clinically expressed by relapses. In the cerebrospinal fluid of MS patients also B cell proliferation is found, but it is unknown at what specific antigen this response is aimed.[7] Next to inflammatory demyelination, axonal injury is a major pathological feature of MS. A number of investigators have hypothesized that dysfunction of sodium-calcium exchangers, triggers calcium mediated injury, leading to neuronal degeneration.[7] Clinically this is expressed by a progressive disease course.
The relationship between inflammatory demyelination and the widespread axonal degeneration is unknown. Many believe that the inflammation itself causes the degeneration, directly or after crossing a certain threshold of damage for which the CNS can not compensate. Others think that inflammatory demyelination and axonal degeneration are two separate entities.

All currently approved treatments of MS are aimed at reducing inflammatory activity. The beta-interferons and glatiramer acetate reduce the number of exacerbations with about one third.[8,9,10,11] Mitoxantrone and natalizumab are more effective but have potentially serious side-effects and can only be used in patients with severe disease activity.[12,13]

In 1999 our research group reported that beta-2 adrenergic receptors on astrocytes in MS patients were deficient.[14] Based on this finding a new hypothesis was presented,[15]

implicating that the resulting decrease in cAMP signaling in the astrocyte might 1) enable T cells to turn astrocytes into antigen –presenting cells, which are necessary to initiate the inflammatory cascade, and 2) decrease the energy supply to the axons, which would lead to axonal degeneration (Figure 1a and Figure 1b[15]).

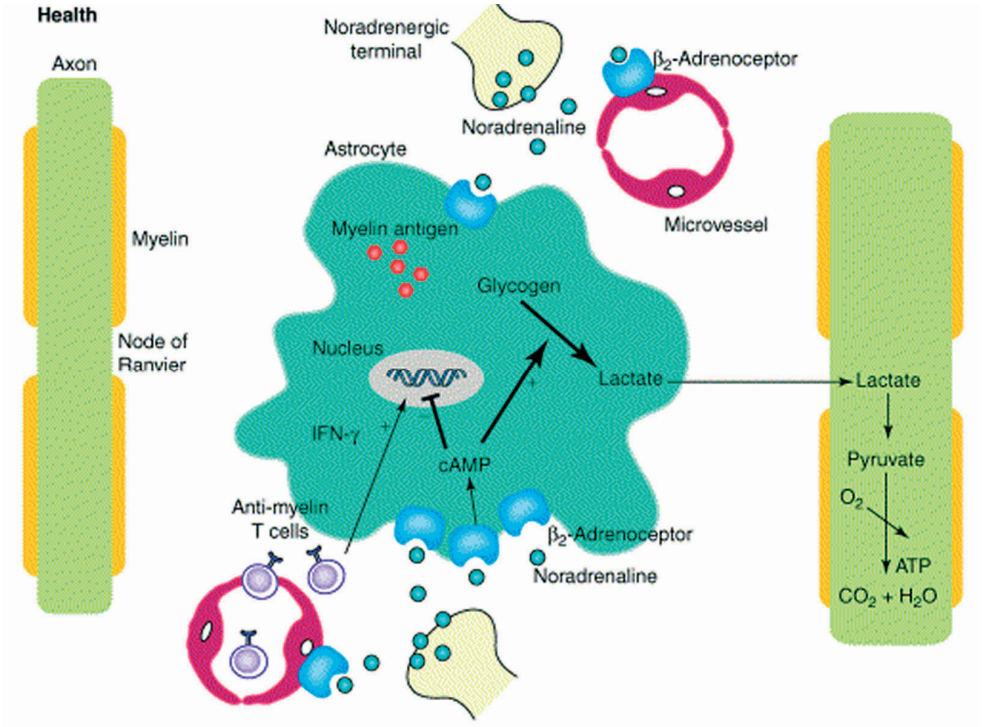

Figure 1a. In healthy individuals, anti-myelin T cells that have been activated in the periphery can penetrate in the CNS and secrete interferon γ (IFN-γ) to induce the expression of major histocompatibility (MHC) class II molecules on putative antigen-presenting cells. In the normal situation, astrocytes are unable to act as antigen-presenting cells because noradrenaline acting at β_2-adrenoceptors, coupled to the generation of cAMP, strongly suppresses IFN-γ-induced expression of MHC class II molecules. Astrocytic β_2-adrenoceptors also play an important role in stimulating the breakdown of astrocytic glycogen into lactate, which is shuttled to axons and converted into ATP.

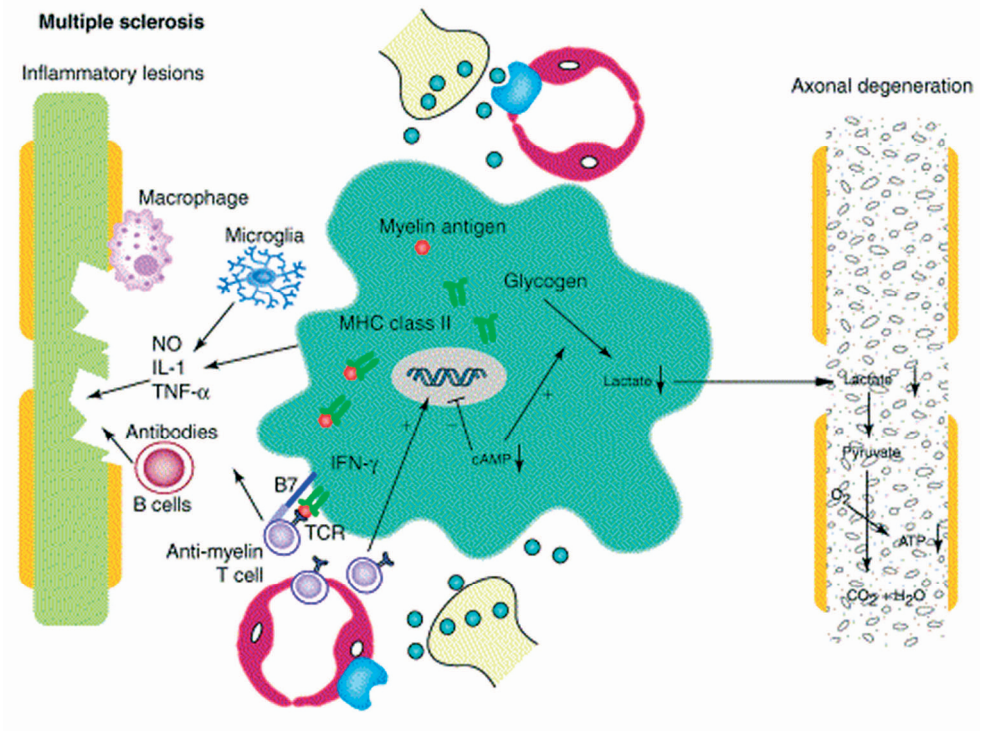

Figure 1b. Hypothetical model that explains both relapses and progressive disability of multiple sclerosis (MS). In MS, astrocytic β_2-adrenoceptors are lost, leading to decreased cAMP signaling. This might facilitate the expression of IFN-γ-induced MHC class II molecules and co-stimulatory factors. Binding of the T-cell receptor (TCR) with the MHC class II–antigen complex and appropriate co-stimulation (B7 molecules) can initiate (auto)immune responses that result in relapses of MS. The concerted attack of immune cells, cytokines [e.g. tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1)] and nitric oxide (NO) released by microglia and astrocytes, and antibodies released from B cells, produces areas of demyelination and injury to oligodendrocytes and axons. Within the inflammatory lesions, macrophages phagocytose pieces of the myelin sheath. The lack of astrocytic β_2-adrenoceptors might also reduce cAMP-mediated glycogenolysis and axonal energy supply, particularly in situations of heightened neuronal activity, resulting in progressive dysfunction and degeneration of axons.

With Magnetic Resonance Imaging (MRI) it is possible to noninvasively 'view' the brain. Different sequences visualize distinctive pathological features of MS. Dual T2 and FLAIR scans show hyperintensities that are typically located in the periventricular white matter, and represent both past (the so-called sclerotic plaques or scars) and current inflammation. On T1 scans the lesions that enhance after the intravenous administration of contrast are representative for active inflammation. Atrophy can be determined on several sequences and is indicative of axonal loss. All these techniques are part of the conventional MRI work-up.

With Proton MR Spectroscopy (^{1}H-MRS), the amount of N-acetyl aspartate (NAA), Creatine (Cr), Choline (Cho) and lactate (Lac) can be measured. NAA is found mainly in neurons and axons and is considered a marker for axonal metabolism and function. Cr is relatively stable in human brain and is commonly used as a reference substance. Cho is a cell membrane marker and increases are indicative of glial proliferation. Lac is the product of anaerobic glycolysis. The concentration of these substances can change in both physiological and pathological conditions, such as MS.[16]
Currently MRI is an important tool in diagnosing MS.[17] The appearance of new lesions on MRI, even without clinical symptoms, and the existence of many 'old' lesions strongly support the diagnosis. In phase 2 trials, the initial trials to investigate whether a drug has the potential to be effective tested on a small group of patients, the cumulative number of enhancing lesions on serial MR scans is used as a surrogate marker for inflammatory activity.[18] Many MRI techniques, including ^{1}H-MRS, are used for research purposes but do not have a common use in clinical practice and clinical trials.

Outline of this thesis

This thesis was initiated after the hypothesis of the loss of the beta-2 adrenergic receptor on astrocytes in patients with MS was put forth. The aim was to study in MS patients effects of drugs that have been shown to increase the amount of cAMP in the astrocytes. We selected the selective serotonin re-uptake inhibitor (SSRI) fluoxetine (Prozac®), which is since 1987 registered for the treatment of depression. Fluoxetine was chosen because it is cheap, well-tolerated, safe to use long-term, and has many "neuroprotective" effects that might be beneficial in neurodegenerative disorders. In experimental allergic encephalomyelitis (EAE), an animal model of MS, fluoxetine-treated mice had less inflammation and axonal damage than placebo treated mice.[19] Since MS is a chronic disease with infrequent disease activity and slow progression of disability, effects of drugs are difficult to measure on clinical scales. On cerebral MRI disease activity can be found without clinical correlates. Therefore we evaluated different MRI methods to assess efficacy of treatment.

This thesis is written in three parts. In the first part (Chapter 2) we review the effects of fluoxetine in neurological disorders.
In the second part we look at conventional MRI methods to measure effects of fluoxetine in MS. Chapter 3 described a placebo-controlled study evaluating the effects of fluoxetine on new enhancing lesions in patients with MS. In Chapter 4 distinct methods to measure brain atrophy are compared. Since the relevance of measuring T2 lesions and brain atrophy is unknown, we look at the predictive value of the number and size of T2 lesions and two measures of brain atrophy, to the development of secondary progression (Chapter 5) and to the occurrence of progression of disability (Chapter 6) after median follow-up times of 14 and 15 years.
In the third part we perform several studies with ^{1}H-MRS to evaluate effects of fluoxetine. One component of the hypothesis of the absence of the beta-2 adrenergic receptor implies that in patients with MS the production of lactate is decreased. Lactate is produced by astrocytes and transported to axons as energy source during periods of intense neural activity.[20] In Chapter 7 we look at the feasibility of detecting lactate formation in the internal capsule of healthy subjects following contralateral motor activity. If we could detect lactate, we wanted to apply this technique to MS patients. In Chapter 8 we investigate whether NAA/Cr ratios are stable within 4 weeks. In Chapter 9 we report the effect of two weeks of fluoxetine treatment on the NAA/Cr ratio.

References

1. Minderhoud JM, Zwanikken CF. Increasing prevalence and incidence of multiple sclerosis: an epidemiological study in the province of Groningen, The Netherlands. 1994; 113-121.
2. Compston A, Coles A. Multiple sclerosis. *Lancet*. 2008; 372; 1502-1517.
3. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology*. 1996; 46; 907-911.
4. Weinshenker BG, Bass B, Rice GP et al. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. *Brain*. 1989; 112 (Pt 1); 133-146.
5. Confavreux C, Vukusic S, Moreau T et al. Relapses and progression of disability in multiple sclerosis. *N.Engl.J.Med.* 2000; 343; 1430-1438.
6. Ramsaransing GS, De Keyser J. Benign course in multiple sclerosis: a review. *Acta Neurol.Scand.* 2006; 113; 359-369.
7. Frohman EM, Racke MK, Raine CS. Multiple sclerosis--the plaque and its pathogenesis. *N.Engl.J.Med.* 2006; 354; 942-955.
8. Jacobs LD, Cookfair DL, Rudick RA et al. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). *Ann.Neurol.* 1996; 39; 285-294.
9. Randomised double-blind placebo-controlled study of interferon beta-1a in relapsing/remitting multiple sclerosis. PRISMS (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis) Study Group. *Lancet*. 1998; 352; 1498-1504.
10. Johnson KP, Brooks BR, Cohen JA et al. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. The Copolymer 1 Multiple Sclerosis Study Group. *Neurology*. 1995; 45; 1268-1276.
11. Paty DW, Li DK. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. UBC MS/MRI Study Group and the IFNB Multiple Sclerosis Study Group. *Neurology*. 1993; 43; 662-667.
12. Kappos L, Bates D, Hartung HP et al. Natalizumab treatment for multiple sclerosis: recommendations for patient selection and monitoring. *Lancet Neurol.* 2007; 6; 431-441.
13. Edan G, Miller D, Clanet M et al. Therapeutic effect of mitoxantrone combined with methylprednisolone in multiple sclerosis: a randomised multicentre study of active

disease using MRI and clinical criteria. *J.Neurol.Neurosurg.Psychiatry*. 1997; 62; 112-118.

14. De Keyser J, Wilczak N, Leta R et al. Astrocytes in multiple sclerosis lack beta-2 adrenergic receptors. *Neurology*. 1999; 53; 1628-1633.
15. De Keyser J, Zeinstra E, Mostert J et al. Beta 2-adrenoceptor involvement in inflammatory demyelination and axonal degeneration in multiple sclerosis. *Trends Pharmacol.Sci.* 2004; 25; 67-71.
16. De Stefano N, Filippi M, Miller D et al. Guidelines for using proton MR spectroscopy in multicenter clinical MS studies. *Neurology*. 2007; 69; 1942-1952.
17. Polman CH, Reingold SC, Edan G et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann.Neurol.* 2005; 58; 840-846.
18. Miller DH, Albert PS, Barkhof F et al. Guidelines for the use of magnetic resonance techniques in monitoring the treatment of multiple sclerosis. US National MS Society Task Force. *Ann.Neurol.* 1996; 39; 6-16.
19. Traugott U, Velia Trego. Fluoxetine greatly inhibits disease progression in Chronic Experimental Allergic EcephaloMyelitis (EAE) in SJL/J mice. *Neurology*. 1997; 48; A 422.
20. Brown AM, Ransom BR. Astrocyte glycogen and brain energy metabolism. *Glia*. 2007; 55; 1263-1271.

Part 1

Effects of fluoxetine in neurological disorders

Chapter 2

Therapeutic Potential of Fluoxetine in Neurological Disorders

J.P. Mostert, M.W. Koch, M. Heerings, D.J. Heersema, J. De Keyser

Author's affiliation:
Department of Neurology, University Medical Centre Groningen,
University of Groningen, The Netherlands.

CNS Neuroscience Therapeutics
2008 Jun;14(2):153-64.

Abstract

The selective serotonin reuptake inhibitor (SSRI) fluoxetine, which is registered for a variety of psychiatric disorders, has been found to stimulate the cAMP responsive element binding protein (CREB), increase the production of brain-derived neurotrophic factor and the neurotrophic peptide S100β, enhance glycogenolysis in astrocytes, block voltage-gated calcium and sodium channels, and decrease the conductance of mitochondrial voltage-dependent anion channels. These mechanisms of actions suggest that fluoxetine may also have potential for the treatment of a number of neurological disorders. We performed a Pubmed search to review what is known about possible therapeutic effects of fluoxetine in animal models and patients with neurological disorders. Beneficial effects of fluoxetine have been noted in animal models of stroke, multiple sclerosis and epilepsy. Fluoxetine was reported to improve neurological manifestations in patients with Alzheimer's disease, stroke, Huntington's disease, multiple sclerosis, traumatic brain injury and epilepsy. Clinical studies so far were small and often poorly designed. Results were inconclusive and contradictory. However, the available preclinical data justify further clinical trials to determine the therapeutic potential of fluoxetine in neurological disorders.

Introduction

The selective serotonin reuptake inhibitor (SSRI) fluoxetine is widely used to treat depression, obsessive-compulsive disorder, bulimia and panic disorder. It became FDA approved in 1987.[1] Fluoxetine increases extracellular serotonin (5-HT), which activates 5-HT receptors. The 5-HT receptors are classified into 7 classes (5-HT 1 to 7) with many subclasses. The effect of activation of 5-HT receptors is divers and dependent on the class of receptor. The 5-HT system is complex and subject to continuing research.[2] Fluoxetine was also found to stimulate 5-HT2 receptors directly.[3,4]
Although the precise mechanism for its beneficial effects in psychiatric disorders is uncertain, fluoxetine has been shown to modulate important cellular functions that are thought to be important for neuronal cell survival and neuroplasticity, including the regulation of the transcription factor cAMP response element binding protein (CREB), the production of neurotrophic factors, the regulation of neuronal energy supply, and the opening and closing of ion channels.
The aim of this article is to give an overview of the neurobiological effects of fluoxetine that could be useful for the treatment of neurological disorders, and to review the reported effects of fluoxetine on neurological disorders and their animal models. We performed a PubMed search with the words fluoxetine, and neurologic(al), neuroprotection, Alzheimer's disease, dementia, Parkinson's disease, multiple sclerosis, stroke, Huntington's disease, epilepsy and brain injury. Articles only reporting effects on psychiatric symptoms were excluded. All available English written reports of animal and patient studies published online before May 2007 were included.

Neurobiological effects

An overview of the articles reporting on the neurobiological effects of fluoxetine that could be useful for the treatment of neurological disorders is shown in table 1 (page 20).

Effects on CREB and neurotrophic factors
Chronic treatment with fluoxetine upregulates cerebral CREB expression and phosphorylation in rats and mice.[5,6,7] CREB is a transcription factor, which induces the expression of genes with roles in cell survival, energy metabolism and regeneration.[8] This transcription factor is so important that the search for drugs that increase CREB levels has been called the search for the 'Holy Grail of neurological therapeutics'.[9]

One of the CREB regulated genes is coding for brain-derived neurotrophic factor (BDNF). BDNF is important for the normal development of the human brain and has a critical role in neural plasticity.[10,11] Decreased levels of BDNF may play a pivotal role in the

neurodegeneration associated with aging, Huntington's disease, and Alzheimer's disease.[10] Increased BDNF expression was found in multiple sclerosis lesions and proposed as a mechanism for neuroprotection.[12] Fluoxetine elevates the concentrations of BDNF in rat brain,[13] and enhances the production of S100β in astrocytes.[14,15] S100β, which is mainly produced in astrocytes, has paracrine and autocrine effects on neurons and glia. It enhances neurogenesis, but leads to apoptosis in high concentration.[16] S100β elevation is associated with treatment response in multiple sclerosis patients on interferon-β, and S100β administration in an in vitro model of traumatic brain injury reduced delayed neuronal injury.[17,18]

Effect of fluoxetine on neuronal energy supply

The energy supply of neurons is complex and incompletely understood. According to the astrocyte-neuron lactate shuttle hypothesis, lactate produced from astrocyte glycogenolysis is shuttled to neurons and axons and serves as metabolic fuel, especially during neuronal activation.[19,20,21] Fluoxetine is able to enhance glycogenolysis in cultured astrocytes and could thus theoretically improve energy supply of axons and neurons.[3,4,22]

Effect of fluoxetine on electrolyte channels

Fluoxetine inhibits voltage-gated calcium channels in rat cerebral cells and sodium channels in bovine adrenal cells.[23,24] This may prevent neurotoxic intracellular calcium overload in neurons, which is a key mechanism in neuronal death in both acute conditions, such as ischemia and hypoxia, and neurodegenerative processes.[25,26,27] In ischemic rat spinal cord, white matter inhibition of the Na^+/Ca^{2+}-exchanger was found to be neuroprotective.[28]

Fluoxetine decreased the conductance of the mitochondrial voltage-dependent anion channel (VDAC) in mitochondria isolated from rat liver.[29] VDAC has an important role in the release of cytochrome c, an important step in apoptosis. Inhibition of the VDAC by fluoxetine protected against staurosporine-induced apoptotic cell death in human U-937 cells.[29,30]

Studies in neurological disorders and their animal models

An overview of the articles reporting effects of fluoxetine in animal models of neurological disorders and in patients with neurological disorders is given in tables 2 (page 22) and 3 (page 25).

Parkinson's disease

In patients with Parkinson's disease neuronal destruction of the substantia nigra reduces the amount of dopamine in the striatum, which impairs motor function. Neuronall cell death may be caused by mitochondrial dysfunction resulting in decreased energy production and increased intracellular Ca^{2+} levels.[31] Fluoxetine might be neuroprotective by preventing elevations of intracellular Ca^{2+} levels, promoting neuronal energy supply and the release of neurotrophic factors by astrocytes.

In a rat model of Parkinson's disease, fluoxetine reduced the availability of extracellular dopamine after L-DOPA administration, and it was suggested that SSRIs might worsen motor function in patients with Parkinson's disease.[32]

In agreement with this observation, a number of case reports and small studies suggested that fluoxetine may worsen motor symptoms in Parkinson's disease.[33,34] However, an open pilot study in 14 patients who used fluoxetine 20 mg daily for 1 month found no change in rigidity and bradykinesia scores, but a decrease in tremor severity was observed.[35] Another open label study of 62 depressed patients with Parkinson's disease showed that SSRIs were well tolerated and did not change motor symptoms as measured with the Unified Parkinson's Disease Rating Scale (UPDRS) after 6 months of treatment.[36]

In three reports the effects of repetitive transcranial magnetic stimulation (rTMS) and fluoxetine in depressed patients with Parkinson's disease were compared. Both fluoxetine and rTMS improved the Stroop (coloured words and interference card) and Hooper and Wisconsin (perseverative errors) test performances. Increases in regional cerebral blood flow (rCBF) in the posterior cingulate gyrus and decreases in the right medial frontal gyrus were noted with both fluoxetine and rTMS. Compared with rTMS, fluoxetine intake was associated with a relative rCBF increase in the occipital lobe.[37,38] The Mini Mental State Examination (MMSE),[39] which is an 11-item examination of cognitive functions with higher scores indicating better cognition, improved when both groups were analyzed together after 8 weeks. The motor score did not change significantly although there was a trend towards worsening in the fluoxetine group.[40]

Alzheimer's disease

In Alzheimer's disease amyloid-β and tau make up the plaques and tangles that are believed to cause the progressive neurodegeneration, which leads to dementia. Impaired energy metabolism is found in Alzheimer's disease, and an increase in CREB phosphorylation has been suggested to offer promise as therapeutic intervention for counteracting neuronal damage in Alzheimer's disease.[41,42]

In a small randomized, double-blind trial, 18 patients with Alzheimer's disease and major depression were treated with fluoxetine 10 mg/day and 19 with amitriptyline 25 mg/day during 6 weeks.[43] Scores on the MMSE increased significantly with treatment when both

groups were analyzed together. In the fluoxetine group the MMSE increased from 20.0 at baseline to 21.4 at day 45. Dropout rates were very high; 55% for amitriptyline and 22% for fluoxetine. A randomized, double-blind trial compared the use of fluoxetine 20 mg/day, haloperidol 3 mg/day and placebo in 15 non-depressed patients with disruptive agitated behaviors (5 per group) over a period of 6 weeks.[44] Besides more side-effects in the active treatment groups no significant differences were found.
No improvement of MMSE was noticed in a randomized, double-blind, placebo-controlled trial of 15 depressed patients on fluoxetine up to 40 mg/day during 6 weeks.[45]

In a 8-week, double-blind, placebo-controlled study of 58 non-depressed patients with mild cognitive impairment, which may be a prodromal state of Alzheimer's disease, fluoxetine improved memory and cognition, measured with the MMSE and subtests from the Persian standardized Wechsler Memory Scale III (WMS-III).[46]

Stroke
In ischemic stroke neurons die when blood supply falls below the infarction threshold of 8-10 mL/100 mg/ min. Neurons in the so-called penumbra, where the blood flow is between the infarction threshold and the functional threshold of 18-22 mL / 100g/ min, can die due to lethal biochemical processes or be rescued by vessel recanalisation or neuroprotective interventions. Recovery after stroke is not only dependent on the survival of the neurons in the penumbra, but also on brain plasticity. Fluoxetine could be neuroprotective in the acute phase (ion channel blocking, enhanced energy metabolism and neurotrophic factor release) and improve brain plasticity during stroke rehabilitation (neurotrophic factors).

After induction of focal ischemia in rats, fluoxetine did not alter the degree of recovery of function compared to non-treated rats after 4 weeks of treatment.[47] In another study, fluoxetine administered 7 days before and up to 28 days after induction of focal cerebral ischemia did not influence sensorimotor recovery in rats.[48] However, low dose fluoxetine given during 7 days post-partum reduced functional deficits in rats with neonatal hypoxic ischemic brain injury.[49]

In 8 non-depressed stroke patients, a single dose of fluoxetine appeared to improve motor skills of the affected side.[50] During rehabilitation 1 to 6 months after stroke, severely disabled patients showed significantly more often good recovery after 3 months of fluoxetine treatment, compared to placebo and the norepinephrine reuptake inhibitor maprotiline.[51] Two other randomized, double-blind, placebo-controlled trials including 104 and 31 stroke patients were mainly focused on the antidepressant effects of fluoxetine and found no benefit on functional recovery after respectively 45 days and 12 weeks of treatment.[52,53]

Spalletta et al. looked at the effect of sertraline (n=21) and fluoxetine 20 mg (n=29) on patients with and without alexithymia, a condition in which patients have problems identifying and coping with feelings. A significant increase of MMSE after 8 weeks of treatment in the 32 patients without alexithymia was noticed.[54]

Huntington's disease

In patients with Huntington's disease the slowly progressive neuronal loss in the basal ganglia causes a movement disorder (characteristic chorea) together with a cognitive and affective disorder. An altered energy metabolism is hypothesized to be important in the pathophysiology of Huntington's disease.[55] Fluoxetine might have a neuroprotective effect by increasing energy metabolism and the production of BDNF.

Two patients with Huntington's disease responded well to fluoxetine treatment. Both showed motor improvement and one patient's cognitive functions also improved. Beneficial effects did take 4-6 months to develop and lasted several years.[56] A randomized, double-blind, placebo-controlled trial in non-depressed Huntington's disease patients failed to show substantial clinical benefits of fluoxetine treatment after 4 months, although a slight reduction in agitation and in the need for routine care was found.[57]

Multiple sclerosis

In the beginning of their disease about 80% of the patients with multiple sclerosis have symptoms that come and go (relapses) resulting from focal inflammatory demyelination in the central nervous system (CNS). After 10 to 20 years most patients experience gradual increasing disability, which is caused by a more diffuse progressive axonal loss. Mitochondrial failure, which gives dysfunction of electrolyte channels and leads eventually to toxic intracellular calcium overload, is suspected to play a pivotal role in the axonal dysfunction and degeneration in multiple sclerosis.[58] By improving energy metabolism and by blocking sodium channels, fluoxetine might protect axons in patients with multiple sclerosis.

In mice with chronic relapsing experimental allergic encephalomyelitis (EAE), an animal model for the inflammatory lesions of multiple sclerosis, fluoxetine prevented worsening of neurological signs, prolonged survival, and reduced CNS inflammation and axonal damage compared to untreated animals.[59]

In a letter to the editor a psychiatrist reported a patient with multiple sclerosis who suffered a worsening of symptoms after initiating treatment with fluoxetine.[60] A number of psychiatrists replied that multiple sclerosis patients on treatment with fluoxetine on the contrary remained quite stable.[61]

In a preliminary open study of 11 patients with multiple sclerosis, 2 weeks of fluoxetine administration increased cerebral white matter N-acetylaspartate levels on magnetic resonance spectroscopy, suggesting an improvement in axonal mitochondrial energy production.[62] Trends towards an improvement of walking ability and fatigue were also noted.

Traumatic brain injury
Trauma to the head causes permanent and reversible damage to neurons. Improved energy metabolism and increased production of neurotrophic factors by the administration of fluoxetine might prevent irreversible loss of neurons and promote plasticity in patients with traumatic brain injury.

In a rat model of moderate to severe traumatic brain injury, fluoxetine treatment during 15 days did not improve motor performance.[63]

In an open-label investigation of 5 head-injured patients, fluoxetine not only improved mood, but had also a beneficial effect on several measures of cognition after 8 months of treatment.[64]

Epilepsy
Epilepsy is caused by a reduced membrane stability of neurons. Both genetic predisposition and neuronal damage increase the susceptibility for epileptic seizures. Treatment is aimed at increasing the membrane stability. By blocking sodium and calcium channels fluoxetine might improve membrane stability.
Fluoxetine reduced seizure activity in many animal models of epilepsy.[65,66,67,68,69,70] However, one study reported an increase in epileptic activity after treatment with fluoxetine in a rat epilepsy model.[71]

In an open-label, add-on trial of fluoxetine in patients with complex partial seizures with and without secondary generalization, 6 patients showed complete disappearance of their seizures and the remaining 11 patients had a 30% reduction in seizure frequency.[72]
It is stated that despite some case reports of worsening of seizure activity, antidepressant drugs can have anticonvulsant effects when used in usual dosages.[73,74]

Discussion

Caution should be taken to extrapolate the results of in vitro studies to in vivo effects. In cell cultures the concentration of fluoxetine used (1-50 μM) mostly exceed therapeutic plasma levels in patients (1-3 μM) and the effect of fluoxetine might be overestimated.

However, drug concentrations of fluoxetine in the human brain are reported to be 20 fold higher than plasma levels[75] and concentrations of up to 50 μM might thus be reached in the human brain.

Beneficial effects of fluoxetine were noted in animal models of stroke, multiple sclerosis and epilepsy. In these studies higher dosages of the medication (1.0 -20 mg/kg/day) were used than in clinical use (20-80 mg/day; = 0.25 – 1.0 mg/kg/day) and the results must therefore also be regarded cautiously. In patients with Parkinson's disease, fluoxetine was well tolerated but no positive effects on symptoms of the disease process were reported. In Alzheimer's disease one positive study was found in mild cognitive impairment. The other studies had only 6 weeks of follow-up and could not find beneficial effects. In stroke patients initial claims of a beneficial effect of fluoxetine on motor recovery could not be confirmed in a larger study with longer follow-up. In Huntington's disease a relatively large, well-designed trial with 4 months of follow-up could not find better performance of patients treated with fluoxetine compared to placebo-treated patients. In epilepsy, multiple sclerosis and traumatic brain injury good studies are lacking.

Many clinical studies were performed in depressive patients, and it is uncertain whether improvement of neurological symptoms was influenced by improvement of the underlying depression. Also it is difficult to measure effects in neurodegenerative disorders since progression is slow, clinical scales are insensitive and good surrogate markers are lacking. Underestimation of therapeutic effect is possible since at least several weeks of treatment are necessary before plasma levels of fluoxetine become stable.[76]

Small studies with a number of other SSRIs have also shown an indication for a possible beneficial effect in some neurological disorders: paroxetine and citalopram in patients with Parkinson's disease,[77,78] sertraline in patients with traumatic brain injury,[79] and citalopram and fluvoxamine in patients with epilepsy.[80,81,82,83,84] Since distinct SSRIs have different affinities for the serotonin receptors it is not possible to generalize the results of fluoxetine to all other SSRIs.

Although clinical studies so far are inconclusive, the preclinical findings justify further trials with fluoxetine and perhaps other SSRIs in patients with neurological disorders.

Table 1 Overview of in vitro studies examining neurobiological effects of fluoxetine.

Article	Cells/animals	Intervention	Duration of treatment/ follow-up	Results
Chen et al, 1995[3]	Cultures of astrocytes of mice	Fluoxetine 10^{-7} to 10^{-4} M Serotonin 10^{-11} to 10^{-5} M	Acute to 1 week	Acute increase in glycogenolysis with both fluoxetine and serotonin; chronic no change glycogenolysis with fluoxetine and increase with serotonin
Deak et al, 2000[23]	Hippocampal pyramidal cells of rats	Fluoxetine 3 μM	Acute	Inhibition of voltage gated calcium channels
Haring et al, 1993[14]	15 male rats	5 rats fluoxetine 35 mg/kg/day; 5 rats parachlorophenylalanine (5-HT inhibitor); 5 rats placebo	1 week	About 85% reduction in S100β with parachlorophenylalanine and 7% increase of S100β with fluoxetine
Kong et al 2002[4]	Cultures of astrocytes of mice	Fluoxetine 10 μM	Short term (1 week) long term (2-3 weeks)	Short term decrease glycogenolysis, long term increase glycogenolysis
Manev et al, 2001[15]	Rats, hippocampus	Fluoxetine 5 mg/kg /day	21 days	Increase S100β
Mercier et al, 2004[13]	Cultures of astrocytes of rats	Fluoxetine 40 μM	2 hours	Increase BDNF lasting for several days

Table 1 continued

Article	Cells/animals	Intervention	Duration of treatment/ follow-up	Results
Nahon et al, 2005[29]	Mitochondria from rat liver	Fluoxetine 10, 20 and 50 μM	Acute	Dose related decrease of the VDAC, inhibition of opening of the mitochondrial permeability pore, inhibition of the release of cytochrome c and protection against staurosporine-induced apoptotic cell death
Nibuya et al 1996[7]	Rats, hippocampus	Fluoxetine 5 mg/kg /day	21 days	Increased expression CREB mRNA and expression of BDNF
Pancrazio et al, 1998[24]	Bovine adrenal chromaffin cells	Fluoxetine 20 μM	Acute	Decrease of voltage gated Na^+ current by 61%
Thome et al, 2000[5]	Transgenic mice with a CRE-LacZ reporter gene construct	Fluoxetine 10 mg/kg/day	14 days	Increase in CRE mediated gene expression and phophorylation of CREB in cerebral cortex, hippocampus, amygdale and hypothalamus
Tiraboschi et al, 2004[6]	12 male rats, hippocampus and whole frontal lobe	Fluoxetine 10 mg/kg/day	Acute (3hrs) chronic (14 days)	Chronic treatment increased the phosphorylation of CREB
Zhang et al, 1993[22]	Cultures of astrocytes of mice	Fluoxetine 10 μM	10 min	Increase glycogenolysis

BDNF = Brain derived neurotrophic factor; VDAC = voltage-dependent anion channel; CREB = cAMP response element binding protein

Table 2 overview of studies reporting results of the effects of fluoxetine in animal models of neurological disorders.

Article	Animal model	Intervention	Follow-up	Results
Chang et al, 2006[49]	Neonatal hypoxic-ischemic brain injury model of rat pups	Fluoxetine 5, 15 mg/kg/day for 7 days, at day 7 hypoxic-ischemic injury	33-35 days	5 mg/kg fluoxetine treatment reduced functional deficits and increased levels of phosphorylated CREB and BDNF gene expression in hippocampus and cortex; 15 mg/kg had no effect
Kecskemeti et al, 2005[70]	Pentylenetetrazol-induced mouse epilepsy model	Fluoxetine 5, 10, 20 mg/kg; norfluoxetine 5, 10, 20 mg/kg; phenytoin 30 mg/kg; clonazepam 0.1 mg/kg; control	60 minutes	Norfluoxetine and fluoxetine 20mg/kg increase survival compared to controls; survival is comparable to effect of phenytoin
Peričić et al, 2005[67]	Epilepsy mouse model (Convulsions elicited with picrotoxin)	Fluoxetine 20 mg/kg/day both after stress and no stress	1 - 5 days	Both acute and prolonged administration of fluoxetine increased the convulsion threshold in stressed and unstressed mice
Prendiville et al, 1993[68]	Rat model of focally evoked complex partial seizures secondary generalized	Fluoxetine 5, 10, 20 mg/kg	1 day	Fluoxetine 5 mg/kg 50% protection, higher protection with higher doses
Richman et al, 2007[66]	Seizure susceptible El mice	Fluoxetine 10 mg/kg/day	3 - 7 days	No effect after 3 days; after 7 days no seizures in fluoxetine treated mice compared to 40% of mice with seizures in control groups

Table 2 continued

Article	Animal model	Intervention	Follow-up	Results
Traugott and Trejo, 1997[59]	Mice with established chronic Experimental Allergic Encephalomyelitis	Fluoxetine 1 mg/kg/day	3 months	Fluoxetine treated mice showed less worsening of neurological signs, survived longer and had less CNS inflammation and axonal damage
Ugale et al, 2004[69]	Pentylenetetrazol-induced mouse epilepsy model	Fluoxetine 1, 5, 10, 20 mg/kg	60 min	Dose dependent effect of protection against seizures (small effect 5 mg/kg [20% protection]; large effect in 20 mg/kg [100% protection])
Wada et al, 1995[65]	Hippocampal seizures elicited by electrical stimulation in a rat model	Fluoxetine 10 mg/kg/day: single dose and injection after 21 days treatment followed by 7 days no drug	1-28 days	After discharge threshold increased after 21 days pretreatment with fluoxetine, acute no effect
Wilson and Hamm, 2002[63]	Rat model of traumatic brain injury	Fluoxetine 2.5, 5.0, 10.0 mg/kg/day	1-15 days postinjury	No effect on motor and cognitive function
Windle and Corbett, 2005[47]	Focal induced ischemia in rats	Fluoxetine 10 mg/kg/day	4 weeks	No effect on functional recovery
Yamato et al, 2001[32]	Rats with nigrostriatal denervation	Fluoxetine 10 mg/kg	300 min	41% reduction in cumulative amount of extracellular dopamine

Table 2 continued

Article	Animal model	Intervention	Follow-up	Results
Zhao et al, 2005[48]	Focal induced ischemia in rats	Fluoxetine 5 mg/kg/day (7 days before ischemia and 28 days after)	28 days	No effect on histological and behavioral outcome
Zienowicz et al, 2005[71]	Pentylenetetrazol-induced mouse epilepsy model	Fluoxetine 10 mg/kg	30 minutes	Number of rats with seizures higher in fluoxetine treated group (100% versus 50%)

CREB = cAMP response element binding protein; BDNF = Brain derived neurotrophic factor; CNS = central nervous system

Table 3 overview of clinical studies with fluoxetine in patients with neurological disorders.

Article	Subjects	Intervention	Study design	Follow-up time	Results (on neurological function)
Auchus et al. 1997[44]	15 non-depressed pt with Alzheimer's disease and disruptive agitated behaviors	5 pt fluoxetine20 mg/day; 5 pt haldol 3 mg/day; 5 pt placebo	Double-blind	6 weeks	No effect on Cohen-Mansfield Agitation Inventory and no effect on sum of scores of sections C, D and E of the behavioral Pathology in Alzheimer's Disease Rating Scale (BEHAVE-AD) and total score on the Caregiver Stress Inventory (CSI)
Boggio et al. 2005*[37]	31 pt with Parkinson's disease and depression	13 pt active rTMS and placebo; 12 pt sham rTMS and fluoxetine 20 mg/day; 6 pt no treatment	Double-blind	8 weeks	Significant improvement of Stroop and Hooper and Wisconsin test performances in the pt on treatment
Browning 1990[60]	1 pt with multiple sclerosis	unknown	Observational	4 days	Severe worsening symptoms of multiple sclerosis
Como et al. 1997[57]	30 non-depressed pt with Huntington's disease	17 pt fluoxetine 20 mg/day; 13 pt placebo	Double-blind, placebo-controlled	4 months	No differences in total functional capacity (TFC), neurological and cognitive ratings

Table 3 continued

Article	Subjects	Intervention	Study design	Follow-up time	Results (on neurological function)
Dam et al. 1996[51]	52 pt with hemiplegic stroke (< 6 months)	18 pt fluoxetine 20 mg/day; 17 pt maprotiline 150 mg/day; 17 pt placebo	Double-blind, placebo-controlled	3 months	Trends towards more improvement in walking and activities of daily living capacities in fluoxetine group; more pt with good recovery in fluoxetine group
Dell' Agnello et al. 2001[36]	62 pt with Parkinson's disease and depression	16 pt fluoxetine 20 mg/day; 15 pt citalopram 20 mg/day; 16 pt fluvoxamine 150 mg/day; 15 pt sertraline 50 mg/day	Open	6 months	No change UPDRS
De Marchi et al. 2001[56]	2 pt with Huntington's disease	Fluoxetine 20 mg	Observational	6 years and 2 year	Improvement in choreatic movements and stability/improvement in MMSE. Improvements did take up to 6 months to appear
Favale et al. 2003[80]	17 pt with complex partial epileptic seizures	Fluoxetine 20 mg/day	Open	14 ± 1.1 months	Complete disappearance of seizures in 6 pt, lowering in seizure frequency by 30% in other patients
Flax et al. 1991[61]	20 pt with multiple sclerosis	unknown	Observational	2-21 months	No worsening symptoms, several patients with improvement in neurological function

Table 3 continued

Article	Subjects	Intervention	Study design	Follow-up time	Results (on neurological function)
Fregni et al. 2004*[40]	42 pt with Parkinson's disease and depression	21 pt active rTMS and placebo; 21 pt sham rTMS and fluoxetine 20 mg/day	Double-blind	8 weeks	MMSE improvement in both groups; tendency for worse motor UPDRS scores in fluoxetine group
Fregni et al. 2006*[38]	26 pt with Parkinson's disease and depression; 29 healthy age-matched controls	13 pt active rTMS and placebo; 13 pt sham rTMS and fluoxetine 20 mg/day	Double-blind	8 weeks	Increase in rCBF in the posterior cingulated gyrus and decreases in the right medial frontal gyrus with both treatments; a relative rCBF increase in the occipital lobe with fluoxetine
Horsfield et al. 2002[64]	5 pt with traumatic brain injury with no or moderate depression	Fluoxetine 20-60 mg/day	Open	8 months	Better performance on Trail Making Test Part A, an attentional-motor speed task and the letter-number sequencing subtest of WAIS-III, reflecting working memory
Montastruc et al. 1995[35]	14 pt with Parkinson's disease	Fluoxetine 20 mg/day	Open	1 month	No change UPDRS, reduction of tremor
Mostert et al. 2006[62]	11 pt with multiple sclerosis	Week 1 fluoxetine 20 mg; Week 2 fluoxetine 40 mg	Open	2 weeks	Increase of NAA/Cr on MRS; trends towards improvement of walking ability and fatigue

Table 3 continued

Article	Subjects	Intervention	Study design	Follow-up time	Results (on neurological function)
Mowla et al. 2007[46]	58 non-depressed pt with mild cognitive impairment	33 pt fluoxetine 20 mg/day; 25 pt placebo	Double-blind, placebo-controlled	8 weeks	High drop out rate (10 pt on fluoxetine group, 4 in placebogroup). Significant improvement of MMSE and logical memory (from the Persian standardized Wechsler Memory Scale III) in fluoxetine group
Pariente et al. 2001[50]	8 non-depressed pt with pure motor stroke	Single dose fluoxetine 20 mg and single dose placebo	Double-blind, crossover, placebo-controlled	5 hours	Under fluoxetine, during active motor task, hyperactivation in the ipsilesional primary motor cortex shown with fMRI and improvement of motor skills of the affected side
Petracca et al. 2001[45]	41 pt with probable Alzheimer's disease and depression	17 pt fluoxetine 40 mg/day; 24 pt placebo	Double-blind, placebo-controlled	6 weeks	No effect of fluoxetine on MMSE and FIM
Robinson et al. 2000[52]	104 pt with acute stroke (< 6 months), 56 were depressed	40 pt fluoxetine 40 mg/day; 31 pt nortriptyline 100 mg/day; 33 pt placebo	Double-blind, placebo-controlled	12 weeks	Nortriptyline improved FIM compared to fluoxetine; no differences in change MMSE
Simons 1996[34]	5 pt with Parkinson's disease	4 pt fluoxetine 20 mg/day; 1 pt fluoxetine 10 mg/day	Observational	1 month	UPDRS increase of 20-25% in 2 pt

Table 3 continued

Article	Subjects	Intervention	Study design	Follow-up time	Results (on neurological function)
Spalletta et al. 2006[54]	50 pt with poststroke major depression, 18 with alexithymia	29 pt fluoxetine 20-40 mg/day; 21 pt sertraline 50-100 mg/day	Open	8 weeks	Pt without alexithymia had a significant increase in MMSE
Steur 1993[33]	4 pt with Parkinson's disease and depression	Fluoxetine 20 mg/day	Observational	8-11 weeks	Significant increase UPDRS during treatment
Taragano et al. 1997[43]	37 pt with Alzheimer's disease and major depression	18 pt fluoxetine 10 mg/day; 19 pt amitriptyline 25 mg/day	Double-blind	45 days	For total group significant increase in MMSE, no difference between fluoxetine and amitriptyline
Wiart et al. 2000[53]	31 pt with hemiplegic stroke (< 3 months) with major depression	16 pt fluoxetine 20mg/day; 15 pt placebo	Double-blind, placebo-controlled	6 weeks	no difference in change in MMSE and change in FIM

pt = patients; UPDRS = unified Parkinson's disease rating scale; * = reports on the same patients; rCBF = regional cerebral blood flow; rTMS = repetitive transcranial magnetic stimulation; MMSE = mini mental state examination; fMRI = functional magnetic resonance imaging; FIM = Functional Independence Measure

References

1. Wong DT, Perry KW, Bymaster FP. Case history: the discovery of fluoxetine hydrochloride (Prozac). *Nat.Rev.Drug Discov.* 2005; 4; 764-774.
2. Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol.Biochem.Behav.* 2002; 71; 533-554.
3. Chen Y, Peng L, Zhang X et al. Further evidence that fluoxetine interacts with a 5-HT2C receptor in glial cells. *Brain Res.Bull.* 1995; 38; 153-159.
4. Kong EK, Peng L, Chen Y et al. Up-regulation of 5-HT2B receptor density and receptor-mediated glycogenolysis in mouse astrocytes by long-term fluoxetine administration. *Neurochem.Res.* 2002; 27; 113-120.
5. Thome J, Sakai N, Shin K et al. cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. *J.Neurosci.* 2000; 20; 4030-4036.
6. Tiraboschi E, Tardito D, Kasahara J et al. Selective phosphorylation of nuclear CREB by fluoxetine is linked to activation of CaM kinase IV and MAP kinase cascades. *Neuropsychopharmacology*. 2004; 29; 1831-1840.
7. Nibuya M, Nestler EJ, Duman RS. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *J.Neurosci.* 1996; 16; 2365-2372.
8. Lonze BE, Ginty DD. Function and regulation of CREB family transcription factors in the nervous system. *Neuron*. 2002; 35; 605-623.
9. Ratan RR. cAMP response element binding protein family transcription factors: the Holy Grail of neurological therapeutics? *Ann.Neurol.* 2004; 56; 607-608.
10. Mattson MP, Maudsley S, Martin B. BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends Neurosci.* 2004; 27; 589-594.
11. Larsson E, Nanobashvili A, Kokaia Z et al. Evidence for neuroprotective effects of endogenous brain-derived neurotrophic factor after global forebrain ischemia in rats. *J.Cereb.Blood Flow Metab*. 1999; 19; 1220-1228.
12. Stadelmann C, Kerschensteiner M, Misgeld T et al. BDNF and gp145trkB in multiple sclerosis brain lesions: neuroprotective interactions between immune and neuronal cells? *Brain*. 2002; 125; 75-85.
13. Mercier G, Lennon AM, Renouf B et al. MAP kinase activation by fluoxetine and its relation to gene expression in cultured rat astrocytes. *J.Mol.Neurosci.* 2004; 24; 207-216.
14. Haring JH, Hagan A, Olson J et al. Hippocampal serotonin levels influence the expression of S100 beta detected by immunocytochemistry. *Brain Res*. 1993; 631; 119-123.

15. Manev R, Uz T, Manev H. Fluoxetine increases the content of neurotrophic protein S100beta in the rat hippocampus. *Eur.J.Pharmacol.* 2001; 420; R1-R2.
16. Rothermundt M, Peters M, Prehn JH et al. S100B in brain damage and neurodegeneration. *Microsc.Res Tech.* 2003; 60; 614-632.
17. Petzold A, Brassat D, Mas P et al. Treatment response in relation to inflammatory and axonal surrogate marker in multiple sclerosis. *Mult.Scler.* 2004; 10; 281-283.
18. Willoughby KA, Kleindienst A, Muller C et al. S100B protein is released by in vitro trauma and reduces delayed neuronal injury. *J.Neurochem.* 2004; 91; 1284-1291.
19. Brown AM, Tekkok SB, Ransom BR. Glycogen regulation and functional role in mouse white matter. *J.Physiol*. 2003; 549; 501-512.
20. Tekkok SB, Brown AM, Westenbroek R et al. Transfer of glycogen-derived lactate from astrocytes to axons via specific monocarboxylate transporters supports mouse optic nerve activity. *J.Neurosci.Res*. 2005; 81; 644-652.
21. Magistretti PJ, Pellerin L, Rothman DL et al. Energy on demand. *Science*. 1999; 283; 496-497.
22. Zhang X, Peng L, Chen Y et al. Stimulation of glycogenolysis in astrocytes by fluoxetine, an antidepressant acting like 5-HT. *Neuroreport*. 1993; 4; 1235-1238.
23. Deak F, Lasztoczi B, Pacher P et al. Inhibition of voltage-gated calcium channels by fluoxetine in rat hippocampal pyramidal cells. *Neuropharmacology*. 2000; 39; 1029-1036.
24. Pancrazio JJ, Kamatchi GL, Roscoe AK et al. Inhibition of neuronal Na+ channels by antidepressant drugs. *J.Pharmacol.Exp.Ther.* 1998; 284; 208-214.
25. Stys PK. General mechanisms of axonal damage and its prevention. *J.Neurol.Sci.* 2005; 233; 3-13.
26. Choi DW. Calcium: still center-stage in hypoxic-ischemic neuronal death. *Trends Neurosci.* 1995; 18; 58-60.
27. Mattson MP. Apoptosis in neurodegenerative disorders. *Nat.Rev.Mol.Cell Biol.* 2000; 1; 120-129.
28. Ouardouz M, Zamponi GW, Barr W et al. Protection of ischemic rat spinal cord white matter: Dual action of KB-R7943 on Na+/Ca2+ exchange and L-type Ca2+ channels. *Neuropharmacology*. 2005; 48; 566-575.
29. Nahon E, Israelson A, Abu-Hamad S et al. Fluoxetine (Prozac) interaction with the mitochondrial voltage-dependent anion channel and protection against apoptotic cell death. *FEBS Lett.* 2005; 579; 5105-5110.
30. Shimizu S, Narita M, Tsujimoto Y. Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC. *Nature*. 1999; 399; 483-487.

31. Mandemakers W, Morais VA, De SB. A cell biological perspective on mitochondrial dysfunction in Parkinson disease and other neurodegenerative diseases. *J.Cell Sci.* 2007; 120; 1707-1716.
32. Yamato H, Kannari K, Shen H et al. Fluoxetine reduces L-DOPA-derived extracellular DA in the 6-OHDA-lesioned rat striatum. *Neuroreport*. 2001; 12; 1123-1126.
33. Steur EN. Increase of Parkinson disability after fluoxetine medication. *Neurology*. 1993; 43; 211-213.
34. Simons JA. Fluoxetine in Parkinson's disease. *Mov Disord.* 1996; 11; 581-582.
35. Montastruc JL, Fabre N, Blin O et al. Does fluoxetine aggravate Parkinson's disease? A pilot prospective study. *Mov Disord.* 1995; 10; 355-357.
36. Dell'Agnello G, Ceravolo R, Nuti A et al. SSRIs do not worsen Parkinson's disease: evidence from an open-label, prospective study. *Clin.Neuropharmacol.* 2001; 24; 221-227.
37. Boggio PS, Fregni F, Bermpohl F et al. Effect of repetitive TMS and fluoxetine on cognitive function in patients with Parkinson's disease and concurrent depression. *Mov Disord.* 2005; 20; 1178-1184.
38. Fregni F, Ono CR, Santos CM et al. Effects of antidepressant treatment with rTMS and fluoxetine on brain perfusion in PD. *Neurology*. 2006; 66; 1629-1637.
39. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J.Psychiatr.Res.* 1975; 12; 189-198.
40. Fregni F, Santos CM, Myczkowski ML et al. Repetitive transcranial magnetic stimulation is as effective as fluoxetine in the treatment of depression in patients with Parkinson's disease. *J.Neurol.Neurosurg.Psychiatry*. 2004; 75; 1171-1174.
41. Chong YH, Shin YJ, Suh YH. Cyclic AMP inhibition of tumor necrosis factor alpha production induced by amyloidogenic C-terminal peptide of Alzheimer's amyloid precursor protein in macrophages: involvement of multiple intracellular pathways and cyclic AMP response element binding protein. *Mol.Pharmacol.* 2003; 63; 690-698.
42. Beal MF. Mitochondria take center stage in aging and neurodegeneration. *Ann.Neurol.* 2005; 58; 495-505.
43. Taragano FE, Lyketsos CG, Mangone CA et al. A double-blind, randomized, fixed-dose trial of fluoxetine vs. amitriptyline in the treatment of major depression complicating Alzheimer's disease. *Psychosomatics*. 1997; 38; 246-252.
44. Auchus AP, Bissey-Black C. Pilot study of haloperidol, fluoxetine, and placebo for agitation in Alzheimer's disease. *J.Neuropsychiatry Clin.Neurosci.* 1997; 9; 591-593.

45. Petracca GM, Chemerinski E, Starkstein SE. A double-blind, placebo-controlled study of fluoxetine in depressed patients with Alzheimer's disease. *Int.Psychogeriatr.* 2001; 13; 233-240.
46. Mowla A, Mosavinasab M, Pani A. Does fluoxetine have any effect on the cognition of patients with mild cognitive impairment? A double-blind, placebo-controlled, clinical trial. *J.Clin.Psychopharmacol.* 2007; 27; 67-70.
47. Windle V, Corbett D. Fluoxetine and recovery of motor function after focal ischemia in rats. *Brain Res*. 2005; 1044; 25-32.
48. Zhao CS, Puurunen K, Schallert T et al. Behavioral and histological effects of chronic antipsychotic and antidepressant drug treatment in aged rats with focal ischemic brain injury. *Behav.Brain Res*. 2005; 158; 211-220.
49. Chang YC, Tzeng SF, Yu L et al. Early-life fluoxetine exposure reduced functional deficits after hypoxic-ischemia brain injury in rat pups. *Neurobiol.Dis.* 2006; 24; 101-113.
50. Pariente J, Loubinoux I, Carel C et al. Fluoxetine modulates motor performance and cerebral activation of patients recovering from stroke. *Ann.Neurol.* 2001; 50; 718-729.
51. Dam M, Tonin P, De Boni A et al. Effects of fluoxetine and maprotiline on functional recovery in poststroke hemiplegic patients undergoing rehabilitation therapy. *Stroke*. 1996; 27; 1211-1214.
52. Robinson RG, Schultz SK, Castillo C et al. Nortriptyline versus fluoxetine in the treatment of depression and in short-term recovery after stroke: a placebo-controlled, double-blind study. *Am.J.Psychiatry*. 2000; 157; 351-359.
53. Wiart L, Petit H, Joseph PA et al. Fluoxetine in early poststroke depression: a double-blind placebo-controlled study. *Stroke*. 2000; 31; 1829-1832.
54. Spalletta G, Ripa A, Bria P et al. Response of emotional unawareness after stroke to antidepressant treatment. *Am.J.Geriatr.Psychiatry*. 2006; 14; 220-227.
55. Walker FO, Raymond LA. Targeting energy metabolism in Huntington's disease. *Lancet*. 2004; 364; 312-313.
56. De Marchi N, Daniele F, Ragone MA. Fluoxetine in the treatment of Huntington's disease. *Psychopharmacology (Berl)*. 2001; 153; 264-266.
57. Como PG, Rubin AJ, O'Brien CF et al. A controlled trial of fluoxetine in nondepressed patients with Huntington's disease. *Mov Disord.* 1997; 12; 397-401.
58. Waxman SG. Ions, energy and axonal injury: towards a molecular neurology of multiple sclerosis. *Trends Mol.Med.* 2006; 12; 192-195.
59. Traugott U, Velia Trego. Fluoxetine greatly inhibits disease progression in Chronic Experimental Allergic EcephaloMyelitis (EAE) in SJL/J mice. *Neurology*. 1997; 48; A 422.
60. Browning WN. Exacerbation of symptoms of multiple sclerosis in a patient taking fluoxetine. *Am.J.Psychiatry*. 1990; 147; 1089.

61. Flax JW, Gray J, Herbert J. Effect of fluoxetine on patients with multiple sclerosis. *Am.J.Psychiatry*. 1991; 148; 1603.
62. Mostert JP, Sijens PE, Oudkerk M et al. Fluoxetine increases cerebral white matter NAA/Cr ratio in patients with multiple sclerosis. *Neurosci.Lett.* 2006; 402; 22-24.
63. Wilson MS, Hamm RJ. Effects of fluoxetine on the 5-HT1A receptor and recovery of cognitive function after traumatic brain injury in rats. *Am.J.Phys.Med.Rehabil.* 2002; 81; 364-372.
64. Horsfield SA, Rosse RB, Tomasino V et al. Fluoxetine's effects on cognitive performance in patients with traumatic brain injury. *Int.J.Psychiatry Med.* 2002; 32; 337-344.
65. Wada Y, Shiraishi J, Nakamura M et al. Prolonged but not acute fluoxetine administration produces its inhibitory effect on hippocampal seizures in rats. *Psychopharmacology (Berl)*. 1995; 118; 305-309.
66. Richman A, Heinrichs SC. Seizure prophylaxis in an animal model of epilepsy by dietary fluoxetine supplementation. *Epilepsy Res.* 2007; 74; 19-27.
67. Pericic D, Lazic J, Svob SD. Anticonvulsant effects of acute and repeated fluoxetine treatment in unstressed and stressed mice. *Brain Res.* 2005; 1033; 90-95.
68. Prendiville S, Gale K. Anticonvulsant effect of fluoxetine on focally evoked limbic motor seizures in rats. *Epilepsia*. 1993; 34; 381-384.
69. Ugale RR, Mittal N, Hirani K et al. Essentiality of central GABAergic neuroactive steroid allopregnanolone for anticonvulsant action of fluoxetine against pentylenetetrazole-induced seizures in mice. *Brain Res.* 2004; 1023; 102-111.
70. Kecskemeti V, Rusznak Z, Riba P et al. Norfluoxetine and fluoxetine have similar anticonvulsant and Ca2+ channel blocking potencies. *Brain Res Bull.* 2005; 67; 126-132.
71. Zienowicz M, Wislowska A, Lehner M et al. The effect of fluoxetine in a model of chemically induced seizures--behavioral and immunocytochemical study. *Neurosci.Lett.* 2005; 373; 226-231.
72. Favale E, Rubino V, Mainardi P et al. Anticonvulsant effect of fluoxetine in humans. *Neurology*. 1995; 45; 1926-1927.
73. Dailey JW, Naritoku DK. Antidepressants and seizures: clinical anecdotes overshadow neuroscience. *Biochem.Pharmacol.* 1996; 52; 1323-1329.
74. Jobe PC, Browning RA. The serotonergic and noradrenergic effects of antidepressant drugs are anticonvulsant, not proconvulsant. *Epilepsy Behav.* 2005; 7; 602-619.
75. Karson CN, Newton JE, Livingston R et al. Human brain fluoxetine concentrations. *J.Neuropsychiatry Clin.Neurosci.* 1993; 5; 322-329.

76. Bergstrom RF, Lemberger L, Farid NA et al. Clinical pharmacology and pharmacokinetics of fluoxetine: a review. *Br.J.Psychiatry Suppl.* 1988; 47-50.
77. Rampello L, Chiechio S, Raffaele R et al. The SSRI, citalopram, improves bradykinesia in patients with Parkinson's disease treated with L-dopa. *Clin.Neuropharmacol.* 2002; 25; 21-24.
78. Chung KA, Carlson NE, Nutt JG. Short-term paroxetine treatment does not alter the motor response to levodopa in PD. *Neurology*. 2005; 64; 1797-1798.
79. Fann JR, Uomoto JM, Katon WJ. Cognitive improvement with treatment of depression following mild traumatic brain injury. *Psychosomatics*. 2001; 42; 48-54.
80. Favale E, Audenino D, Cocito L et al. The anticonvulsant effect of citalopram as an indirect evidence of serotonergic impairment in human epileptogenesis. *Seizure.* 2003; 12; 316-318.
81. Specchio LM, Iudice A, Specchio N et al. Citalopram as treatment of depression in patients with epilepsy. *Clin.Neuropharmacol.* 2004; 27; 133-136.
82. Harmant J, van Rijckevorsel-Harmant K, de BT et al. Fluvoxamine: an antidepressant with low (or no) epileptogenic effect. *Lancet*. 1990; 336; 386.
83. Kim KY, Craig JM, Hawley JM. Seizure possibly associated with fluvoxamine. *Ann.Pharmacother.* 2000; 34; 1276-1278.
84. Nakahira H, Tomotake M, Ohmori T. Fluvoxamine induced complex partial seizure in the treatment of bulimia nervosa. *Gen.Hosp.Psychiatry*. 2005; 27; 148-150.

Part 2

Conventional MRI studies

Chapter 3

Effects of fluoxetine on disease activity in relapsing multiple sclerosis: a double-blind, placebo-controlled, exploratory study

J.P. Mostert[1], F. Admiraal-Behloul[2], J.M. Hoogduin[3], J. Luyendijk[2], D.J. Heersema[1], M.A. van Buchem[2], J. De Keyser[1]

Authors' affiliations:
[1] Department of Neurology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands
[2] Department of Radiology, Leiden University Medical Centre, Leiden, The Netherlands
[3] NeuroImaging Centre, University of Groningen, Groningen, The Netherlands

Journal of Neurology, Neurosurgery and Psychiatry
2008 Sep;79(9):1027-31.

Abstract

By enhancing intracellular cAMP signalling in astrocytes fluoxetine might be able to suppress the antigen presenting capacity of glial cells in multiple sclerosis (MS). This study was designed to evaluate the effects of fluoxetine on new lesion formation in patients with relapsing MS.

In a double-blind, placebo-controlled exploratory trial, forty non-depressed patients with relapsing remitting or relapsing secondary progressive MS were randomised to oral fluoxetine 20 mg or placebo daily for 24 weeks. New lesion formation was studied by assessing the cumulative number of gadolinium-enhancing lesions on brain MRI performed on weeks 4, 8, 16 and 24.

Nineteen patients in both groups completed the study. The mean (SD) cumulative number of new enhancing lesions during the 24 weeks of treatment was 1.84 (2.9) in the fluoxetine group and 5.16 (8.6) in the placebo group (p=0.15). The number of scans showing new enhancing lesions was 25 % in the fluoxetine group vs 41 % in the placebo group (p=0.04). Restricting the analysis to the last 16 weeks of treatment showed that the cumulative number of new enhancing lesions was 1.21 (2.6) in the fluoxetine group and 3.16 (5.3) in the placebo group (p=0.05). The number of patients without enhancing lesions was 63 % in the fluoxetine group vs 26 % in the placebo group (p=0.02).

This proof-of-concept study shows that fluoxetine tends to reduce the formation of new enhancing lesions in patients with MS. Further studies with this compound are warranted.

Trial registration: Current Controlled Trials, ISRCTN65586975

Introduction

Focal inflammatory demyelinating lesions in multiple sclerosis (MS) are believed to result from autoreactive T cell-mediated processes.[1,2] The inflammatory cascade and the resultant lesion formation in MS is contingent upon the ability of activated anti-myelin T cells to recognise their specific antigen in the context of class II major histocompatability complex (MHC) molecules expressed on the membrane of antigen presenting glial cells.[1,2] Medications currently used or under development for the treatment of MS target the peripheral immune system. Inhibition of MHC class II expression on glial cells would represent a novel therapeutic approach to suppress disease activity in MS.

Whether microglia or astrocytes represent the principal CNS antigen presenting cells in MS remains a controversial issue. Many neuroimmunologists consider microglia as the primary immunoeffector cells in MS because they constitutively express MHC class II antigens.[3] An alternative hypothesis proposes that astrocytes play an important role as facultative antigen presenting cells.[4] In contrast to microglia, MHC class II expression on astrocytes under normal conditions is severely restricted by regulatory influences, some of which are mediated by intracellular cAMP signalling pathways.[5,6] A role of astrocytes as facultative antigen presenting cells in MS is supported by the findings that scattered astrocytes in active MS lesions express MHC class II and B-7 costimulatory molecules.[7,8] Our group has postulated the hypothesis that this may be attributed to reduced cAMP signalling in astrocytes caused by a loss of beta-2 adrenergic receptors.[4,9,10]

The objective of this study was to provide proof of concept that enhancing intracellular cAMP signalling pathways in astrocytes in patients with MS reduces inflammatory disease activity. We selected fluoxetine, which is a selective serotonin-reuptake inhibitor (SSRI) widely prescribed for depression, bulimia and obsessive compulsive disorders. Astrocytes, including those in MS lesions, contain serotonin receptors and reuptake sites, and serotonin has been shown to elevate cAMP levels in astrocytes.[11,12,13] Furthermore, prolonged exposure to SSRIs activates intracellular cAMP signalling in the CNS of animals.[14,15] We conducted a double-blind, placebo-controlled, exploratory study to assess the effects of fluoxetine on inflammatory disease activity in patients with a relapsing form of MS by using serial gadolinium-enhanced brain MRI.

Methods

Subjects

The local medical ethics committee approved the protocol. All patients provided written informed consent. Eligible patients were aged 18 to 65 years with clinically definitive

relapsing remitting or relapsing secondary progressive MS.[16,17] Additional inclusion criteria were an Expanded Disability Status Score (EDSS) of less or equal to 6, and at least one relapse in the preceding year, or two relapses in the preceding two years, or one gadolinium-enhancing lesion on the screening MRI of the brain. Exclusion criteria were the use of immunomodulatory, immunosuppressive or antidepressants drugs in the previous 6 months, the use of corticosteroids in the previous 8 weeks, depression defined as a score of 19 or higher on Beck's Depression Inventory II,[18] bipolar disorder, contraindication to MRI, other neurological or systemic disorder that would interfere with the assessments, and pregnancy or unwillingness to use acceptable birth control.

Study design

In this single-centre, double-blind, placebo-controlled study 40 patients were randomised to receive a single tablet of fluoxetine 20 mg or identical placebo daily in the morning for 24 weeks. After a screening visit and brain MRI 4 weeks prior to start of the study medication, patients visited the clinic for brain MRI and clinical evaluation at weeks 0, 4, 8, 16 and 24. Disability status was assessed at baseline and week 24. The hospital pharmacy produced the study medication and performed the randomisations. The code was revealed to the researchers once the follow-up of all patients and the MRI analyses were completed. One physician was responsible for all clinical assessments. Relapses could be treated with high dose corticosteroids for 5 consecutive days.

MRI protocol and processing

All scans were performed on a 3.0 Tesla scanner (Philips). Brain transaxial Dual TSE (repetition time, 3000 msec; echo times, 26.7 and 120 msec), FLAIR (repetition time, 11,000 msec; echo time, 100 msec) and T1-weighted (repetition time, 700 msec; echo time, 8.4 msec) images before and after intravenous administration of gadolinium (0.1 mmol/kg) with 51 contiguous slices of 3 mm thickness were obtained at each visit.

The scans were blindly analysed in the Department of Radiology of the Leiden University Medical Center. T1 enhancing lesions were determined according to published guidelines.[19] T2 lesion volume was segmented with a semi-automated home-developed software program called SNIPER.[20]

Outcome measures

The primary outcome measure was the cumulative number of new gadolinium-enhancing lesions during the treatment phase. Other MRI outcome measures included the number of scans showing new enhancing lesions, the number of scans showing enhancing lesions, the number of patients with no enhancing lesions, the change in T2 lesion volume and cumulative new T1 gadolinium enhancing lesion volume.

Secondary clinical endpoints were number of relapses, number of patients with relapses, and changes in EDSS and Multiple Sclerosis Functional Composite (MSFC). The MSFC is a multidimensional test consisting of a task for leg function (timed 25-foot walk), arm function (9-hole peg test) and cognition (paced auditory serial addition test). Its score represents the mean of the z- scores of the three tests, which are calculated in comparison to a pooled reference population.[21] Lower scores indicate more disability. Exploratory analyses of MRI data in the first 8 weeks and last 16 weeks were performed separately to assess possible time dependent effects of fluoxetine treatment.

Statistical analysis

Because this was an exploratory study and because it is difficult to define a relevant effect of fluoxetine we did not perform a power calculation. The aim of our study was to collect information about an effect size, and if any to use this effect size in the design of future studies.

All data were tested for normality. Baseline and between treatment comparisons of the number and volume of T1 enhancing lesions, T2 lesion load, and EDSS were evaluated with the Wilcoxon-Mann-Whitney rank-sum test. For baseline and between treatment comparisons of the MSFC the independent samples t-test was used. The χ^2 test and Fisher's exact test were used to compare differences in categorical variables. Analyses were performed with the Statistical Package for the Social Sciences (SPSS 14.0 for Windows, Chicago, Illinois). All reported p values are two-tailed. Significance was taken at 0.05.

Results

Patients

Between April 2004 and August 2006, 65 patients were screened, after which 40 were found eligible for inclusion. Figure 1 shows the flow of the patients. In the first week after randomisation one patient in the fluoxetine group and one patient in the placebo group withdrew because of nausea. All other patients completed the study and were used in the analyses. There were no significant differences between the two groups in baseline MRI, clinical or demographic characteristics (Table 1).

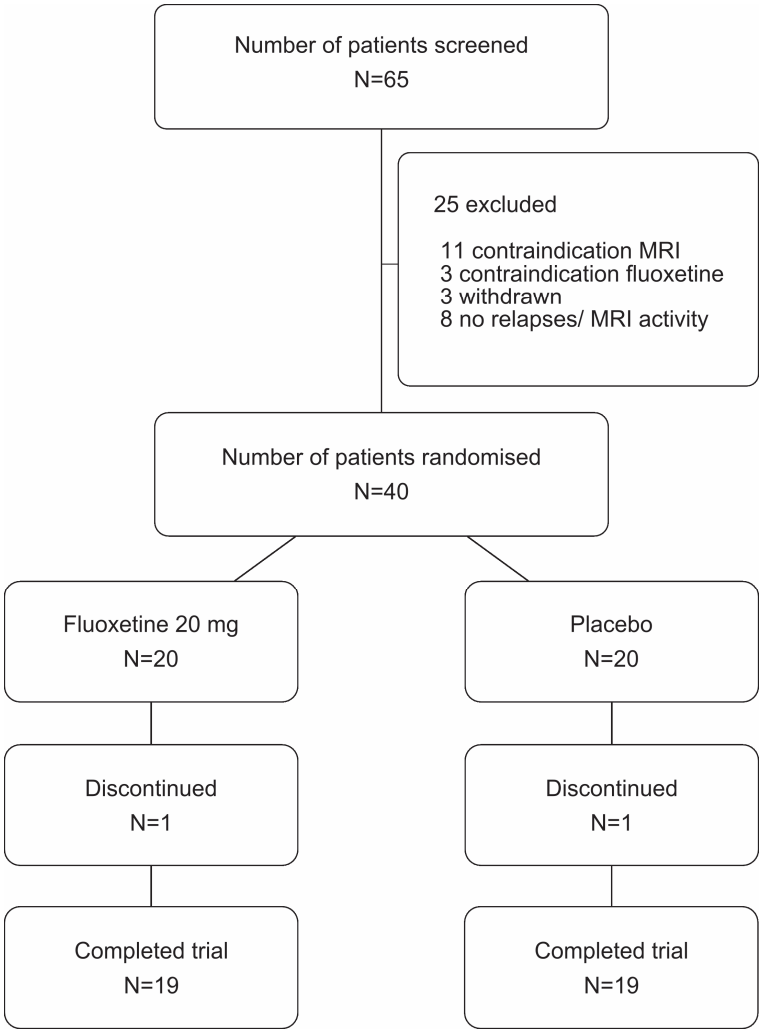

Figure 1 Patient flowchart.

Table 1 Patient Characteristics.

	Fluoxetine (n=19)	Placebo (n=19)	p
Mean age (y) (SD)	41 (10)	38 (9)	0.34
Sex (M/F)	9/10	9/10	1.00
Disease course: RR/SP	18/1	16/3	0.60
Mean time from first symptoms (y) (SD)	11 (7)	11 (8)	0.97
Median number of exacerbations in the last 2 years (range)	2 (1 – 3)	2 (0 – 3)	0.62
Median EDSS (range)	3.0 (0.0 – 6.0)	3.0 (1.0 – 5.5)	0.95
Mean MSFC (SD)	0.18 (0.6)	0.10 (0.6)	0.70
Number of new enhancing lesions at baseline			0.56
Mean (SD)	0.63 (1.3)	0.58 (0.8)	
Median (range)	0 (0 – 5)	0 (0 – 3)	
Scans showing enhancing lesions baseline	7 (37%)	8 (42%)	0.74
T2 lesion load (mm^3)			0.54
Mean (SD)	4761 (6414)	5527 (6891)	
Median (range)	1894 (670 –20829)	2946 (80 –28496)	

RR = relapsing remitting; SP = secondary progressive; EDSS = Expanded Disability Status Scale; MSFC = Multiple Sclerosis Functional Composite

Efficacy

Table 2 shows MRI outcomes over the 24-week study period.

The mean cumulative number of new enhancing lesions tended to be lower in the fluoxetine group than in the placebo group, but this was not significant (p=0.15). However, compared to the placebo group, there were significantly less scans with new enhancing lesions in the fluoxetine group (p=0.04). The fluoxetine group also showed a trend toward a reduction in the cumulative volume of new enhancing lesions, the number of scans with enhancing lesions, and increase of the T2 lesion load.

Figure 2 shows the time-dependent changes in mean cumulative number of new enhancing lesions from the start of treatment.

Table 2 MRI outcomes over the 24-week study period.

	Fluoxetine (n=19)	Placebo (n=19)	p
Cumulative number of new enhancing lesions			0.15
Mean (SD)	1.84 (2.9)	5.16 (8.6)	
Median (range)	1 (0 – 12)	2 (0 – 35)	
Cumulative volume of new enhancing lesions (mm^3)			0.16
Mean (SD)	124 (278)	398 (745)	
Median (range)	22 (0 – 1191)	77 (0 – 3063)	
Number of patients with no new enhancing lesions	6 (32%)	4 (21%)	0.71
Scans showing new enhancing lesions	19 (25%)	31 (41%)	0.04
Scans showing enhancing lesions	22 (29%)	33 (43%)	0.06
Change in T2 lesion load (mm^3)			0.10
Mean (SD)	444 (958)	531 (1004)	
Median (range)	128 (-506 – 2930)	475 (-1907 – 2391)	

Exploratory analysis revealed no differences in MRI outcome measures between the two groups up to the first 8 weeks of treatment. Analysis of disease activity during the last 16 weeks (Table 3) showed a nearly significant reduction in the cumulative number (p=0.05) and volume (p=0.06) of new enhancing lesions, and a lower number of scans with enhancing (p=0.03) or new enhancing lesions (p=0.03) in the fluoxetine group compared to the placebo group. The fluoxetine group contained a higher number of patients with no new enhancing lesions (p=0.02).

Number of relapses, and changes in EDSS and MSFC were comparable between the two groups (Table 4).

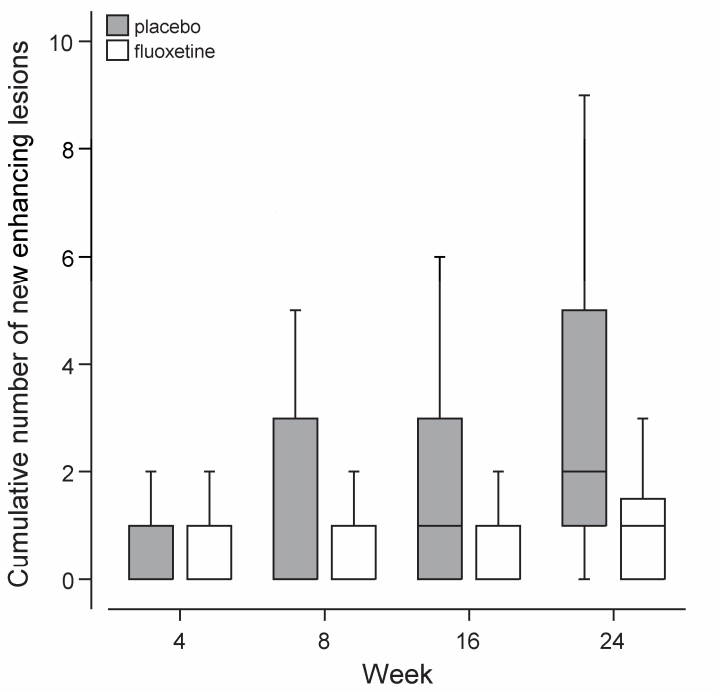

Figure 2 Boxplots of the cumulative number of new enhancing lesions per treatment group over time.

Table 3 MRI outcomes during the last 16 weeks.

	Fluoxetine (n=19)	Placebo (n=19)	p
Cumulative number of new enhancing lesions			0.05
Mean (SD)	1.21 (2.6)	3.16 (5.3)	
Median (range)	0 (0 – 11)	1 (0 – 22)	
Cumulative volume of new enhancing lesions (mm^3)			0.06
Mean (SD)	90 (231)	227 (485)	
Median (range)	0 (0 – 961)	35 (0 – 2095)	
Number of patients with no new enhancing lesions	12 (63%)	5 (26%)	0.02
Scans showing new enhancing lesions	9 (24%)	18 (47%)	0.03
Scans showing enhancing lesions	9 (24%)	18 (47%)	0.03

Table 4 Clinical outcomes.

	Fluoxetine (n=19)	Placebo (n=19)
Number of exacerbations	4	6
Number of patients with exacerbations	4 (21%)	5 (26%)
Median change in EDSS (range)	0.0 (-2.0 – 1.5)	0.0 (-1.5 – 1.0)
Mean change in MSFC (SD)	0.075 (0.38)	0.049 (0.14)

EDSS = Expanded Disability Status Scale; MSFC = Multiple Sclerosis Functional Composite

Safety and tolerability

In general fluoxetine was well tolerated. Patients using fluoxetine suffered more often from nausea and drowsiness (Table 5). Most adverse events were at the start of the study medication and diminished after a few days to weeks.

Table 5 Adverse events.

	Fluoxetine (n=20)	Placebo (n=20)	p
Nausea	13 (65%)	6 (30%)	0.03
Headache	4 (20%)	6 (30%)	0.72
Dizziness	5 (25%)	7 (35%)	0.49
Drowsiness	11 (55%)	6 (30%)	0.11
Insomnia	2 (10%)	2 (10%)	1.00
Transpiration	2(10%)	1 (5%)	1.00
Palpitations	2 (10%)	-	0.49
Loss of appetite	2 (10%)	-	0.49

Discussion

In this study, patients with the relapsing form of MS treated with fluoxetine showed a trend toward a reduction in the number of new enhancing lesions over time. This effect became apparent after 8 weeks of treatment, suggesting that it takes several weeks before fluoxetine becomes effective. Fluoxetine plasma concentrations are gradually build up and achieve steady-state conditions only after several weeks,[22] and it is well known that patients with depression need treatment for a number of weeks before effects become clinically evident.

Conclusions from our results must be made with caution because of the small sample size and exploratory design. Retrospective power calculation shows that we were only able to detect treatment effects above 80% with a statistical power of 80%.[23] The treatment with fluoxetine was shown to be safe and well tolerated.

The results lend support to the hypothesis that elevating cAMP signalling in astrocytes may reduce inflammatory disease activity in patients with MS.
An increase in cAMP signalling in astrocytes downregulates the class II transactivator protein,[24] thereby suppressing the induction by proinflammatory cytokines of MHC class II molecules.[25] This mechanism is thought to prevent the deviation of astrocytes to function as facultative immunocompetent CNS antigen presenting cells that can mediate inflammatory events. Possible influences of fluoxetine on microglia or cells of the peripheral immune system cannot be dismissed, but are unlikely. In vitro studies showed that elevation of intracellular cAMP levels suppresses interferon-γ induction of MHC class II in astrocytes, but not in microglial cells.[6] In vitro experiments on human lymphocyte suspensions showed that fluoxetine decreases lymphocyte proliferation and suppresses interferon-γ production.[26] However, these effects were only found with fluoxetine concentrations of 10-50 μM, which are far in excess of therapeutic plasma concentrations. Serum levels of fluoxetine with daily doses ranging from 20 to 40 mg vary between 0.26 and 0.65 μM,[27] making it unlikely that immunomodulatory effects of fluoxetine on T cells were involved in our study.

An interesting aspect of considering fluoxetine as a candidate drug for the treatment of MS are its additional mechanisms of action that might be relevant for reducing axonal loss, leading to progressive disability. These include the production by astrocytes of neurotrophic factors such as brain-derived neurotrophic factor,[28] stimulation of astrocyte glycogenolysis,[29] and blockage of sodium channels.[30] Sodium channel activation,[31] and reduced axonal energy metabolism caused by impaired glycogenolysis in astrocytes,[10] have been hypothesized to play a role in the axonal degeneration in MS. In patients with MS, fluoxetine improved the cerebral white matter N-acetylaspartate/creatine ratio, which can be regarded as a measure of axonal energy metabolism.[32]

The results of our exploratory trial are sufficiently encouraging to justify further studies with fluoxetine in patients with MS. Higher doses of fluoxetine and combination treatment with immunomodulatory drugs should be considered.

References

1. Frohman EM, Racke MK, Raine CS. Multiple sclerosis--the plaque and its pathogenesis. *N.Engl.J.Med.* 2006; 354; 942-955.
2. Steinman L. Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. *Cell*. 1996; 85; 299-302.
3. Ulvestad E, Williams K, Bo L et al. HLA class II molecules (HLA-DR, -DP, -DQ) on cells in the human CNS studied in situ and in vitro. *Immunology*. 1994; 82; 535-541.
4. De Keyser J, Zeinstra E, Frohman E. Are astrocytes central players in the pathophysiology of multiple sclerosis? *Arch.Neurol.* 2003; 60; 132-136.
5. Frohman EM, Vayuvegula B, Gupta S et al. Norepinephrine inhibits gamma-interferon-induced major histocompatibility class II (Ia) antigen expression on cultured astrocytes via beta-2-adrenergic signal transduction mechanisms. *Proc.Natl.Acad.Sci.U.S.A*. 1988; 85; 1292-1296.
6. Lee SC, Collins M, Vanguri P et al. Glutamate differentially inhibits the expression of class II MHC antigens on astrocytes and microglia. *J.Immunol.* 1992; 148; 3391-3397.
7. Zeinstra E, Wilczak N, Streefland C et al. Astrocytes in chronic active multiple sclerosis plaques express MHC class II molecules. *Neuroreport*. 2000; 11; 89-91.
8. Zeinstra E, Wilczak N, De Keyser J. Reactive astrocytes in chronic active lesions of multiple sclerosis express co-stimulatory molecules B7-1 and B7-2. *J.Neuroimmunol.* 2003; 135; 166-171.
9. De Keyser J, Wilczak N, Leta R et al. Astrocytes in multiple sclerosis lack beta-2 adrenergic receptors. *Neurology*. 1999; 53; 1628-1633.
10. De Keyser J, Zeinstra E, Mostert J et al. Beta 2-adrenoceptor involvement in inflammatory demyelination and axonal degeneration in multiple sclerosis. *Trends Pharmacol.Sci.* 2004; 25; 67-71.
11. Anderson EJ, McFarland D, Kimelberg HK. Serotonin uptake by astrocytes in situ. *Glia*. 1992; 6; 154-158.
12. Krzan M, Wu VW, Schwartz JP. Serotonin regulation of nerve growth factor synthesis in neonatal and adult astrocytes: comparison to the beta-adrenergic agonist isoproterenol. *J.Neurosci.Res.* 2001; 64; 261-267.
13. Zeinstra E, te Riele P, Langlois X et al. Aminergic receptors in astrogliotic plaques from patients with multiple sclerosis. *Neurosci.Lett.* 2002; 331; 87-90.
14. Duman RS, Heninger GR, Nestler EJ. A molecular and cellular theory of depression. *Arch.Gen.Psychiatry*. 1997; 54; 597-606.

15. Thome J, Sakai N, Shin K et al. cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. *J.Neurosci.* 2000; 20; 4030-4036.
16. Poser CM, Paty DW, Scheinberg L et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann.Neurol.* 1983; 13; 227-231.
17. McDonald WI, Compston A, Edan G et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann.Neurol.* 2001; 50; 121-127.
18. Beck A, Steer RA, Brown GK. Manual for Beck Depression Inventory-2. 1996.
19. Barkhof F, Filippi M, van Waesberghe JH et al. Improving interobserver variation in reporting gadolinium-enhanced MRI lesions in multiple sclerosis. *Neurology*. 1997; 49; 1682-1688.
20. Admiraal-Behloul F, van den Heuvel DM, Olofsen H et al. Fully automatic segmentation of white matter hyperintensities in MR images of the elderly. *Neuroimage.* 2005; 28; 607-617.
21. Fischer J, Jack A, Knicker J et al. Administration and scoring manual for the Multiple Sclerosis Functional Composite Measure (MSFC). 1999.
22. Bergstrom RF, Lemberger L, Farid NA et al. Clinical pharmacology and pharmacokinetics of fluoxetine: a review. *Br.J.Psychiatry Suppl*. 1988; 47-50.
23. Sormani MP, Miller DH, Comi G et al. Clinical trials of multiple sclerosis monitored with enhanced MRI: new sample size calculations based on large data sets. *J.Neurol.Neurosurg.Psychiatry*. 2001; 70; 494-499.
24. Li G, Harton JA, Zhu X et al. Downregulation of CIITA function by protein kinase a (PKA)-mediated phosphorylation: mechanism of prostaglandin E, cyclic AMP, and PKA inhibition of class II major histocompatibility complex expression in monocytic lines. *Mol.Cell Biol.* 2001; 21; 4626-4635.
25. Dong Y, Rohn WM, Benveniste EN. IFN-gamma regulation of the type IV class II transactivator promoter in astrocytes. *J.Immunol.* 1999; 162; 4731-4739.
26. Diamond M, Kelly JP, Connor TJ. Antidepressants suppress production of the Th1 cytokine interferon-gamma, independent of monoamine transporter blockade. *Eur.Neuropsychopharmacol.* 2006; 16; 481-490.
27. Lundmark J, Reis M, Bengtsson F. Serum concentrations of fluoxetine in the clinical treatment setting. *Ther.Drug Monit.* 2001; 23; 139-147.
28. Mercier G, Lennon AM, Renouf B et al. MAP kinase activation by fluoxetine and its relation to gene expression in cultured rat astrocytes. *J.Mol.Neurosci.* 2004; 24; 207-216.
29. Kong EK, Peng L, Chen Y et al. Up-regulation of 5-HT2B receptor density and receptor-mediated glycogenolysis in mouse astrocytes by long-term fluoxetine administration. *Neurochem.Res.* 2002; 27; 113-120.

30. Pancrazio JJ, Kamatchi GL, Roscoe AK et al. Inhibition of neuronal Na+ channels by antidepressant drugs. *J.Pharmacol.Exp.Ther.* 1998; 284; 208-214.
31. Bechtold DA, Kapoor R, Smith KJ. Axonal protection using flecainide in experimental autoimmune encephalomyelitis. *Ann.Neurol.* 2004; 55; 607-616.
32. Mostert JP, Sijens PE, Oudkerk M et al. Fluoxetine increases cerebral white matter NAA/Cr ratio in patients with multiple sclerosis. *Neurosci.Lett.* 2006; 402; 22-24.

Chapter 4

Brain atrophy correlations in relapsing multiple sclerosis

J.P. Mostert[1], I.G. Vos[1], M.W. Koch[1], C. Steen[1], J. Luyendijk[2], J.M. Hoogduin[3], M.A. van Buchem[2], J.H.A. De Keyser[1,4]

Authors' affiliations:
[1] Department of Neurology, University Medical Centre Groningen, University of Groningen, The Netherlands
[2] Department of Radiology, Leiden University Medical Centre, Leiden, The Netherlands
[3] BCN Neuroimaging Centre, University Medical Centre Groningen, University of Groningen, The Netherlands
[4] Department of Neurology, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Brussels, Belgium

Submitted

Abstract

The objective of this study was to compare different measures of brain atrophy in patients with relapsing multiple sclerosis (MS).

We determined in a cross-sectional study in 33 patients with relapsing MS the bicaudate ratio (BCR), third ventricle width (TVW), corpus callosum area (CCA), brain parenchymal fraction (BPF), grey matter fraction (GMF), white matter fraction (WMF), and T2 lesion load. Correlations with the Expanded Disability Status Scale (EDSS) and Multiple Sclerosis Functional Composite (MSFC) were performed.

Except for CCA, all atrophy measurements correlated with each other. Only TVW and BCR did not correlate with the T2 lesion load. The EDSS correlated with TVW (r=0.45). The MSFC correlated significantly with TVW, BPF, WMF and GMF (r= 0.46 to r= 0.53).

We conclude that the BPF, WMF, GMF and TVW show a moderate correlation with clinical disability. BPF might be best to use in clinical trials. The TVW is of particular interest because it does not seem to be influenced by the T2 lesion load.

Introduction

Brain atrophy, which is a normal process of aging, is accelerated in individuals with multiple sclerosis (MS).[1,2,3,4,5] Progressive brain volume loss in relapsing MS can already be detected on magnetic resonance imaging (MRI) over a period of one year, and early in the disease course.[3,6,7,8,9,10] Progressive atrophy seems to be caused by the typical focal lesions (plaques) and an apparently focal lesion-independent diffuse axonal degeneration.[6]
Brain atrophy has been proposed as a surrogate marker to monitor disease progression and treatment efficacy in MS.[11,12,13] In the many studies evaluating brain atrophy in MS different methods have been used.[6,14,15]
It is unclear whether the measured degree of atrophy in patients with MS is dependent on the technique used and, if so, what technique is most suitable to use in clinical trials. Therefore we compared several techniques to quantify brain atrophy in MS on brain MRI: the bicaudate ratio (BCR), third ventricle width (TVW), corpus callosum area (CCA), brain parenchymal fraction (BPF), and grey and white matter fraction (GMF and WMF). We assessed the correlations of the different atrophy measurements with each other, clinical disability and T2 lesion load.

Methods

Subjects
The study population consisted of 33 patients with clinically definite MS with relapses (28 relapsing-remitting, 5 relapsing secondary progressive). None of the patients was using immunomodulating medication and all patients were relapse free for at least 8 weeks at the time of scanning. Disease duration was defined as the time from first symptoms attributable to the MS.

Disability measurements
Disability was measured with the Expanded Disability Status Scale (EDSS) and Multiple Sclerosis Functional Composite (MSFC).[16,17] The EDSS ranges from 0 to 10, with higher scores indicating more severe disease. The MSFC is a multidimensional clinical outcome measure that includes quantitative tests of leg function/ambulation (timed 25-foot walk; T25W), arm function (9-hole peg test; 9HPT) and cognitive function (paced auditory serial addition test; PASAT). The score on each assessment is converted to a Z score and the composite score is computed according to a standardised formula.[18]

MRI protocol
MRI scans were performed on a 3.0-T unit (Philips Medical Systems, Best, The Netherlands). In the same session transaxial Dual TSE (repetition time, 3000 milliseconds;

echo times, 26.7 and 120 milliseconds), FLAIR (repetition time, 11000 milliseconds; echo time, 100 milliseconds) with 51 contiguous slices of 3mm, and high resolution 3D T1-weighted (HR3D-T1; repetition time, 7.5 milliseconds; echo time, 3.4 milliseconds) with 160 contiguous slices of 1 mm of the brain were obtained.

MRI analysis

T2 lesion load was quantified by fully automatic segmentation on axial dual fast spin echo sequences using fuzzy clustering. Details are described elsewhere.[19]

Atrophy was determined by three manual linear or area measurements: BCR, TVW, CCA, and two semi-automated brain volume measurement: the BPF, and the GMF -WMF. The three manual measures were performed on the T1 weighted images; the dual echo scan was used to determine the anterior commissure (AC)-posterior commissure (PC) plane.

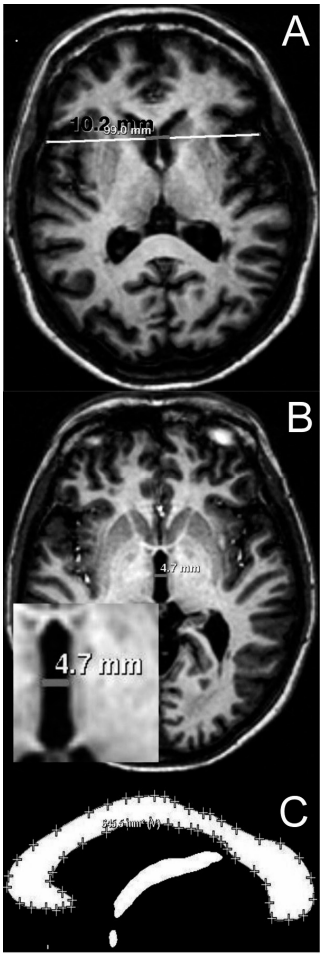

Figure 1
A) Calculation of BCR: length of grey line divided by length of white line.
B) Determination of TVW.
C) Assessment of CCA: by adjusting the grey scale the corpus callosum area is highlighted.

BCR is defined as the minimum intercaudate distance divided by brain width along the same line (Fig. 1A). Compared to other MS studies which use the slice where the heads of the caudate nuclei are most visible and closest to another,[20] we measured the BCR on the slice 10 mm superior to the AC-PC plane.[21] This way of determining the slice is also used in Huntington's disease studies and is more objective.

TVW was measured by drawing a linear region of interest perpendicular to the long axis of the third ventricle parallel to the interhemispheric fissure in the section wherein the third ventricle was most visible (Fig. 1B).[22]

CCA was determined by outlining the margins on the mid-sagittal slice (Fig. 1C).
To determine intra-observer and inter-observer reproducibility of the three linear measurements, 17 scans were analysed twice by the same observer (IV) and 6 by a second observer (JM).

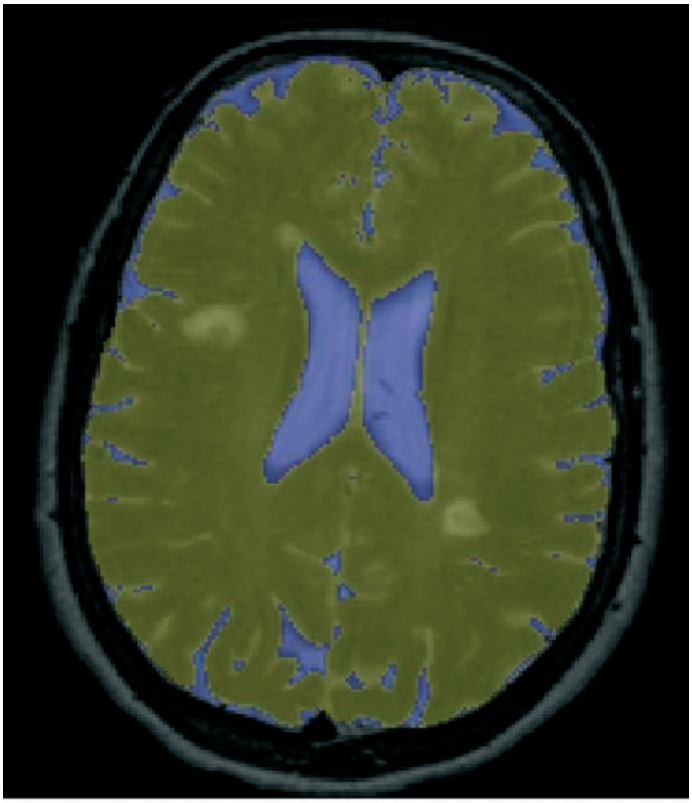

Figure 2 Semi-automatic measurement of BPF: the parenchyma (yellow) volume is divided by the intracranial (yellow and blue) volume. The intracranial volume is manually edited when necessary.

BPF is a normalised measure, which is calculated as total brain volume divided by total intracranial volume (Fig. 2). Fully automated segmentation of whole brain and intracranial volume was performed on the dual T2 and FLAIR images, using fuzzy C-means with in-house developed software (SNIPER: Software for Neuro Image Processing in Experimental Research, Laboratory for Clinical and Experimental Image Processing, Department of Radiology, LUMC). Brain and non-brain structures were manually edited or removed. Details are described elsewhere.[19]

The GMF and WMF were determined using SPM2 software. The HR3D-T1 images were spatially normalized and segmented in native space into GM and WM images.[23,24] The GM and WM segmentation maps were visually checked for misclassification and adjusted when necessary.

Statistics

Pearsons' correlation coefficients were used to calculate correlations between different atrophy measurements.

Relations between atrophy measurements and the MSFC were assessed with Pearsons' correlation coefficients, the relationship between atrophy measurements and EDSS and T2 lesions load were analysed with Spearman rank correlation coefficients.

Inter-observer en intra-observer reproducibility of the three linear measurements was assessed with the coefficient of variation (COV = 100 * standard deviation of the mean difference / [√2 *pooled mean values]).[25]

To correct for multiple testing, significance was taken at a p value < 0.01. All statistical analyses were done using SPSS statistical software package version 14.0 (SPSS Inc, Chicago, USA).

Results

Baseline characteristics

Demographic, clinical and MRI characteristics of the MS patients are shown in Table 1.

Table 1 Characteristics of the patients.

Number of patients	33
Gender: Male (%)	14 (42)
Age (years): mean (SD)	39.4 (9.4)
Disease duration (years): mean (SD)	11.5 (7.2)
EDSS score: Mean (SD)/ median(range)	3.1 (1.5) / 3.0 (0 – 6.0)
MSFC score: mean (SD)	0.154 (0.629)
T2 lesion volume (cc): mean (SD)	5.4 (6.8)
BCR: mean (SD)	0.114 (0.035)
TVW (mm): mean (SD)	3.8 (2.2)
CCA (mm^2): mean (SD)	593 (131.2)
BPF: mean (SD)	0.88 (0.02)
WMF: mean (SD)	0.27 (0.021)
GMF: mean (SD)	0.37 (0.027)

EDSS = Expanded Disability Status Scale; MSFC = Multiple Sclerosis Functional Composite; BCR = bicaudate ratio; TVW = third ventricle width; CCA = corpus callosum area; BPF = brain parenchymal fraction; WMF = white matter fraction, GMF = grey matter fraction

Table 2 Correlations among atrophy measurements.

		r	P
BCR	Vs. TVW	0.80	<0.001
	Vs. CCA	-0.17	0.33
	Vs. BPF	-0.51	0.003
	Vs. GMF	-0.55	0.001
	Vs. WMF	-0.49	0.004
TVW	Vs. CCA	-0.38	0.03
	Vs. BPF	-0.47	0.006
	Vs. GMF	-0.46	0.007
	Vs. WMF	-0.61	< 0.001
CCA	Vs. BPF	0.48	0.005
	Vs. nGMF	0.37	0.04
	Vs. nWMF	0.61	< 0.001
BPF	Vs. GMF	0.76	< 0.001
	Vs. WMF	0.76	< 0.001
GMF	Vs. WMF	0.47	0.005

BCR = bicaudate ratio; TVW = third ventricle width; CCA = corpus callosum area; BPF = brain parenchymal fraction; WMF = white matter fraction, GMF = grey matter fraction. The level of significance was taken as a p value < 0.01.

Correlations between atrophy measurements

Table 2 summarises correlations between different brain atrophy measures. CCA correlated well with WMF, moderately with BPF, but not with the other atrophy measures. All other atrophy values correlated significantly with each other. The strongest correlations were found between TVW and BCR, and between BPF and both GMF and WMF.

Table 3 Correlations between atrophy measures and clinical scores, age, disease duration and T2 lesion load.

		r	P			r	P
BCR	Vs. EDSS	0.27	0.13	BPF	Vs. EDSS	-0.37	0.032
	Vs. MSFC	-0.43	0.014		Vs. MSFC	0.53	0.001
	Vs. age	0.47	0.006		Vs. age	-0.36	0.037
	Vs. disease duration	0.41	0.017		Vs. disease duration	-0.28	0.11
	Vs. T2 lesion load	0.23	0.20		Vs. T2 lesion load	-0.47	0.005
TVW	Vs. EDSS	0.45	0.009	GMF	Vs. EDSS	-0.41	0.017
	Vs. MSFC	-0.51	0.003		Vs. MSFC	0.46	0.006
	Vs. age	0.35	0.047		Vs. age	-0.42	0.016
	Vs. disease duration	0.36	0.042		Vs. disease duration	-0.49	0.004
	Vs. T2 lesion load	0.26	0.14		Vs. T2 lesion load	-0.45	0.009
CCA	Vs. EDSS	-0.41	0.019	WMF	Vs. EDSS	-0.34	0.056
	Vs. MSFC	0.33	0.063		Vs. MSFC	0.47	0.006
	Vs. age	-0.31	0.079		Vs. age	-0.23	0.20
	Vs. disease duration	-0.20	0.27		Vs. disease duration	-0.15	0.41
	Vs. T2 lesion load	-0.61	< 0.001		Vs. T2 lesion load	-0.46	0.007

BCR = bicaudate ratio; TVW = third ventricle width; CCA = corpus callosum area; BPF = brain parenchymal fraction; WMF = white matter fraction, GMF = grey matter fraction; EDSS = Expanded Disability Status Scale; MSFC = Multiple Sclerosis Functional Composite.
The level of significance was taken as a p value < 0.01.

Relation between clinical disability, T2 lesion load and atrophy measurements

As shown in Table 3, correlations were found between T2 lesion volume and BPF, GMF, WMF and CCA, but not with TVW and BCR. The EDSS was only correlated to TVW. The

MSFC correlated with BPF, GMF, WMF and TVW, but not with the BCR and CCA. The strongest correlation was with the BPF (Fig. 3).

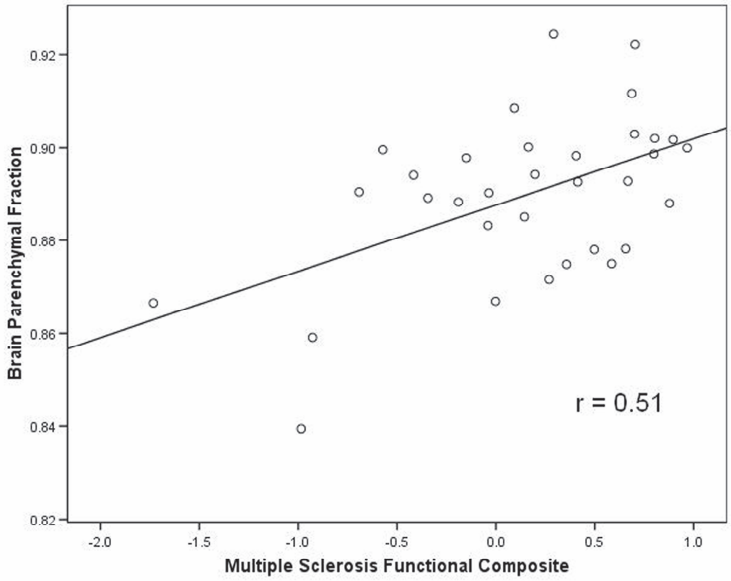

Figure 3 Correlation of the BPF with the MSFC (P = 0.001).

Reproducibility of linear brain atrophy measurements

The coefficients of variation (COV) for intra-observer reliability of BCR, TVW and CCA were 4.4%, 5.3% and 2.5%. The COVs for inter-observer reliability of these linear measurements were 3.6%, 8.9% and 2.4%, respectively.

Discussion

Except for CCA, all atrophy measurements correlated to each other. This suggests that the process that leads to atrophy has global as well as local anatomical impact. It indicates that the measured degree of atrophy is more dependent on the amount of 'true' atrophy than on the technique used.

The strong correlation between BCR and TVW might reflect degeneration of the axons in the internal capsule. These two measurements had no correlation with T2 lesion load, adding support to the hypothesis that the progressive central neurodegeneration in MS runs independently of the development of the focal lesions. In contrast BPF, GMF, WMF and CCA correlated with T2 lesion volume, supporting the idea that central and global brain atrophy may have a different pathophysiological background.[14] CCA correlated best with

WMF and T2 lesion load, indicating that CCA is an appropriate measure of white matter disease burden. A comparable correlation between CCA and T2 lesion load was found previously.[8]

In agreement with Kallmann and colleagues we found a significant correlation between TVW and the EDSS.[26] The third ventricle divides the thalamic hemispheres and the adjacent posterior limbs of the internal capsule, and both thalamic and internal capsule atrophy may give rise to ex vacuo enlargement of the third ventricle. Other atrophy measurements did not correlate significantly with the EDSS, but there were trends toward weak and moderate correlations (Table 2).
These correlations between EDSS and atrophy measurements are in agreement with previous observations,[27,28,29] and may be explained by the fact that this functional score is heavily weighted towards assessment of leg function.[30] Deterioration of ambulation is mainly caused by spinal cord involvement.
The MSFC was developed to overcome the limitations of the EDSS and might thus represent better the overall clinical disability of MS patients. We found a significant correlation between the MSFC and TVW, BPF, GMF and WMF. A previous study reported also that the MSFC correlated better to the BPF than the EDSS did, and a recent study in 117 subjects with secondary progressive MS found similar results.[31,32] In this last study the normalized brain volume (NBV), a measure comparable to BPF, had the strongest correlation with the MSFC score, was a significant independent predictor of the MSFC, and was proposed as the atrophy measurement to use in clinical trials.[32] Since in our study with relapsing MS patients the MSFC was best explained by BPF, we can support the recommendation of the use of techniques like BPF and NBV to measure atrophy in MS trials.

Measurement of the CCA appeared to be the best reproducible manual measurement of brain atrophy in our as well as in previous studies.[8,20,28] TVW measurement was least reproducible. This may be attributed to the way of selecting the slice. Although we agreed in advance to use the middle one of a serial of appropriate slices and in doubt the one with the widest ventricle, there wasn't always consensus.

Linear, manually determined atrophy measures are methodologically simple. Disadvantages are the observer's bias, low accuracy compared to automated techniques, and time consuming analysis. (Semi-) automated methods are faster, more accurate and better reproducible.[14,27]

Main limitations of our study are the low number of patients and the absence of patients with an EDSS higher than 6.0, which may reduce the ability to find stronger correlations between atrophy measurements and clinical scores.

In conclusion, we show that all atrophy measurements except for CCA are correlated. CCA appears to be a good measure of white matter disease burden. The BPF, WMF, GMF and TVW correlate with clinical disability. BPF might be the best atrophy measurement to use in clinical trials. The TVW is of particular interest because there is no correlation with the T2 lesion load, and may represent a more pure surrogate of the diffuse axonal degeneration

References

1. Fox NC, Jenkins R, Leary SM et al. Progressive cerebral atrophy in MS: a serial study using registered, volumetric MRI. *Neurology*. 2000; 54; 807-812.
2. Ingle GT, Stevenson VL, Miller DH et al. Primary progressive multiple sclerosis: a 5-year clinical and MR study. *Brain*. 2003; 126; 2528-2536.
3. Losseff NA, Wang L, Lai HM et al. Progressive cerebral atrophy in multiple sclerosis. A serial MRI study. *Brain*. 1996; 119 (Pt 6); 2009-2019.
4. Stevenson VL, Miller DH, Leary SM et al. One year follow up study of primary and transitional progressive multiple sclerosis. *J.Neurol Neurosurg.Psychiatry*. 2000; 68; 713-718.
5. Courchesne E, Chisum HJ, Townsend J et al. Normal brain development and aging: quantitative analysis at in vivo MR imaging in healthy volunteers. *Radiology*. 2000; 216; 672-682.
6. Miller DH, Barkhof F, Frank JA et al. Measurement of atrophy in multiple sclerosis: pathological basis, methodological aspects and clinical relevance. *Brain*. 2002; 125; 1676-1695.
7. Rudick RA, Fisher E, Lee JC et al. Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. Multiple Sclerosis Collaborative Research Group. *Neurology*. 1999; 53; 1698-1704.
8. Simon JH, Jacobs LD, Campion MK et al. A longitudinal study of brain atrophy in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). *Neurology*. 1999; 53; 139-148.
9. Tiberio M, Chard DT, Altmann DR et al. Gray and white matter volume changes in early RRMS: a 2-year longitudinal study. *Neurology*. 2005; 64; 1001-1007.
10. Zivadinov R, Sepcic J, Nasuelli D et al. A longitudinal study of brain atrophy and cognitive disturbances in the early phase of relapsing-remitting multiple sclerosis. *J.Neurol Neurosurg.Psychiatry*. 2001; 70; 773-780.
11. Filippi M, Rovaris M, Iannucci G et al. Whole brain volume changes in patients with progressive MS treated with cladribine. *Neurology*. 2000; 55; 1714-1718.
12. Frank JA, Richert N, Bash C et al. Interferon-beta-1b slows progression of atrophy in RRMS: Three-year follow-up in NAb- and NAb+ patients. *Neurology*. 2004; 62; 719-725.
13. Paolillo A, Pozzilli C, Giugni E et al. A 6-year clinical and MRI follow-up study of patients with relapsing-remitting multiple sclerosis treated with Interferon-beta. *Eur.J.Neurol*. 2002; 9; 645-655.
14. Bermel RA, Bakshi R. The measurement and clinical relevance of brain atrophy in multiple sclerosis. *Lancet Neurol*. 2006; 5; 158-170.

15. Zivadinov R, Reder AT, Filippi M et al. Mechanisms of action of disease-modifying agents and brain volume changes in multiple sclerosis. *Neurology*. 2008; 71; 136-144.
16. Fischer JS, Rudick RA, Cutter GR et al. The Multiple Sclerosis Functional Composite Measure (MSFC): an integrated approach to MS clinical outcome assessment. National MS Society Clinical Outcomes Assessment Task Force. *Mult.Scler.* 1999; 5; 244-250.
17. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. 1983; 33; 1444-1452.
18. Fischer JS, Jack AJ, Knicker JE et al. Administration and Scoring Manual for the Multiple Sclerosis Functional Composite Measure (MSFC). New York, NY: Demos Medical Publishing, Inc. 1999.
19. Admiraal-Behloul F, van den Heuvel DM, Olofsen H et al. Fully automatic segmentation of white matter hyperintensities in MR images of the elderly. *Neuroimage.* 2005; 28; 607-617.
20. Bermel RA, Bakshi R, Tjoa C et al. Bicaudate ratio as a magnetic resonance imaging marker of brain atrophy in multiple sclerosis. *Arch.Neurol.* 2002; 59; 275-280.
21. Barr AN, Heinze WJ, Dobben GD et al. Bicaudate index in computerized tomography of Huntington disease and cerebral atrophy. *Neurology*. 1978; 28; 1196-1200.
22. Benedict RH, Weinstock-Guttman B, Fishman I et al. Prediction of neuropsychological impairment in multiple sclerosis: comparison of conventional magnetic resonance imaging measures of atrophy and lesion burden. *Arch.Neurol*. 2004; 61; 226-230.
23. Ashburner J, Friston KJ. Nonlinear spatial normalization using basis functions. *Hum.Brain Mapp.* 1999; 7; 254-266.
24. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. *Neuroimage.* 2000; 11; 805-821.
25. van der Worp HB, Claus SP, Bar PR et al. Reproducibility of measurements of cerebral infarct volume on CT scans. *Stroke*. 2001; 32; 424-430.
26. Kallmann BA, Sauer J, Schliesser M et al. Determination of ventricular diameters in multiple sclerosis patients with transcranial sonography (TCS)--a two year follow-up study. *J.Neurol.* 2004; 251; 30-34.
27. Sharma J, Sanfilipo MP, Benedict RH et al. Whole-brain atrophy in multiple sclerosis measured by automated versus semiautomated MR imaging segmentation. *AJNR Am.J.Neuroradiol.* 2004; 25; 985-996.
28. Bermel RA, Sharma J, Tjoa CW et al. A semiautomated measure of whole-brain atrophy in multiple sclerosis. *J.Neurol Sci*. 2003; 208; 57-65.

29. Sanfilipo MP, Benedict RH, Sharma J et al. The relationship between whole brain volume and disability in multiple sclerosis: a comparison of normalized gray vs. white matter with misclassification correction. *Neuroimage.* 2005; 26; 1068-1077.
30. Whitaker JN, McFarland HF, Rudge P et al. Outcomes assessment in multiple sclerosis clinical trials: a critical analysis. *Mult.Scler.* 1995; 1; 37-47.
31. Kalkers NF, Bergers E, Castelijns JA et al. Optimizing the association between disability and biological markers in MS. *Neurology*. 2001; 57; 1253-1258.
32. Furby J, Hayton T, Anderson V et al. Magnetic resonance imaging measures of brain and spinal cord atrophy correlate with clinical impairment in secondary progressive multiple sclerosis. *Mult.Scler.* 2008; 14; 1068-1075.

Chapter 5

Relationship between the extent of T2 lesions and the onset of secondary progression in multiple sclerosis

J.P. Mostert[1], J.C. de Groot[2], G.S.M. Ramsaransing[1], M.W. Koch[1], J. De Keyser[1]

Author's affiliations:
[1] Department of Neurology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands
[2] Department of Radiology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands

European Journal of Neurology
2007 Nov;14(11):1210-5.

Abstract

Patients with relapsing remitting multiple sclerosis (MS) are at risk of converting to a secondary progressive disease course. To assess the relationship between brain MRI findings and onset of secondary progression, we reanalysed the initial brain MRI scans of 90 relapsing remitting MS patients, who were clinically followed for at least 10 years (median 14 years) after their scan, for the number and volume of T2 lesions, and for two measures of brain atrophy (bicaudate ratio and third ventricle width). The relationship to development of secondary progression was studied with Cox regression models and Kaplan-Meier survival analyses. At the end of follow-up 36 patients had become progressive. The presence of more than 10 T2 lesions more than doubled the risk of becoming secondary progressive (hazard ratio: 2.36; 95% CI: 1.19 – 4.66). When at least one of the 10 lesions was confluent the risk increased to 3.51 (1.64 – 7.50). The hazard ratio for an estimated T2 lesion load of more than 3400* mm^3 was 2.11 (1.07 – 4.16). Linear brain atrophy measures were not predictive. Our data show a relationship between the extent of brain T2 lesions and the onset of secondary progression in MS.

* compared to the published article the T2 lesion load was multiplied by $^4/_3\Pi$.

Introduction

The majority of patients with multiple sclerosis (MS) present with relapses, which are followed by variable degrees of recovery, spontaneously or in response to corticosteroids. Within 10 years after the first clinical manifestation approximately 30-40%, and within 20 years around 70%, of the patients convert to a secondary progressive phase, which is characterized by a steady progression of clinical disability independent of relapses.[1,2,3,4] At present, there are no established measures to predict the onset of the secondary progressive phase.

Relapses of MS are caused by focal lesions in eloquent areas of the CNS, whereas secondary progression results from a more diffuse degeneration of axons.[5,6] The mechanism underlying conversion from a relapsing remitting disease course to secondary progressive MS is unknown. The aim of this study was to assess whether findings on the base-line brain MRI are related to a more rapid onset of the secondary progressive phase. Do patients with early onset of secondary progression have early signs of brain atrophy or is there a relationship with the extent of existing white matter lesions? To answer these questions we reanalysed a cohort of patients with relapsing remitting MS who had a brain MRI scan performed between 1987 and 1995 as part of the diagnostic work-up and who were followed up for at least 10 years.

Methods

Patients

The Groningen MS database was established in 1985. Data of patients attending the Groningen MS clinic are systematically entered when a patient is first seen and at each of the regular follow-up visits (from 3 to 12 months).

Of the 454 patients with definite MS with a relapsing disease onset[7] in our database, 249 had a brain MRI-scan before 1996. Seventy-six patients were already progressive at the time of scanning, 81 scans had been destroyed, and 2 patients were lost to follow-up within 10 years, leaving 90 patients for analysis. Patient characteristics were comparable between included and not included patients (Table 1).

Disability was measured with the Expanded Disability Status Scale (EDSS; scores can range from 0 to 10, with higher scores indicating more severe disease).[8] The onset of the secondary progressive phase (the onset of gradual worsening of MS symptoms for at least 12 months unrelated to relapse[4]) was available in the database, and was verified.

Table 1 Characteristics of the patients with relapsing remitting MS in the Groningen MS database (median, IQR).

Characteristics	Participating patients	All other relapsing remitting patients in database	All other relapsing remitting patients with a MRI-scan before 1996
Number of patients	90	364	83
Gender: male (%)/ female (%)	25 (28%) / 65 (72%)	111 (30%) / 253 (70%)	26 (31%) / 57 (69%)
Age at start disease	29.5 (23.75 – 36)	29 (23 – 26)	29 (23 – 33)
Age at time of the MRI scan (years)	33 (26 – 40.25)		31 (28 – 38)
Disease duration at time of scanning (years)	1 (0 – 6.25)		2 (0 – 6)
Number of patients that become progressive	36 (40%)		33 (40%)

MRI

MRI was performed on a 1.5 Tesla scanner which was available in the University Hospital Groningen from 1987 (Philips Gyroscan S15). Slice thickness was 5 mm in most patients (n=67), and 6, 9 and 10 mm in the remainder (1, 3 and 19 patients respectively). Because not all standard sequences were available, only T2-weighted scans were evaluated. The hardcopies of all scans were analyzed by an experienced neuroradiologist (J.C. de G.), who was unaware of the clinical data. Lesions were classified by region (supratentorial, infratentorial) and by size (small < 5 mm; medium 5 – 10 mm; large > 10 mm, or confluent). Confluent lesions were defined as single T2 lesions larger than 20 mm typically located in the periventricular region, or as two or more T2 lesions connected at one or more margins.[9] We estimated the total lesion volume by assuming the lesions were spherical with a fixed diameter size per category.[10]

Bicaudate ratio and third ventricle width were used as measures of brain atrophy.[11,12] The bicaudate ratio is the minimal distance between both caudate nuclei divided by the brain width at the same level, measured on the axial slice where the caudate nuclei are best visible. Third ventricle width is the maximal diameter of the third ventricle.

Statistical analyses

To estimate the predictive value of brain MRI findings on conversion to secondary progression, we performed Cox proportional-hazards regression analyses to calculate hazard ratios with adjustment for age at time of scanning, disease duration at time of scanning, and gender. T2 lesion number, T2 lesion load, third ventricle width and bicaudate ratio were entered as continuous variables. We also studied confluent lesions, infratentorial lesions, and increasing cut-off points of T2 lesion number, T2 lesion load, third ventricle width and bicaudate ratio. The time-to-event was the time between the MRI scan and the start of the progressive phase or to the date of the last follow-up visit if progression had not developed. Time-to-event curves describing the proportion of patients becoming secondary progressive during the whole follow-up period were calculated by the Kaplan-Meier method, and compared using log-rank tests.

Between group comparisons were performed with Mann-Whitney tests or with Chi-Square tests when appropriate. Correlation coefficients were calculated with Spearman's rank correlation test. Significance was taken at the two-tailed 0.05 level. Analyses were performed with the Statistical Package for the Social Sciences (SPSS 12.0 for Windows, Chicago, Illinois).

Results

Patients

Baseline characteristics of the participating patients are listed in Table 2.

Brain MRI data

Double reading was performed for a random set of 24 scans. Intra-class correlation coefficients were excellent for T2 lesion load ($r = 0.96$; $p < 0.001$), T2 lesion number ($r = 0.95$; $p < 0.001$) and third ventricle width ($r = 0.99$, $p < 0.001$), and good for bicaudate ratio ($r = 0.82$; $p < 0.001$).

The number of T2 lesions ranged from 0 to 80. The EDSS score was not documented at the time of the MRI scan in 8 patients and ranged from 0 to 5.0. There was no correlation between the EDSS at time of scanning and either T2 lesion load ($r = 0.056$; $p = 0.62$) or T2 lesion number ($r = 0.13$; $p = 0.24$). Disease duration at time of scanning was not correlated to T2 lesion number ($r = 0.051$; $p = 0.63$) or T2 lesion load ($r = -0.031$; $p = 0.78$). Twenty-eight patients were already diagnosed with clinically definite MS at the time of scanning; the other 62 patients were definitively diagnosed with MS in the year of their initial MRI-scan or in the years thereafter. In 4 patients, the infratentorial part of the brain was missing on the MRI scan. In the analysis the number of infratentorial lesions for these patients was

set to 0. Excluding these 4 patients from the analysis did not change the results (data not shown).

Table 2 Patient characteristics (median, IQR).

Characteristics	All patients	Patients not progressive at follow-up	Patients progressive at follow-up
Number of patients	90	54	36
Gender: male (%)/ female (%)	25 (28%)/ 65 (72%)	13 (24%)/ 41 (76%)	12 (33%)/ 24 (67%)
Age at time of the MRI scan (years)	33 (26 – 40)	32.5 (25 – 39)	34.5 (29 – 44)
Disease duration at time of scanning (years)	2.5 (0 – 6.25)	1.5 (0 – 6)	3 (1 – 7)
Time between scanning and diagnosis (years)	0 (0 – 2)	0 (0 – 2)	0 (0 – 2)
Follow-up after the MRI scan (years)	14 (12 – 16)	14 (12 – 15)	15 (13 – 16)
EDSS at time of scanning[#]	2 (1 – 2.625)	1.5 (1 – 2)	2 (1.5 – 3.5)**
Bicaudate ratio	0.11 (0.10 – 0.13)	0.11 (0.10 – 0.13)	0.11 (0.10 – 0.14)
Third ventricle width (mm)	1.00 (0.50 – 1.25)	1.00 (0.50 – 1.00)	1.00 (0.63 – 1.5)
T2 lesion load (mm^3)	1648 (250 – 3636)	1347 (243 – 2879)	2551 (351 – 4366)
Total number of T2 lesions	9.5 (4 – 19)	7 (4.75 – 14)	14.5 (3.25 – 25.5)
Number of patients with > 10 T2 lesions	40 (44%)	18 (33%)	22 (61%)**
Number of patients with infratentorial lesions[$]	14 (16%)	7 (14%)	7 (21%)
Number of patients with confluent lesions	24 (27%)	10 (19%)	14 (39%)*
Number of patients with > 10 T2 lesions including at least one confluent lesion	20 (22%)	6 (11%)	14 (39%)**

[#]N = 82, [$]N = 86

*p < 0.05, ** p < 0.01: patients progressive at follow-up compared to patients not progressive at follow-up.

Thirty-six patients (40%) had become progressive over a median follow-up period of 14 years. Twenty-three patients (64%) who entered the progressive phase had been treated with immunomodulatory therapy (20 with interferon-β, 2 with monthly methylprednisolon infusions, and 1 with azathioprine) compared to 8 of 54 patients (15%) who remained relapsing remitting (5 with interferon-β, 2 with monthly methylprednisolon infusions, and 1 with azathioprine).

The results of Cox proportional-hazards regression analyses are shown in Table 3. Higher T2 lesion number and load increased the risk of becoming secondary progressive. This risk increased with higher cut-off points of T2 lesion number and T2 lesion load. The presence of more than 10 T2 lesions, a T2 lesion load of more than 3400 mm³, or the presence of at least one confluent lesion more than doubled the risk of becoming secondary progressive. The presence of more than 10 T2 lesions, including at least one confluent lesion, more than tripled the risk of entering the progressive phase of the disease when compared to patients with up to 10 T2 lesions (HR 3.51; 95% CI: 1.64 – 7.50). The presence of infratentorial lesions did not increase the risk of becoming progressive. The relationship between T2

Table 3 Hazard ratios of developing secondary progression for several MRI findings.

	Number of subjects	Hazard Ratio	95% confidence interval	P-value
Bicaudate ratio		0.079	$0 – 2.2*10^4$	0.69
Ratio > 0.11	48	0.71	0.34 – 1.45	0.34
Ratio > 0.12	34	0.1	0.39 – 1.66	0.56
Ratio > 0.14	19	0.89	0.39 – 2.0	0.78
Third ventricle width		1.10	0.65 – 1.87	0.72
> 1.0 mm	21	1.07	0.49 – 2.34	0.87
Number of T2 lesions		1.02	1.01 – 1.04	0.005
> 5 T2 lesions	58	1.49	0.71 – 3.14	0.29
> 10 T2 lesions	40	2.36	1.19 – 4.66	0.01
> 15 T2 lesions	28	2.57	1.33 – 4.98	0.005
> 25 T2 lesions	14	2.99	1.34 – 6.66	0.008
Infratentorial lesions	14	1.70	0.70 – 4.10	0.24
Confluent lesions	24	2.23	1.13 – 4.39	0.02
> 10 T2 lesions including at least one confluent lesion	20	3.10	1.57 – 6.13	0.001
T2 lesion load		1.00	1.000 – 1.001	0.04
> 400 mm^3	63	1.01	0.48 – 2.13	0.98
> 2100 mm^3	40	1.84	0.94 – 3.59	0.08
> 3400 mm^3	25	2.11	1.07 – 4.16	0.03

lesion number or T2 lesion load and the risk of secondary progression was not altered when the EDSS was included as a covariate (data not shown). Brain atrophy measures, disease duration, age and gender were not predictive.

Kaplan Meier survival analyses showed that the probability of entering the progressive phase was significantly higher in patients with more than 10 T2 lesions than in patients with 10 or fewer T2 lesions (P = 0.007, Figure 1A).

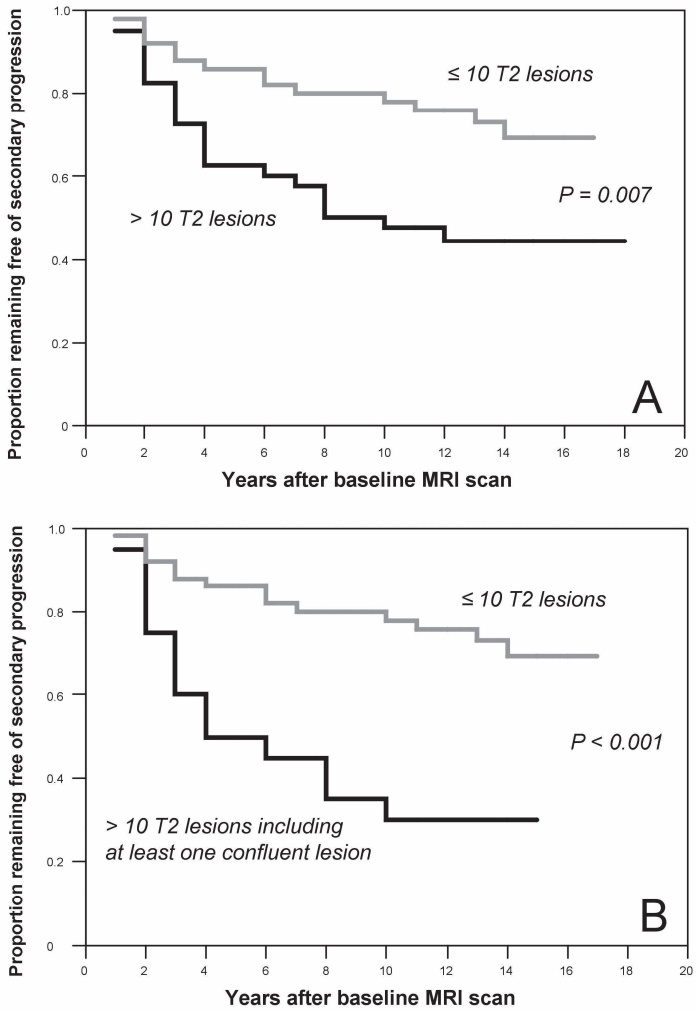

Figure 1 Kaplan-Meier estimates for secondary progression for patients with up to 10 T2 lesions (N=50) compared to (A) patients with more than 10 T2 lesions (N=40) and (B) patients with more than 10 T2 lesions including at least one confluent lesion (N=20) (B).

Of the 50 patients with 10 or fewer T2 lesions, a quarter (13 patients) had become progressive at 13 years, and 28% (14 patients) at the end of follow-up. Of the 40 patients with more than 10 T2 lesions, a quarter (10 patients) had already become progressive at 3 years, half (20 patients) at 8 years and 55% (22 patients) at the end of follow-up. The probability of entering the progressive phase was even more pronounced when comparing patients with more than 10 T2 lesions, of which at least one lesion was confluent, with patients who had 10 or fewer T2 lesions ($P < 0.001$, Figure 1B).

Discussion

In this study we investigated brain MRI characteristics of patients with relapsing remitting MS in relation to subsequent onset of secondary progression. We found a strong relationship between the extent of T2 lesions and the onset of secondary progression. Although signs of brain atrophy can occur early in the disease course of MS,[13] we found no association between early brain atrophy and onset of secondary progression.

In patients with clinically isolated syndromes suggestive of MS, it has been shown that increases in the volume of brain T2 lesions in the first five years correlate with the degree of long-term disability as measured with the EDSS.[14] In another study of 156 patients with clinically isolated syndromes EDSS at 5 years correlated moderately ($r = 0.43$) with the number of baseline T2 lesions.[15] A study on 30 relapsing remitting patients found a correlation between T2 lesion load and disability 13 years later.[16] However, disability can develop from both incomplete recovery from relapses and gradual progression. We did not focus on disability but investigated the onset of the progressive phase.

Some limitations of our study should be noted. The sample size is relatively small and because of the long follow-up it is possible that patients with a benign disease course are underrepresented. However, the percentage of patients that became progressive during follow-up is similar to that reported in natural history studies.[1,3,4] The scans were analysed retrospectively, but interpretation bias is unlikely because the neuroradiologist who scored the scans had no knowledge of the clinical data. Since our patients were scanned for clinical purposes, the scanning protocol was inconsistent and follow-up scans are lacking. Our method of manually estimating T2 lesion load and using linear brain atrophy measures may be less precise than the current automated methods. On the other hand, the measurements performed were highly reproducible. At the time of scanning our cohort had variable disease duration, age and EDSS, but we corrected our analyses for these three variables.

In two studies investigating the conversion of patients with clinically isolated syndromes to definite MS, the median number of T2 lesions at the baseline MRI-scan was 4 in the study with a low conversion rate and 18 in the study with a high conversion rate.[17,18] Thus, the median number of lesions of 9.5 in our study is representative for patients with relapsing MS at the beginning of their disease.

Focal lesions of MS are primarily due to episodes of T-cell-mediated inflammatory activity causing demyelination, axonal injury and gliosis,[5,6,19] while the progressive phase of MS appears to be driven by relentless axonal degeneration. Several observations indicate that different effector mechanisms underlie the development of focal lesions and the progressive phase of MS. First, the progression of disability in secondary progressive MS is not affected by relapses, neither by those occurring before the onset of the progressive phase nor by those occurring during this phase.[1,4] Second, medications such as beta interferons, glatiramer acetate and immunosuppressive drugs, which reduce relapses and suppress the inflammatory component of the disease, are unable to slow down the progressive phase.[2] Third, MRI and neuropathological investigations found no correlation between CNS focal lesion load and the diffuse axonal loss in the spinal cord that characterises the progressive phase of MS.[20,21]
Our finding that the extent of focal T2 lesions predicts the onset of the progressive phase seems in conflict with these observations. A plausible explanation for this apparent contradiction is that a higher number of focal white matter lesions facilitates the clinical expression of the progressive phase. The CNS has a large functional reserve capacity. Even in a tract system with clearly defined and measurable clinical function, as the pyramidal tract, permanent clinical deficit is only seen when more than 60% of the axons in the tract system are lost.[22] If we assume that the slowly progressive axonal degeneration follows a similar course in the majority of MS patients, clinical manifestations of progression will start earlier in those patients who have lost more reserve capacity due to CNS tissue injury in focal lesions. Confluent lesions represent a large area of focal lesions and this might explain the striking association between confluent lesions and transition to the progressive phase.

There is no consensus on the time when treatment with immunomodulatory drugs should be started, or whether every patient should be treated.[23,24] Some neurologists begin treatment in patients with a clinically isolated syndrome and two or more clinically silent lesions suspect for MS on the brain MRI.[24] Others do not treat patients at the time of diagnosis because there is a chance for a benign disease course.[5] The high price, adverse effects and unproven long-term efficacy of immunomodulatory drugs for MS are reasons to refrain from immediate treatment. In our hospital we always discuss the option of immunomodulatory treatment with our patients and many patients decide not to use it.

Immunomodulatory therapies do, however, prevent new T2 lesions and the enlargement of existing T2 lesions. In view of our results, this effect of immunomodulatory drugs may be a worthwhile therapeutic goal.

Our results suggest that the extent of base-line T2 lesions, and their development over time, should be taken into consideration when therapeutic decisions in patients with relapsing-remitting MS are made.

References

1. Confavreux C, Vukusic S, Moreau T et al. Relapses and progression of disability in multiple sclerosis. *N.Engl.J.Med.* 2000; 343; 1430-1438.
2. Rovaris M, Confavreux C, Furlan R et al. Secondary progressive multiple sclerosis: current knowledge and future challenges. *Lancet Neurol.* 2006; 5; 343-354.
3. Weinshenker BG, Bass B, Rice GP et al. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. *Brain*. 1989; 112 (Pt 1); 133-146.
4. Kremenchutzky M, Rice GP, Baskerville J et al. The natural history of multiple sclerosis: a geographically based study 9: observations on the progressive phase of the disease. *Brain*. 2006; 129; 584-594.
5. Compston A, Coles A. Multiple sclerosis. *Lancet*. 2002; 359; 1221-1231.
6. Noseworthy JH, Lucchinetti C, Rodriguez M et al. Multiple sclerosis. *N.Engl.J.Med.* 2000; 343; 938-952.
7. Poser CM, Paty DW, Scheinberg L et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann.Neurol.* 1983; 13; 227-231.
8. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. 1983; 33; 1444-1452.
9. Zivadinov R, Bakshi R. Role of MRI in multiple sclerosis I: inflammation and lesions. *Front Biosci.* 2004; 9; 665-683.
10. de Leeuw FE, de Groot JC, Achten E et al. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *J.Neurol.Neurosurg.Psychiatry*. 2001; 70; 9-14.
11. Bermel RA, Bakshi R, Tjoa C et al. Bicaudate ratio as a magnetic resonance imaging marker of brain atrophy in multiple sclerosis. *Arch.Neurol.* 2002; 59; 275-280.
12. Simon JH, Jacobs LD, Campion MK et al. A longitudinal study of brain atrophy in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). *Neurology*. 1999; 53; 139-148.
13. Chard DT, Brex PA, Ciccarelli O et al. The longitudinal relation between brain lesion load and atrophy in multiple sclerosis: a 14 year follow up study. *J.Neurol.Neurosurg.Psychiatry*. 2003; 74; 1551-1554.
14. Brex PA, Ciccarelli O, O'Riordan JI et al. A longitudinal study of abnormalities on MRI and disability from multiple sclerosis. *N.Engl.J.Med.* 2002; 346; 158-164.
15. Tintore M, Rovira A, Rio J et al. Baseline MRI predicts future attacks and disability in clinically isolated syndromes. *Neurology*. 2006; 67; 968-972.
16. Rudick RA, Lee JC, Simon J et al. Significance of T2 lesions in multiple sclerosis: A 13-year longitudinal study. *Ann.Neurol.* 2006; 60; 236-242.

17. Kappos L, Polman CH, Freedman MS et al. Treatment with interferon beta-1b delays conversion to clinically definite and McDonald MS in patients with clinically isolated syndromes. *Neurology*. 2006; 67; 1242-1249.
18. Korteweg T, Tintore M, Uitdehaag B et al. MRI criteria for dissemination in space in patients with clinically isolated syndromes: a multicentre follow-up study. *Lancet Neurol.* 2006; 5; 221-227.
19. Frohman EM, Racke MK, Raine CS. Multiple sclerosis--the plaque and its pathogenesis. *N.Engl.J.Med.* 2006; 354; 942-955.
20. Bergers E, Bot JC, De Groot CJ et al. Axonal damage in the spinal cord of MS patients occurs largely independent of T2 MRI lesions. *Neurology*. 2002; 59; 1766-1771.
21. Deluca GC, Williams K, Evangelou N et al. The contribution of demyelination to axonal loss in multiple sclerosis. *Brain*. 2006; 129; 1507-1516.
22. Bjartmar C, Kidd G, Mork S et al. Neurological disability correlates with spinal cord axonal loss and reduced N-acetyl aspartate in chronic multiple sclerosis patients. *Ann.Neurol.* 2000; 48; 893-901.
23. Pittock SJ, Weinshenker BG, Noseworthy JH et al. Not every patient with multiple sclerosis should be treated at time of diagnosis. *Arch.Neurol.* 2006; 63; 611-614.
24. Frohman EM, Havrdova E, Lublin F et al. Most patients with multiple sclerosis or a clinically isolated demyelinating syndrome should be treated at the time of diagnosis. *Arch.Neurol.* 2006; 63; 614-619.

Chapter 6

T2 lesions and rate of progression of disability in relapsing remitting and progressive multiple sclerosis

J.P. Mostert[1], M.W. Koch[1], C. Steen[1], D.J. Heersema[1], J.C. de Groot[2], J. De Keyser[1,3]

Author's affiliations:
[1] Department of Neurology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands
[2] Department of Radiology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands
[3] Department of Neurology, University Hospital Brussels, Free University Brussels, Brussels, Belgium

Submitted

Abstract

To assess the predictive value of T2 lesions on the rate of progression of disability in multiple sclerosis (MS), we reanalyzed T2 lesion number and load on brain MRI scans performed before 1997 of 186 MS patients, who were clinically followed. There were 90 patients with progressive MS (35 secondary progressive and 55 primary progressive), and 96 with relapsing remitting MS. The rate of progression of disability was measured by the Multiple Sclerosis Severity Scale (MSSS), and by time to progression of disability (defined as an increase in ≥ 1 point when the EDSS was 5.5 or less and an increase in EDSS of ≥ 0.5 point when the EDSS was 6.0 or higher). During follow-up (median 15 years, IQR 12 – 17 years) 94% of the patients with progressive MS and 50% of the patients with relapsing remitting MS had progression of disability. Higher T2 lesion number and load were associated with a higher rate of disease progression on the MSSS and a shorter time to progression of disability in relapsing remitting MS, but not in progressive MS. Our findings indicate that the amount of T2 lesions has a small predictive value for progression of disability in relapsing remitting MS, but has no influence on the rate of progression in progressive MS.

Introduction

Inflammation in white matter tracts of the central nervous system (CNS) is responsible for the relapses in patients with multiple sclerosis (MS).[1] Twenty years after the first symptom around 70% of the patients with a relapsing remitting disease course have gradual progression of their symptoms, which is termed secondary progressive MS.[2,3] Ten to twenty percent of MS patients experience a gradual progression of symptoms from the onset of their disease, which is called primary progressive MS. Both the secondary and primary progressive phase of MS is caused by a slowly progressive axonal degeneration.
Predicting the rate of progression of disability in an individual MS patient is difficult on clinical grounds.[4,5] In patients with clinically isolated syndromes, a higher number of T2 lesions, which can represent current or past episodes of inflammation, is associated with a more severe disease course.[6,7,8] Previously, we have shown in patients with relapsing remitting MS that a higher number of T2 lesions is associated with a higher risk of becoming secondary progressive.[9] Since epidemiological and pathological studies, as well as clinical trials with immunomodulatory and immunosuppressive drugs, indicate that the progressive phase of MS runs quite independently from inflammation,[3,10,11,12,13] we interpreted this association as an earlier clinical expression of the ongoing axonal degeneration due to a decreased CNS reserve capacity.[9] However, in view of this hypothesis, we would also expect an effect of the amount of T2 lesions on the rate of progression of disability in patients with progressive MS.
The aim of this study was to investigate the influence of T2 lesion number and load on the rate of progression of disability in patients with relapsing remitting and progressive MS.

Methods

Patients
The Groningen MS database was established in 1985. Data from all patients attending the Groningen MS clinic are systematically entered in the database at first visit and after each follow-up visit (at regular intervals between 3 and 12 months). We retrospectively collected the available cerebral MRI scans of all MS patients performed between 1987 (installation of the first MRI scanner in Groningen) and 1997.
Disability was measured with the Expanded Disability Status Scale (EDSS; scores range from 0 to 10 with higher scores indicating more disability).[14] Progression of disability was defined as an increase in $\geq$ 1 point when the EDSS at scanning was 5.5 or less and as an increase in EDSS of $\geq$ 0.5 point when the EDSS at scanning was 6.0 or higher. This definition is commonly used in clinical trials evaluating effects of medication in the progressive phase of the disease.[15,16,17]

Rate of progression of disability was measured with time to progression of disability (in years) and with Multiple Sclerosis Severity Scale (MSSS).[18] This score is based on the combination of EDSS and disease duration, and ranges from 0 to 10. Higher scores indicate a faster progression of disability. The MSSS was calculated at time of scanning and at last follow-up.

A progressive disease course (primary or secondary) was defined as a slowly progressive neurological worsening for at least 12 months. EDSS, disease duration and age at time of scanning, gender, time to progression (in years), and use of immunomodulating therapy (either interferon beta or glatiramer acetate; duration of therapy) were extracted from the database.

MRI

MRI was performed on a 1.5 Tesla scanner (Philips Gyroscan S15) in 179 patients and on a 1.0 Tesla scanner (Magneton Expert) in 7 patients. Slice thickness was 5 mm in most patients (n=126), and 3, 6, 7, 9 and 10 mm in the remainder (1, 8, 1, 11 and 39 patients, respectively). The hardcopies of all scans were analyzed by an experienced neuroradiologist (J.C. de G.), who was unaware of the clinical data. Lesions were classified by region (supratentorial, infratentorial) and by size (small < 5mm; medium 5 – 10 mm; large >10 mm, or confluent). Confluent lesions were defined as single T2 lesions larger than 20 mm typically located in the periventricular region, or as two or more T2 lesions connected at one or more margins.[19] We estimated the total lesion volume by assuming the lesions were spherical with a fixed diameter size per category as described previously.[20]

Statistical analyses

To estimate the predictive value of brain MRI findings on progression of disability, we performed Cox proportional-hazards regression analyses to calculate hazard ratios with adjustment for age at time of scanning, disease duration at time of scanning, EDSS at time of scanning, and gender. We analysed the relapsing remitting and progressive disease courses separately. T2 lesion number and T2 lesion load were entered as continuous variables. We also studied the presence of confluent lesions and infratentorial lesions. The time-to-event was the time between the MRI scan and progression of disability or to the date of the last follow-up visit if progression had not developed.

Between group comparisons were analyzed using Mann-Whitney tests, Kruskal-Wallis tests or Chi-Square tests where appropriate. Correlation coefficients were calculated with Spearman's rank correlation test. Significance was taken at a p value < 0.05. All analyses were performed with the Statistical Package for the Social Sciences (SPSS 14.0 for Windows, SPSS Inc., Chicago, Illinois, USA).

Results

Patients

The MRI scans of 200 patients were available for analysis. Fourteen patients were excluded because no T2 scan was available (n=4) or follow-up was lacking (n=10). The characteristics of the remaining 186 patients are shown in table 1. During a median follow-up time of 15 years, 134 patients had progression of disability. Compared to patients with PPMS, patients with SPMS were younger at the time of their first MRI scan (p=0.033), had longer disease duration (p=0.006) and experienced more disability as measured with the EDSS (p=0.014). Of the 96 patients with relapsing remitting MS, 48 (50%) had progression of disability.

Table 1 Characteristics of the participating patients (median, IQR).

Characteristics	All patients	Relapsing Remitting	Secondary Progressive	Primary Progressive
Number of patients	186	96	35	55
Gender: male (%)/ female (%)	62 (33%)/ 124 (67%)	29 (30%) / 67 (70%)	15 (43%) / 20 (57%)	18 (32%) / 37 (67%)
Age at time of the MRI scan (years)	39 (31 – 48)	34 (27 – 41)	41 (32 – 49)	48 (39 – 55)
Disease duration at time of scanning (years)	4 (1 – 9)	2 (0 – 7)	10 (3 – 21)	4 (2 – 9)
Follow-up after the MRI scan (years)	15 (12 – 17)	15 (13 – 17)	15 (10 – 18)	15 (11 – 18)
EDSS at time of scanning	3.0 (2.0 – 4.5)	2.0 (1.0 – 2.5)	5.0 (4.0 – 6.0)	4.0 (3.0 – 6.0)
EDSS at last follow-up	6.0 (3.0 – 7.5)	3.3 (2.0 –6.0)	7.5 (7.0 – 9.0)	6.5 (6.0 – 8.0)
MSSS at time of scanning	5.8 (3.3 – 8.0)	4.3 (2.4 – 6.1)	6.9 (5.1 – 8.8)	7.7 (5.9 – 8.6)
MSSS at last follow-up	5.6 (2.2 – 8.2)	2.3 (1.0 – 5.3)	8.0 (6.7 – 9.9)	7.5 (5.7 – 9.5)
Progression of disability during follow-up: N (%)	134 (72%)	48 (50%)	33 (94%)	52 (95%)
Time to progression (years)	3 (1 – 5)	3 (2 – 7)	2 (1 – 4)	3 (2 – 5)

Brain MRI

Double reading was performed for a random set of 24 scans. Intra-class correlation coefficients were excellent or good for T2 lesion load ($r = 0.96$; $p < 0.001$) and T2 lesion number ($r = 0.95$; $p < 0.001$).

Table 2 MRI characteristics of the participating patients (median, IQR).

Characteristics	All patients	Relapsing Remitting	Secondary Progressive	Primary Progressive
Number of patients	186	96	35	55
T2 lesion load (ml)	2.0 (0.4 – 4.5)	1.5 (0.3 – 3.7)	4.1 (1.2 – 6.0)	1.8 (0.3 – 4.5)
Total number of T2 lesions	11 (5 – 24)	9 (4 – 19)	20 (10 – 38)	13 (4 – 26)
Confluent lesions: yes Number (%)	51 (27%)	26 (27%)	10 (29%)	15 (27%)
Infratentorial lesions: yes Number (%)*	28 (16%)	13 (14%)	10 (29%)	5 (10%)

* N=177

MRI characteristics of all patients and per disease course are presented in table 2.
Compared to patients with PPMS, those with SPMS had a higher T2 lesion load (p=0.011), higher T2 lesion number (p=0.034), and more frequent infratentorial lesions (p=0.02).
In relapsing remitting MS the 48 patients with progression of disability had a slightly higher T2 lesion load compared to the 48 patients with no progression of disability (median 2.4 ml versus 1.3 ml, p = 0.06). The follow-up time was similar (median of 15 years in both groups).

Influence of T2 lesions on the rate of progression of disability
In patients with relapsing remitting MS, but not with progressive MS, T2 lesion load and T2 lesion number correlated with the MSSS at follow-up (table 3).

Table 3 Correlations of T2 lesion load and T2 lesion number with MSSS scores.

		MSSS at scanning	MSSS at last follow-up
PMS	T2 lesion load	0.06	0.19
	T2 lesion number	0.04	0.04
RRMS	T2 lesion load	0.02	0.29#
	T2 lesion number	0.10	0.32#

# $p < 0.01$; PMS = progressive MS; RRMS = relapsing remitting MS.

In relapsing remitting patients a higher number of T2 lesions, a higher T2 lesion load and the presence of confluent lesions increased the risk of progression of disability (table 4). No other clinical variable predicted the time to progression of disability (all p-values > 0.24). In contrast, in patients with progressive MS, MRI and clinical variables did not predict progression of disability (table 5).
Analysis of PPMS and SPMS separately did not show any significant correlation with MSSS and any significant predictor for progression of disability either (data not shown).

Table 4 Cox regression analyses for progression of disability of the separate MRI variables in patients with relapsing remitting MS controlled for EDSS at time of scanning, age at time of scanning, disease duration at time of scanning and gender.

Variable		Hazard Ratio	95% CI	p-value
T2 lesion load	per ml increase	1.16	1.06 – 1.26	0.001
T2 lesion number	per number increase	1.02	1.003 – 1.03	0.026
Confluent lesions	No	1.0 (reference)		
	Yes	2.58	1.41 – 4.73	0.002
Infratentorial lesions	No	1.0 (reference)		
	Yes	0.72	0.27 – 1.90	0.51

Table 5 Cox regression analyses for progression of disability of the separate MRI variables in patients with progressive MS controlled for EDSS at time of scanning, age at time of scanning, disease duration at time of scanning and gender.

Variable		Hazard Ratio	95% CI	p-value
T2 lesion load	per ml increase	1.01	0.94 – 1.08	0.84
T2 lesion number	per number increaese	1.002	0.99 – 1.011	0.73
Confluent lesions	No	1.0 (reference)		
	Yes	1.16	0.69 – 1.95	0.58
Infratentorial lesions	No	1.0 (reference)		
	Yes	1.21	0.65 – 2.27	0.53

Influence of immunomodulating treatment on progression of disability

Five patients with secondary progressive MS (mean time of treatment 5 years, sd 1.9), 3 patients with primary progressive MS (mean time of treatment 4 years, sd 1), and 23 out of the 96 (24%) patients with relapsing remitting MS received interferon-beta (mean time of treatment: 7.13 years, sd 3.4). Only 8 patients received immunomodulating therapy before progression of disability had occurred. In the other patients progression of disability was the reason for starting treatment. The numbers were too low to evaluate the effect of immunomodulating therapy on the time to progression of disability.

In patients with relapsing remitting MS MSSS scores at last follow-up were higher in patients who had used immunomodulating therapy compared to patients that had not (mean 4.5 and 3.0 respectively, $p = 0.009$). This difference was not found for patients with progressive MS.

Discussion

In relapsing remitting patients a higher T2 lesion load and number increased the risk for earlier and faster progression of disability. In contrast, we found no relationship between the amount of T2 lesions and rate of progression of disability in patients with progressive forms of MS.

T2 lesions represent a diverse pathological substrate, ranging from blood-brain barrier breakdown and inflammatory demyelination to fibrillary astrocytosis and remyelination.[21] T2 lesions are considered to result from inflammatory disease activity, whereas progression in progressive forms of MS is caused by relentless axonal degeneration. The lack of a relation between T2 lesion load and number on progression of disability in the progressive phase indicates that this process of axonal degeneration is largely independent from the focal inflammatory disease activity. This is in agreement with several studies showing a lack of effect of immunomodulatory and immunosuppressive treatment on the progressive phase of the disease,[3] lack of an association between the number of clinical relapses and progression,[10] and a dissociation of axonal loss from lesion load in patients with early multiple sclerosis.[11]

The predictive value of T2 lesions for progression of disability in relapsing remitting patients is in agreement with follow-up studies of different cohorts of patients with clinically isolated syndromes showing a higher level of disability after 5 years, 13 years and 20 years of follow-up in patients with more T2 lesions at baseline MRI.[6,7,8] It also supports our finding that more T2 lesions in relapsing remitting MS are associated with a higher risk of becoming secondary progressive.[9] It is important to mention that the predictive value of T2 lesions for progression of disability in relapsing remitting MS is modest and not absolute.[6,22]

How can we explain that T2 lesions predict progression of disability in relapsing remitting MS but not in progressive MS? In RRMS progression of disability is mainly caused by axonal injury in the context of focal inflammatory demyelination. Assuming that every MS patient has a gradual progressive axonal degeneration and that every MS patient has some clinically silent inflammatory demyelination, decreased CNS reserve capacity due to more T2 lesions can lead to an earlier clinical expression of progression of disability.[9] Once patients have reached a critical threshold of axonal degeneration, they enter the progressive phase of the disease, and from that time the rate of subsequent progression is no longer influenced by the existing reduction in reserve capacity.[23]

The main strength of this study is the fact that we have a large cohort of patients with well-documented clinical follow-up. Since we used Cox-regression models we could include all patients with an available MRI-scan and at least one year of follow-up. Limitations of our study were the lack of follow-up MRI-scans, and the difference in slice thickness and MR field strength between the scans. The EDSS has many limitations, like a poor reproducibility and a tendency to measure mainly pyramidal dysfunction, especially in its higher ranges. Nevertheless, the EDSS is the most frequently used scale to assess disability and alternative scales are currently lacking.
The MSSS is a scale derived from the EDSS with a correction for disease duration. It is relatively unreliable in the first two years of the disease,[18] which might explain the lack of a relationship between the amount of T2 lesions and MSSS at time of scanning in relapsing remitting MS. The similar results of the time to progression of disability and MSSS analyses provide further support that the MSSS is suitable to measure rate of progression of disability in MS.

Studies with follow-up measurements of T2 lesion load should be performed to further investigate whether changes in the amount of T2 lesion load over time are associated with progression of disability in progressive MS.

Our study is of pathophysiological interest and may have practical implications with regard to counselling, in so far that the amount of T2 lesions is a prognostic factor for progression of disability in relapsing remitting MS, but seems to have no prognostic significance in the progressive forms of MS.

References

1. Compston A, Coles A. Multiple sclerosis. *Lancet*. 2008; 372; 1502-1517.
2. Weinshenker BG, Bass B, Rice GP et al. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. *Brain*. 1989; 112 (Pt 1); 133-146.
3. Rovaris M, Confavreux C, Furlan R et al. Secondary progressive multiple sclerosis: current knowledge and future challenges. *Lancet Neurol.* 2006; 5; 343-354.
4. Ramsaransing GS, De Keyser J. Benign course in multiple sclerosis: a review. *Acta Neurol.Scand.* 2006; 113; 359-369.
5. Ramsaransing GS, De Keyser J. Predictive value of clinical characteristics for 'benign' multiple sclerosis. *Eur.J.Neurol.* 2007; 14; 885-889.
6. Fisniku LK, Brex PA, Altmann DR et al. Disability and T2 MRI lesions: a 20-year follow-up of patients with relapse onset of multiple sclerosis. *Brain*. 2008; 131; 808-817.
7. Tintore M, Rovira A, Rio J et al. Baseline MRI predicts future attacks and disability in clinically isolated syndromes. *Neurology*. 2006; 67; 968-972.
8. Rudick RA, Lee JC, Simon J et al. Significance of T2 lesions in multiple sclerosis: A 13-year longitudinal study. *Ann.Neurol.* 2006; 60; 236-242.
9. Mostert JP, de Groot JC, Ramsaransing GS et al. Relationship between the extent of T2 lesions and the onset of secondary progression in multiple sclerosis. *Eur.J.Neurol.* 2007; 14; 1210-1215.
10. Confavreux C, Vukusic S, Moreau T et al. Relapses and progression of disability in multiple sclerosis. *N.Engl.J.Med.* 2000; 343; 1430-1438.
11. Pascual AM, Martinez-Bisbal MC, Bosca I et al. Axonal loss is progressive and partly dissociated from lesion load in early multiple sclerosis. *Neurology*. 2007; 69; 63-67.
12. Deluca GC, Williams K, Evangelou N et al. The contribution of demyelination to axonal loss in multiple sclerosis. *Brain*. 2006;
13. Bergers E, Bot JC, De Groot CJ et al. Axonal damage in the spinal cord of MS patients occurs largely independent of T2 MRI lesions. *Neurology*. 2002; 59; 1766-1771.
14. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. 1983; 33; 1444-1452.
15. Placebo-controlled multicentre randomised trial of interferon beta-1b in treatment of secondary progressive multiple sclerosis. European Study Group on interferon beta-1b in secondary progressive MS. *Lancet*. 1998; 352; 1491-1497.

16. Hommes OR, Sorensen PS, Fazekas F et al. Intravenous immunoglobulin in secondary progressive multiple sclerosis: randomised placebo-controlled trial. *Lancet*. 2004; 364; 1149-1156.
17. Panitch H, Miller A, Paty D et al. Interferon beta-1b in secondary progressive MS: results from a 3-year controlled study. *Neurology*. 2004; 63; 1788-1795.
18. Roxburgh RH, Seaman SR, Masterman T et al. Multiple Sclerosis Severity Score: using disability and disease duration to rate disease severity. *Neurology*. 2005; 64; 1144-1151.
19. Zivadinov R, Bakshi R. Role of MRI in multiple sclerosis I: inflammation and lesions. *Front Biosci.* 2004; 9; 665-683.
20. de Leeuw FE, de Groot JC, Achten E et al. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *J.Neurol.Neurosurg.Psychiatry*. 2001; 70; 9-14.
21. Guttmann CR, Ahn SS, Hsu L et al. The evolution of multiple sclerosis lesions on serial MR. *AJNR Am.J.Neuroradiol.* 1995; 16; 1481-1491.
22. Filippi M. Predictive value of MRI findings in multiple sclerosis. *Lancet Neurol.* 2002; 1; 9.
23. Bjartmar C, Wujek JR, Trapp BD. Axonal loss in the pathology of MS: consequences for understanding the progressive phase of the disease. *J.Neurol.Sci.* 2003; 206; 165-171.

Part 3

^{1}H-MRS studies

Chapter 7

^{1}H Magnetic resonance spectroscopy of the internal capsule in human brain: a feasibility study to detect lactate following contralateral motor activity

J. P. Mostert[1], P.E. Sijens[2], M. Oudkerk[2], J. De Keyser[1]

Authors' affiliations:
[1] Department of Neurology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands
[2] Department of Radiology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands

European Radiology
2005 Jul;15(7):1349-52.

Abstract

Animal experiments suggest that astrocytic glycogen may act as an energy source for axons especially during heightened activity. In this model astrocytic glycogen breaks down to lactate that is shuttled to axons where it is metabolised oxidatively to generate ATP. The aim of this study was to investigate whether ^{1}H-magnetic resonance spectroscopy could be used to detect a rise in lactate levels in human white matter during enhanced axonal activation. Six healthy volunteers (4 women and 2 men; age range 21-38 years) participated in the study. We were unable to detect any significant MR spectral change, i.e. neither in the peak areas of inositol, choline, creatine, glutamate and N-acetylaspartate nor in the lactate level, in the contralateral posterior limb of the internal capsule during intense motor activation of the hand (4 successive episodes of squeezing a soft ball for 7 minutes followed by 7 min rest). Possible explanations are that the technique is not sensitive enough to detect a small rise in lactate, or lactate turnover is too fast to be detected, or that another monocarboxylate different from lactate may be involved in axonal energy metabolism.

Introduction

Lactate produced by the breakdown of glycogen in astrocytes plays an important role in energy metabolism of axons, especially during increased physiological activity.[1,2] With in vivo ^{1}H magnetic resonance spectroscopy (MRS) of the human brain we have detected lactate in pathological conditions such as cancer.[3] With exception of a phenomenon not uncommon with aging beyond 60 years,[4] healthy persons do not have brain tissue lactate levels beyond a level of 0.5 mM, generally considered to be the MRS detection limit. A number of studies with magnetic resonance spectroscopy (MRS) in human volunteers claim to have detected transient increases in lactate levels in the relevant brain areas following either motor, visual, auditory or cognitive stimulation.[5,6,7,8,9,10,11,12]

Because axons can extend over great distances from their cell bodies, they depend on local production of ATP to maintain ion gradients and sustain other energy-consuming functions, such as axonal transport. Along axons in the white matter, astrocytes extend processes that directly abut nodes of Ranvier where axonal energy metabolism primarily takes place.[13] Astrocytic glycogen is converted to glucose phosphate, and then via pyruvate to lactate, which is released in the extracellular space. In this model, the astrocyte to neuron lactate shuttle hypothesis based on studies of murine cells,[14] astrocytes produce lactate in response to synaptically released glutamate. Lactate can be taken up by neurons through monocarboxylate transporters and then converted to pyruvate. Pyruvate enters the mitochondria where it is metabolized via the oxidative metabolism to generate ATP.[1,2] The purpose of this investigation was to use ^{1}H MRS to evaluate motor stimulus-dependent changes in lactate level, and in the other MRS detectable metabolites (inositol, choline, creatine, glutamate and N-acetylaspartate), in human white matter.

Methods

Six right-handed healthy volunteers (4 women and 2 men; age range 21-38 years) participated. The ethical committee of the Academic Hospital Groningen approved the project and all subjects signed an informed consent paper before participation. All scans were performed at 1.5 Tesla. Automated hybrid PRESS (point resolved spectroscopy) 2D-chemical shift imaging (CSI) measurements with a repetition time (RT) of 1500 ms and an echo time of 135 ms were performed. Hybrid-CSI includes pre-selection of a volume of interest (VOI) that is located within the brain to prevent the strong interference from subcutaneous fat and is smaller than the phase-encode field of view (FOV) that must be large enough to prevent wraparound artefacts.[15]

The CSI sequence produced a 16x16 transversely oriented matrix that was defined by phase encoding with a FOV of varying dimensions to allow for optimal measurement in the posterior limb of the internal capsule (Figure 1A). The field homogeneity achieved in automated non-

localized multiple angle projection (MAP) shimming resulted in water peak line widths of less than 8 Hz in the VOI. Excitation with with 2.56 ms sinc-Hanning shaped radio frequency (RF) pulses preceded by 25.6 ms Gaussian shaped RF pulses for chemical shift selective excitation (CHESS) and subsequent spoiling of the resultant water signal, was followed by collection of the second spin echo using 1024 data points and a spectral width of 500 Hz. All 16x16 2D-CSI measurements were 1 acquisition per phase encoded step with 4 pre-scans and RTs of 1500 ms (acquisition time 7 min). Time domain data were multiplied with a Gaussian function (center 0 ms, half width 256 ms), 2D-Fourier transformed, phase and baseline corrected and quantified by means of frequency domain curve fitting with the assumption of Gaussian line shapes, using the standard "Numaris-3" software package provided with the MR system. Sixth order polinomial lines with a 0-4.3 ppm calculation range were used for baseline correction. In the curve fitting the number of peaks fitted included the chemical shift ranges restricted to 3.4-3.6 ppm for inositol, 3.1-3.3 ppm for choline (Cho), 2.9-3.1 for creatine (Cr), 2.2-2.4 for glutamate (Glu), 1.9-2.1 for N-acetylaspartate (NAA), and 1.2-1.5 ppm for lactate, and their line widths and peak intensities unrestricted. We acknowledge that inositol and Glu are best detected at TE's shorter than the 135 ms used here and that in our case one might fail to detect a small change in the level of these particular metabolites. Part of our TE-135 ms CSI measurements was therefore repeated at TE 40 ms. Using standard post-processing protocols the raw data were thus processed automatically, allowing for operator-independent quantifications.
In all subjects a T2 weighted MRI series was used as guidance for defining the volume of interest for MRS. The VOI typically was a transversely oriented volume of approximately 8x8x2 cm3, phase encoded into 64 voxels of 2 cm3 each, extending over both hemispheres and including 24 voxels containing caudate nucleus, posterior limb of the internal capsule, putamen and thalamus (Figure 1C and D). The MRS measurements were performed at rest (7 min) and subsequently while the subject was squeezing a soft ball at a frequency of 1/1.5sec with the dominant right hand for 7 min. A total of 4 successive measurements at rest and during activation were performed. The investigator verified that hand squeezing was performed correctly.

Results

A representative example of MRS measurements is shown in Figure 1B. No lactate was detected either in rest or during right hand motor stimulation in any of the 12 voxels shown in Figure 1C and D. No significant differences in other metabolites (inositol, Glu, NAA, Cr, Cho) were found between the resting state and during motor activation. Upon repeating part of our TE-135 ms CSI measurements at TE 40 ms we still failed to detect any lactate or any change in the peak areas of inositol and Glu, compounds with comparatively short T2's and thus more accurately quantified at shorter TE, during motor activation. Table 1 illustrates that at motoric activation the levels of Cr and NAA are not affected. The trend of

comparative Cho decrease on the contralateral (=left) side is not significant, neither is any change in any MRS voxel when considered separately.

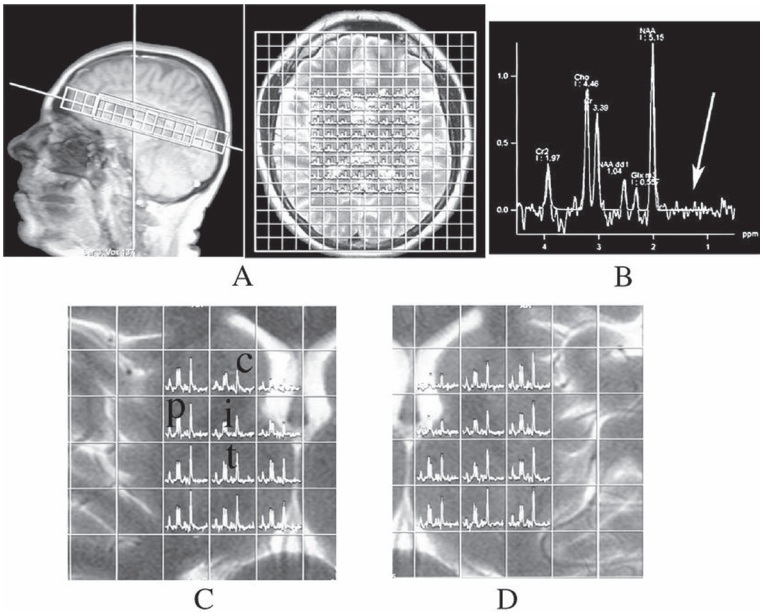

Figure 1 ^{1}H-magnetic resonance spectroscopy. CSI performed at TE=135ms yields a map of 64 spectra (A) showing peaks of Cho, Cr, Glu and NAA (B). The white arrow points to the spectrum where lactate should appear. Only some noice is seen. Below are 12 spectra containing caudate nucleus (c), posterior limb of the internal capsule (i), putamen (p), and thalamus (t), of the left (C) and right (D) hemisphere.

Table 1 Metabolite peak areas at right hand motor activation, in % of the areas at rest ±SEM, in two volumes in the left and right brain hemisphere containing capsula/caudate nucleus/putamen.

	Cho	Cr	NAA	Lactate
Left (sum of 12 voxels=24 ml)	98±5	100±7	99±7	_____*
Right (sum of 12 voxels=24 ml	103±4	100±5	99±7	_____*
Total VOI (64 voxels)	103±4	100±5	99±5	_____*

*Not detected

Discussion

There are a number of possible explanations why we were unable to detect an activation-induced increase in lactate levels in the corticospinal tract at the level of the posterior limb of the internal capsule. First, the theory concerning lactate as energy resource for axons may be wrong. Brown and colleagues found that intense neural activity reduced glycogen content in adult mouse optic nerve, a prototypic white matter preparation.[1] Axon function was quantified by measuring the compound action potential (CAP) area. The CAP declined more rapidly during high frequency stimulation if monocarboxylate transport out of astrocytes or into axons was inhibited. Although most probably lactate, the exact nature of the monocarboxyate has not been demonstrated with certainty. Thus, another monocarboxyate might be involved. Second, lactate buildup is not a necessary condition of the astrocyte to neuron lactate shuttle hypothesis. In fact the key event leading to glycogen breakdown may be a fall in baseline lactate precipitated by increased axonal lactate uptake as local glucose is depleted. This event may be 'sensed' by neighboring astrocytes which would then increase their lactate export through glycogen breakdown. The sum of these shifts might not increase measurable lactate concentration. Third, the motor stimulus may have been inadequate. This may be true, although in our experiment the motor activity was much stronger than in the previous MRS study of motor activation by Kuwabari and co-workers who reported a rise in lactate in the basal ganglia (putamen, globus pallidus) following finger opposition movements.[6] Fourth, lactate produced by astrocytes is readily converted into pyruvate and oxidatively metabolised and cannot be detected because it does not accumulate.

To our knowledge the above mentioned study of Kuwabari is the only published MRS study of *motoric* activation. The claim of lactate increases in the basal ganglia brain region following finger opposition movements is not reproduced in our study in which a much stronger stimulus was applied. Our failure to detect true lactate does not reflect inadequate sensitivity of the MRI equipment used in this study; to the contrary, in terms of signal–to-noise ratio and resolution the spectra shown here (Fig.1) are not inferior to those published by others. Considering the appearance of the "Lactate" in the spectrum shown in reference 6, it appears likely that artefacts, such as out-of-phase lipid signals, were erroneously interpreted as lactate. In a recent paper we have shown how out of phase lipid signals from outside the VOI are easily mistaken for lactate.[16] An other explanation for the discrepancy is partial volue effect due to the substantial ventricular space included in the large MRS voxels measured by Kuwabara et al[6]; whereas in normal brain tissue the lactate levels are in the order of 0.3 mM and up to a couple of mMs in severe hypoxia or pathology, in cerebrospinal fluid the lactate level can easily be even higher as well as more visible in the MR spectrum because of a longer T2 relaxation time.[17] Although in our study we zoomed in on the motoric activity brain areas with a spatial resolution of 2 cm^2, this may still have

been too crude for being able to detect the metabolism in the corticospinal tracts involved. A (maybe still optimistic) filling of the relevant MRS voxels by 10% with the corticospinal tracts involved with hand movement would require a tract lactate level of 10 x 0.5 mM = 5 mM to yield a clear lactate level in the MR spectrum.

The trend of comparative contralateral (= left side) Cho decrease upon (right hand) motoric stimulation, though not significant and unexplained, resembles the trends of Cho decrease observed recently in occipital brain tissue upon optic stimulation.[16]

In conclusion, MRS of the internal capsule in humans is not a suitable method for studying the so-called lactate shuttle from astrocytes to axons during physiologic activation of the corticospinal tract. With the use of higher field MRS eqiupment (3T or higher) it might be possible to detect any subtle changes in lactate level that might happen in the concentration range up to 0.5 mM (or higher if one takes partial volume effects into account).

References

1. Brown AM, Tekkok SB, Ransom BR. Glycogen regulation and functional role in mouse white matter. *J.Physiol*. 2003; 549; 501-512.
2. Wender R, Brown AM, Fern R et al. Astrocytic glycogen influences axon function and survival during glucose deprivation in central white matter. *J.Neurosci.* 2000; 20; 6804-6810.
3. Sijens PE, Oudkerk M. 1H chemical shift imaging characterization of human brain tumor and edema. *Eur.Radiol.* 2002; 12; 2056-2061.
4. Sijens PE, den HT, de Leeuw FE et al. MR spectroscopy detection of lactate and lipid signals in the brains of healthy elderly people. *Eur.Radiol.* 2001; 11; 1495-1501.
5. Kuwabara T, Watanabe H, Tanaka K et al. Mitochondrial encephalomyopathy: elevated visual cortex lactate unresponsive to photic stimulation--a localized 1H-MRS study. *Neurology*. 1994; 44; 557-559.
6. Kuwabara T, Watanabe H, Tsuji S et al. Lactate rise in the basal ganglia accompanying finger movements: a localized 1H-MRS study. *Brain Res.* 1995; 670; 326-328.
7. Frahm J, Kruger G, Merboldt KD et al. Dynamic uncoupling and recoupling of perfusion and oxidative metabolism during focal brain activation in man. *Magn Reson.Med.* 1996; 35; 143-148.
8. Prichard J, Rothman D, Novotny E et al. Lactate rise detected by 1H NMR in human visual cortex during physiologic stimulation. *Proc.Natl.Acad.Sci.U.S.A*. 1991; 88; 5829-5831.
9. Richards TL, Corina D, Serafini S et al. Effects of a phonologically driven treatment for dyslexia on lactate levels measured by proton MR spectroscopic imaging. *AJNR Am.J.Neuroradiol.* 2000; 21; 916-922.
10. Richards TL, Gates GA, Gardner JC et al. Functional MR spectroscopy of the auditory cortex in healthy subjects and patients with sudden hearing loss. *AJNR Am.J.Neuroradiol.* 1997; 18; 611-620.
11. Sappey-Marinier D, Calabrese G, Fein G et al. Effect of photic stimulation on human visual cortex lactate and phosphates using 1H and 31P magnetic resonance spectroscopy. *J.Cereb.Blood Flow Metab*. 1992; 12; 584-592.
12. Urrila AS, Hakkarainen A, Heikkinen S et al. Metabolic imaging of human cognition: an fMRI/1H-MRS study of brain lactate response to silent word generation. *J.Cereb.Blood Flow Metab*. 2003; 23; 942-948.
13. Black JA, Waxman SG. The perinodal astrocyte. *Glia*. 1988; 1; 169-183.
14. Magistretti PJ. Cellular bases of functional brain imaging: insights from neuron-glia metabolic imaging. *Brain Res.* 2000; 886; 108-112.

15. Sijens PE, van den Bent MJ, Nowak PJ et al. 1H chemical shift imaging reveals loss of brain tumor choline signal after administration of Gd-contrast. *Magn Reson.Med.* 1997; 37; 222-225.
16. Boucard CC, Mostert JP, Cornelissen FW et al. Visual stimulation, 1H MR spectroscopy and fMRI of the human visual pathways. *Eur.Radiol.* 2005; 15; 47-52.
17. Braun KPJ, Gooskens RHJM, Vandertop PW et al. ^{1}H magnetic resonance spectroscopy in human hydrocephalus. *J Magn Reson Imaging.* 2003; 17; 291-299.

Chapter 8

Reproducibility over a one month period of ^{1}H-MR spectroscopic imaging NAA/Cr ratios in clinically stable multiple sclerosis patients

JP Mostert[1], Y Blaauw[1], MW Koch[1], AJ Kuiper[2], JM Hoogduin[2], J De Keyser[1]

Authors' affiliations:
[1] Department of Neurology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands
[2] NeuroImaging Centre, University of Groningen, Groningen, The Netherlands

European Radiology
2008 Aug;18(8):1736-40.

Abstract

N-acetylaspartate / creatine (NAA/Cr) ratios, assessed with proton magnetic resonance spectroscopy, are increasingly used as a surrogate marker for axonal dysfunction and degeneration in multiple sclerosis (MS). The purpose of this study was to test short-time reproducibility of NAA/Cr ratios in patients with clinically stable MS.

In 35 MS patients we analysed NAA/Cr ratios obtained with ^{1}H-MR spectroscopic imaging at the centrum semiovale either with lateral ventricles partially included (group 1; n=15) or more cranially with no ventricles included (group 2; n=20). To test short-term reproducibility of the NAA/Cr measurements, patients were scanned twice 4 weeks apart. We determined mean NAA/Cr and Cho/Cr ratios of 12 grey matter and 24 white matter voxels.

Mean NAA/Cr ratios of both the white and grey matter did not change after 4 weeks. Overall four week reproducibility of the NAA/Cr ratio, expressed as coefficient of variation, was 4.8 % for grey matter and 3.5 % for white matter. Reproducibility of scanning cranially of the ventricles was slightly better than with cerebrospinal fluid included.

Our study shows good short-term reproducibility of NAA/Cr ratio measurements in the centrum semiovale, which supports the reliability of this technique for longitudinal studies.

Introduction

Multiple sclerosis (MS) is a chronic disorder of the central nervous system with inflammatory demyelination and axonal degeneration.[1] The disease usually starts between the ages of 20 and 40 years, and by the age of 55 years, about half of the patients needs unilateral assistance to walk.[2] The disease progresses slowly and clinical scales currently used are not sensitive enough to quantify progression.[3] Using surrogate markers of progression can shorten the duration of clinical trials in progressive MS.

^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) of the brain in MS patients can detect changes in cell metabolites in both focal lesions and normal appearing white matter (NAWM).[4] Compared to controls, N-acetylaspartate (NAA) concentrations in MS patients are lower both in the lesions and in NAWM, whereas creatine (Cr) concentrations are equal to slightly increased.[5] NAA is a marker of neuroaxonal metabolism and integrity, and reductions in the white matter are due to axonal dysfunction or loss. It has been suggested that whole brain NAA decline rates in patients with MS may predict future disease course.[6] Cr is present in neurons, astrocytes and oligodendrocytes, and elevations in white matter of MS patients can be due to axonal transection, astrocytic proliferation, and demyelination. Because absolute concentrations of metabolites with ^{1}H-MRS are difficult to interpret, the NAA/Cr ratio is often used as a valid surrogate marker of 'cerebral tissue integrity'.[5] Decreases in NAA/Cr, which in MS patients are found in lesions and NAWM, are indicative of neuroaxonal disturbance, oligodendroglial disturbance, or astrocytic proliferation. The third metabolite well observable in long TE ^{1}H-MRS is Choline (Cho) which is regarded as a marker of glial proliferation.[7]

Next to validity, good surrogate markers need to be reliable, especially when used for monitoring white matter integrity in longitudinal studies. In this study we investigated the reproducibility of NAA/Cr ratios obtained with 2D spectroscopic imaging at two different levels of the centrum semiovale within a time span of 4 weeks in patients with clinically stable MS.

Methods

Patients

All patients co-operated in the study with informed consent and local medical ethical committee approval. Thirty-five patients participated in the study. Patients were randomly divided in two groups. All patients had clinically definitive MS with a relapsing remitting or secondary progressive disease course.[8,9] None of the patients were using immunomodulatory medication. Patients were relapse free for at least 4 weeks and had not

taken corticosteroids 4 weeks prior to the first MRI and during the study period. Disability was assessed with the Expanded Disability Status Scale (EDSS), which gives a score ranging from 0 to 10 with higher scores indicating more disability.[10]

^{1}H-MR Spectroscopy

MRI scans of the brain were obtained at baseline and after 4 weeks at a 3.0 Tesla unit (Philips, Best, The Netherlands). ^{1}H-MR spectroscopic imaging was preceded by the acquisition of a transverse multiple slice MRI series in order to position the volume of interest (VOI). The standard transmitter/receive coil was used and the volume localisation was done by a combination of slice selection and 2D phase encoding with outer volume suppression with 12 rest slabs. All scan parameters were the same: TE=144 ms, TR=2.5s, FOV=230mm, slice thickness=20mm, matrix= 24x24, turbo factor=3. Total scan time was around 8 minutes.

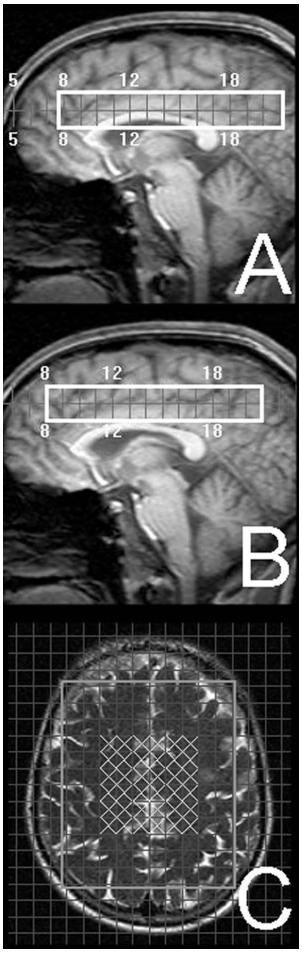

Figure 1 Sagittal MRI image showing the volume of interest scannedin grop 1 (A) and group 2 (B) with an axial image (C) displaying the grey (inner 12) and white matter (outer 12 in both hemispheres) voxels.

Group 1 was scanned around the lateral ventricles with some cerebrospinal fluid included in the VOI (figure 1A). Group 2 was scanned above the lateral ventricles with no cerebrospinal fluid included in VOI (figure 1B). Individual anatomic landmarks were used for voxel repositioning.
To select voxels that mainly contained grey matter 12 spectroscopic imaging (SI) voxels were selected in the midline grey matter in each subject (figure 1C). To select voxels that mainly contained white matter 24 SI voxels were selected (12 in each hemisphere) in the deep white matter in each subject (figure 1C). Metabolite peak areas of NAA and Cho were determined with the manufacturers software (Philips SPECTROVIEW) and are expressed as ratios to Cr. All voxels were visually checked for quality of the spectra and quality of the fitting of the peak areas and rejected if inaccurate according to the opinion of the two observers.
In every patient the NAA/Cr and Cho/Cr ratios per voxel were determined and average ratios of the voxels in grey and white matter were calculated.

Statistical analysis
Reproducibility was tested by a coefficient of variation (CV = 100 * standard deviation of the mean difference / [√2 * pooled mean values]).[11] Changes in grey matter and white matter after 4 weeks and differences between baseline grey and white matter NAA/Cr ratios in patients were tested with paired t-tests. Differences between baseline grey matter and white matter NAA/Cr ratios of the 2 VOIs were tested with unpaired t-tests.

Results

Baseline characteristics of the patients are shown in table 1.

Table 1 Characteristics of the patients.

	All	Group 1	Group 2
Number	35	15	20
Gender: Male	16 (46%)	8 (53%)	8 (40%)
Disease course: Relapsing Remitting / Secondary Progressive	31 / 4	13 / 2	18 / 2
Age: mean (sd) in years	40 (9)	43 (10)	37 (8)
Disease Duration: mean (sd) in years	11 (7)	12 (9)	11 (6)
EDSS: median (IQR)	3.0 (1.5 – 4.0)	3.0 (1.5 – 4.5)	3.0 (2.0 – 4.0)

sd = standard deviation; IQR = interquartile range

Patients were scanned after a mean ± standard deviation of 27.3 ± 2.2 days. The mean number of excluded voxels was 1.51 ± 2.93 for NAA/Cr and 4.54 ± 5.40 for Cho/Cr (out of 36 voxels). White matter NAA/Cr and Cho/Cr ratios were higher than grey matter ratios (table 2). The mean NAA/Cr did not change in either white matter or grey matter in both groups after 4 weeks (table 2).

Table 2 The NAA/Cr ratios in the centrum semiovale at week 0 and week 4: Mean (sd).

		Week 0	Week 4	p- value*
All	NAA/Cr white matter	2.27 (0.26)	2.26 (0.25)	0.60
(N=35)	NAA/Cr grey matter	1.90 (0.23)#	1.88 (0.21)	0.42
	Cho/Cr white matter	1.05 (0.13)	1.03 (0.15)	0.11
	Cho/Cr grey matter	0.98 (0.12)#	0.98 (0.13)	0.60
Group 1	NAA/Cr white matter	2.45 (0.26)	2.45 (0.25)	0.82
(N=15)	NAA/Cr grey matter	2.08 (0.23)#	2.03 (0.20)	0.30
Group 2	NAA/Cr white matter	2.14 (0.15)	2.13 (0.14)	0.53
(N=20)	NAA/Cr grey matter:	1.76 (0.11)#	1.77 (0.12)	0.87

* p- value: comparison week 0 with week 4

# $p < 0.001$: comparison grey matter with white matter

sd = standard deviation

The NAA/Cr ratios in grey and white matter were significantly higher in the group scanned with the ventricles partially included ($p < 0.001$). Mean Cho/Cr in white and grey matter was 1.14 and 1.07 in group 1 and 0.98 and 0.92 in group 2 ($p<0.001$). Overall the CV for NAA/Cr and Cho/Cr was 3.5% and 4.1% for white matter and 4.8% and 5.2% for grey matter. In group 1 the CV of NAA/Cr in white matter and grey matter were 4.3 % and 5.7 %, and in group 2 2.5 % and 3.7 %.

Discussion

Several small clinical trials in MS patients have used NAA/Cr ratios at the centrum semiovale as surrogate outcome measure.[12,13,14,15,16] Narayanan et al reported an increase in NAA/Cr in 10 relapsing MS patients after being treated with interferon β for 12 months.[12] Khan et al. reported an elevation of NAA/Cr in 18 patients in a large central brain volume after 2 years of treatment with glatiramer acetate.[15] An increase in NAA/Cr at the centrum semiovale in 11 MS patients after 2 weeks of treatment with fluoxetine was observed.[16] These studies suggest beneficial effects of therapy on axonal functioning or integrity. However, interpretation of the results is hampered by a lack of studies assessing reproducibility of NAA/Cr measurements in MS patients.

One study in 5 healthy subjects, assessing intraday and interday reproducibility of NAA/Cr ratios in the centrum semiovale found CV's ranging between 5.1% and 7.2%.[17] Another study found a mean CV of 3.7 % in 6 healthy subjects scanned twice, within 15 days apart, at a large volume of supratentorial brain.[18] Our study for the first time shows good short-term (4-week apart) reproducibility of NAA/Cr ratio measurements in the centrum semiovale of patients with MS.

In agreement with previous studies, NAA/Cr ratios in grey matter were lower than in white matter.[19,20] Patients scanned cranially of the ventricles had lower NAA/Cr and Cho/Cr ratios compared to patients scanned with the ventricles partially included. Since the corpus callosum has the highest white matter NAA/Cr and Cho/Cr ratios, the larger area of the corpus callosum included in the VOI when scanning more caudally might explain this difference.[21] This underlines the importance of correct positioning of the VOI and voxel selection in longitudinal MRS studies.

Our study indicates that NAA/Cr ratio measurements with ^{1}H-MRS at the centrum semiovale can be used as a reliable surrogate marker of cerebral tissue integrity in longitudinal MS studies. Selecting the VOI above the ventricles is preferred above placing the VOI partially in the ventricles.

References

1. Compston A, Coles A. Multiple sclerosis. *Lancet*. 2002; 359; 1221-1231.
2. Confavreux C, Vukusic S. Age at disability milestones in multiple sclerosis. *Brain*. 2006; 129; 595-605.
3. Filippi M, Rocca MA, Comi G. The use of quantitative magnetic-resonance-based techniques to monitor the evolution of multiple sclerosis. *Lancet Neurol.* 2003; 2; 337-346.
4. Filippi M, Rocca MA, Comi G. The use of quantitative magnetic-resonance-based techniques to monitor the evolution of multiple sclerosis. *Lancet Neurol.* 2003; 2; 337-346.
5. Caramanos Z, Narayanan S, Arnold DL. 1H-MRS quantification of tNA and tCr in patients with multiple sclerosis: a meta-analytic review. *Brain*. 2005; 128; 2483-2506.
6. Gonen O, Oberndorfer TA, Inglese M et al. Reproducibility of three whole-brain N-acetylaspartate decline cohorts in relapsing-remitting multiple sclerosis. *AJNR Am.J.Neuroradiol.* 2007; 28; 267-271.
7. Chard DT, Griffin CM, McLean MA et al. Brain metabolite changes in cortical grey and normal-appearing white matter in clinically early relapsing-remitting multiple sclerosis. *Brain*. 2002; 125; 2342-2352.
8. McDonald WI, Compston A, Edan G et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann.Neurol.* 2001; 50; 121-127.
9. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology*. 1996; 46; 907-911.
10. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. 1983; 33; 1444-1452.
11. van der Worp HB, Claus SP, Bar PR et al. Reproducibility of measurements of cerebral infarct volume on CT scans. *Stroke*. 2001; 32; 424-430.
12. Narayanan S, De SN, Francis GS et al. Axonal metabolic recovery in multiple sclerosis patients treated with interferon beta-1b. *J.Neurol.* 2001; 248; 979-986.
13. Parry A, Corkill R, Blamire AM et al. Beta-Interferon treatment does not always slow the progression of axonal injury in multiple sclerosis. *J.Neurol.* 2003; 250; 171-178.
14. Schubert F, Seifert F, Elster C et al. Serial 1H-MRS in relapsing-remitting multiple sclerosis: effects of interferon-beta therapy on absolute metabolite concentrations. *MAGMA*. 2002; 14; 213-222.

15. Khan O, Shen Y, Caon C et al. Axonal metabolic recovery and potential neuroprotective effect of glatiramer acetate in relapsing-remitting multiple sclerosis. *Mult.Scler.* 2005; 11; 646-651.
16. Mostert JP, Sijens PE, Oudkerk M et al. Fluoxetine increases cerebral white matter NAA/Cr ratio in patients with multiple sclerosis. *Neurosci.Lett.* 2006; 402; 22-24.
17. Rotondo E, Bruschetta G, Sacca A et al. Straightforward relative quantitation and age-related human standards of N-acetylaspartate at the centrum semiovale level by CSI (1)H-MRS. *Magn Reson.Imaging*. 2003; 21; 1055-1060.
18. Pelletier D, Nelson SJ, Grenier D et al. 3-D echo planar (1)HMRS imaging in MS: metabolite comparison from supratentorial vs. central brain. *Magn Reson.Imaging*. 2002; 20; 599-606.
19. Safriel Y, Pol-Rodriguez M, Novotny EJ et al. Reference values for long echo time MR spectroscopy in healthy adults. *AJNR Am.J.Neuroradiol.* 2005; 26; 1439-1445.
20. Lundbom N, Barnett A, Bonavita S et al. MR image segmentation and tissue metabolite contrast in 1H spectroscopic imaging of normal and aging brain. *Magn Reson.Med.* 1999; 41; 841-845.
21. Cecil KM, Hills EC, Sandel ME et al. Proton magnetic resonance spectroscopy for detection of axonal injury in the splenium of the corpus callosum of brain-injured patients. *J.Neurosurg.* 1998; 88; 795-801.

Chapter 9

Fluoxetine increases cerebral white matter NAA/Cr ratio in patients with multiple sclerosis

J.P. Mostert[1], P.E. Sijens[2], M. Oudkerk[2], J. De Keyser[1]

Authors' affiliations:
[1] Department of Neurology, University Medical Centre Groningen, University of Groningen, The Netherlands.
[2] Department of Radiology, University Medical Centre Groningen, University of Groningen, The Netherlands.

Neuroscience letters
2006 Jul 10;402(1-2):22-4.

Abstract

Axonal degeneration in multiple sclerosis (MS) may be caused by mitochondrial dysfunction and is associated with decreased levels of N-acetylaspartate (NAA) as measured with ^{1}H-magnetic resonance spectroscopy (MRS). Fluoxetine stimulates astrocytic glycogenolysis, which serves as an energy source for axons. Eleven patients with MS received fluoxetine orally 20 mg a day during the first week, and 40 mg a day during the second week. The mean NAA/Creatine ratio in cerebral white matter of the MS patients increased from 1.77 at baseline to 1.84 at the end of the second week ($p = 0.007$). These findings show evidence for a reversible axonal dysfunction in patients with MS and provide a rationale for investigating whether fluoxetine has neuroprotective effects in MS.

Introduction

Multiple sclerosis (MS) is a chronic disease of the central nervous system characterized by inflammation, demyelination and axonal degeneration. Current immunomodulatory therapies reduce inflammation but are unsuccessful in stopping the progressive axonal loss.[1] The pathogenesis of axonal degeneration is unclear but likely involves mitochondrial dysfunction and decreased energy supply.[2] Reduced ATP formation impairs the ATP-dependent ion-pumps, which results in accumulation of Ca^{2+} in axons. This Ca^{2+} overload will inappropriately stimulate a variety of Ca^{2+} -dependent enzyme systems (e.g., calpains, phospholipases), leading to axonal injury.[2,3] There is evidence from in vitro studies that glycogen in astrocytes represents a major energy source for axons.[4] The loss of beta-2 adrenergic receptors on astrocytes in MS may impair astrocytic glycogenolysis, and thus be responsible for reduced energy supply to the axons.[3] It has been shown that the selective serotonin re-uptake inhibitor fluoxetine stimulates glycogenolysis in cultured astrocytes.[5] In this study we used ^{1}H-Magnetic Resonance Spectroscopy (^{1}H-MRS) of the brain to assess the effects of fluoxetine administration in MS patients on the levels of N-acetyl-aspartate (NAA), which is produced in axonal mitochondria and reflects energy production.[6,7]

Methods

All patients co-operated in the study with informed consent and local medical ethical committee approval. Five man and six women (age range 31-58 years) with definite MS participated. Seven patients had relapsing remitting MS and four secondary progressive MS. None of them used immunomodulatory drugs. One patient had an exacerbation three weeks prior to start of the study, but the others were exacerbation-free for the last six months. Their Expanded Disability Status Scale (EDSS) ranged from 0 to 6 (median 2.5).
Patients received fluoxetine 20 mg once a day for seven days, followed by seven days of fluoxetine 20 mg twice a day. MRI scans of the brain were obtained at baseline, week 1 and week 2 on a 1.5 T unit (Magnetom Sonata; Siemens Medical Solutions, Erlangen, Germany). ^{1}H-MRS was preceded by the acquisition of a transverse multiple slice MRI series in order to position the volume of interest (VOI) containing 8 by 8 voxels of 2 cm³ each above the lateral ventricles. Individual anatomic landmarks were used for voxel repositioning. Automated hybrid chemical shift imaging with point resolved spectroscopy (PRESS; echo time 135 ms, repetition time 1500 ms) was used with chemical shift selective excitation (CHESS) water suppression.[8] Metabolite peak areas were analyzed bilaterally in the 12 voxels corresponding to the centrum semiovale and determined with Numaris software and expressed as ratios to the main creatine (Cr) signal (the 3.0 ppm peak).

We also assessed the Timed 25-foot Walk Test (TWT, the time it takes for the patient to walk 25 feet), and the Abbreviated Fatigue Questionnaire (AFQ), which consists of four questions that have to be answered on a 7-point Likert scale.[9] A higher score indicates a higher degree of subjective fatigue (range 4-28).
In the Statistical Package for the Social Sciences (SPSS 12.0 for Windows, Chicago, Illinois) the general linear model of repeated measures was used to evaluate the effects of fluoxetine on NAA/Cr ratio and Choline/Creatine (Cho/Cr) ratio. When the Wilks' Lambda was significant means were compared with paired samples t-tests. Paired t-tests were used to assess the effect of fluoxetine on TWT and AFQ. P-values < 0.05 were considered to be significant.

Results

The results of the effects on NAA/Cr and Cho/Cr are summarized in table 1.

Table 1 The effects of fluoxetine administration in MS patients on NAA/Cr and Cho/Cr.

	Baseline	Week 1	Week 2	P-value
NAA/Cr ratio	1.77	1.79	1.84	0.031
mean ± SD	± 0.183	± 0.178	± 0.203	
Cho/Cr ratio	1.06	1.07	1.06	0.841
mean ± SD	± 0.133	± 0.154	± 0.134	

NAA/Cr = N-acetyl-aspartate/Creatine, Cho/Cr = Choline/Creatine

The Cho/Cr ratio did not change (p=0.841). The mean NAA/Cr ratio in the cerebral white matter increased from 1.77 at baseline to 1.79 at week 1, and to 1.84 at week 2 (p=0.031). Comparison of means showed that the difference between week 0 and week 2 was significant (p=0.007, 95% CI of change: 0.02-0.11). All patients' scores of NAA/Cr, AFQ and TWT at baseline and week 2 are shown in table 2. Both AFQ and TWT improved after two weeks of fluoxetine administration, but this was not significant.

Table 2 The effect of 2 weeks fluoxetine administration on NAA/Cr, AFQ and TWT in eleven MS patients.

Patient Number	NAA/Cr Week 0	NAA/Cr Week 2	AFQ Week 0	AFQ Week 2	TWT (sec) Week 0	TWT (sec) Week 2
1	1.57	1.71	10	11	5.27	4.96
2	1.99	2.06	28	24	5.31	5.78
3	1.63	1.72	4	4	5.67	4.91
4	1.76	1.76	19	18	11.05	11.73
5	1.76	1.76	14	15	5.95	6.08
6	2.06	2.07	19	21	5.04	4.73
7	1.53	1.54	19	21	6.26	6.11
8	1.73	1.76	12	6	7.47	7.14
9	1.62	1.67	28	22	7.69	7.04
10	1.85	1.93	20	14	7.15	7.02
11	2.01	2.21	14	11	4.84	3.80
Mean	1.77	1.84*	17.00	15.18[#]	6.52	6.26[$]

Comparison week 0 with week 2: * p=0.007; [#] p= 0.096; [$] p=0.145

NAA/Cr = N-acetyl-aspartate/Creatine, AFQ = Abbreviated Fatigue Questionnaire, TWT = Timed 25-feet Walk Test, sec = seconds.

Discussion

In this study we showed that short-term fluoxetine administration significantly elevated the NAA/Cr ratio in cerebral white matter of patients with relapsing remitting and secondary progressive MS. This reflects increases in NAA levels, as Cr is assumed to be relatively stable,[10] which was confirmed by the stable Cho/Cr ratio in our patients. The method of measuring NAA/Cr displays good intra-individual reproducibility.[11] After one week of 20 mg fluoxetine administration NAA/Cr increased slightly, but with fluoxetine 40 mg given during the second week the elevation was significant, indicating a dose or time dependent response. Also fatigue, which was assessed as symptom of altered axonal metabolism, and walking improved but these changes did not reach significance. In MS, NAA concentrations in lesions as well as normal appearing white matter are lower than in controls,[12] indicating axonal loss or axonal metabolic dysfunction. NAA has been shown to be almost exclusively within neuronal cell bodies and axons, where it is synthesized by mitochondria. Its levels reflect the energetic state of neurons as shown, for example, by the concurrent decrease and recovery of NAA and ATP in an animal model of traumatic brain injury,[7] Further evidence relating NAA formation to mitochondrial energy production was obtained in studies with inhibitors of different complexes of the mitochondrial respiratory chain.[6] The underlying mechanism by which fluoxetine improves axonal mitochondrial

energy production needs to be further explored, but may be due to its stimulatory effect on astrocytic glycogenolysis.[5]

Fluoxetine stimulates glycogenolysis in astrocytes by directly activating 5-HT2 receptors, which induces hydrolysis of glycogen probably via the phosphoinositol second messenger system,[5] and by blocking serotonin re-uptake, leading to elevated extracellular concentrations of serotonin, which also stimulates glycogenolysis.[13] Besides the effect on glycogenolysis, fluoxetine induces the production of astrocytic brain-derived neurotrophic factor (BDNF), which may also improve axonal functions in MS patients.[14,15]

Limitations of our study are the lack of a control group, the heterogeneous patient group and the use of single slice MRS, which covers the brain only partially. A placebo-controlled long-term follow-up study evaluating the effects of fluoxetine on axonal degeneration is recommended. Brain atrophy, which is a prominent feature in MS patients, could be used as surrogate marker. This proof-of-concept study shows that fluoxetine might be able to improve the metabolic function of axons in MS. Further research is necessary to evaluate whether fluoxetine has neuroprotective properties in MS.

References

1. Compston A, Coles A. Multiple sclerosis. *Lancet*. 2002; 359; 1221-1231.
2. Stys PK. General mechanisms of axonal damage and its prevention. *J.Neurol.Sci.* 2005; 233; 3-13.
3. De Keyser J, Zeinstra E, Mostert J et al. Beta 2-adrenoceptor involvement in inflammatory demyelination and axonal degeneration in multiple sclerosis. *Trends Pharmacol.Sci.* 2004; 25; 67-71.
4. Brown AM, Tekkok SB, Ransom BR. Glycogen regulation and functional role in mouse white matter. *J.Physiol*. 2003; 549; 501-512.
5. Kong EK, Peng L, Chen Y et al. Up-regulation of 5-HT2B receptor density and receptor-mediated glycogenolysis in mouse astrocytes by long-term fluoxetine administration. *Neurochem.Res.* 2002; 27; 113-120.
6. Bates TE, Strangward M, Keelan J et al. Inhibition of N-acetylaspartate production: implications for 1H MRS studies in vivo. *Neuroreport*. 1996; 7; 1397-1400.
7. Signoretti S, Marmarou A, Tavazzi B et al. N-Acetylaspartate reduction as a measure of injury severity and mitochondrial dysfunction following diffuse traumatic brain injury. *J.Neurotrauma*. 2001; 18; 977-991.
8. Sijens PE, van den Bent MJ, Nowak PJ et al. 1H chemical shift imaging reveals loss of brain tumor choline signal after administration of Gd-contrast. *Magn Reson.Med.* 1997; 37; 222-225.
9. Alberts M, Smets EM, Vercoulen JH et al. ['Abbreviated fatigue questionnaire': a practical tool in the classification of fatigue]. *Ned.Tijdschr.Geneeskd.* 1997; 141; 1526-1530.
10. De Stefano N, Narayanan S, Mortilla M et al. Imaging axonal damage in multiple sclerosis by means of MR spectroscopy. *Neurol.Sci.* 2000; 21; S883-S887.
11. Rotondo E, Bruschetta G, Sacca A et al. Straightforward relative quantitation and age-related human standards of N-acetylaspartate at the centrum semiovale level by CSI (1)H-MRS. *Magn Reson.Imaging*. 2003; 21; 1055-1060.
12. Filippi M, Rocca MA, Comi G. The use of quantitative magnetic-resonance-based techniques to monitor the evolution of multiple sclerosis. *Lancet Neurol.* 2003; 2; 337-346.
13. Cambray-Deakin M, Pearce B, Morrow C et al. Effects of neurotransmitters on astrocyte glycogen stores in vitro. *J.Neurochem.* 1988; 51; 1852-1857.
14. Mercier G, Lennon AM, Renouf B et al. MAP kinase activation by fluoxetine and its relation to gene expression in cultured rat astrocytes. *J.Mol.Neurosci.* 2004; 24; 207-216.

15. Stadelmann C, Kerschensteiner M, Misgeld T et al. BDNF and gp145trkB in multiple sclerosis brain lesions: neuroprotective interactions between immune and neuronal cells? *Brain*. 2002; 125; 75-85.

Chapter 10

Summary & Future perspectives

Multiple sclerosis is a chronic disorder of the central nervous system, characterized by inflammation and axonal degeneration. All current therapies modulate the peripheral immune system. Treatments for patients in the progressive phase of the disease, which is characterized by axonal degeneration, are lacking. Many MS researchers believe that early and strong suppression of inflammation prevents axonal degeneration. However, so far strong immunosuppressive treatments failed to stop disease progression.
In 2004 a hypothesis was presented in which the lack of the beta-2 adrenergic receptor on astrocytes of patients with MS might explain both the inflammatory responses and the progressive axonal degeneration. The loss of beta-2 adrenergic receptors on astrocytes reduces the formation of intracellular cAMP which 1) enables astrocytes to express interferon gamma induced MHC-2 and co-stimulatory B7 molecules, which turns astrocytes into antigen presenting cells, and 2) decreases astrocyte lactate production that is transported to axons as energy source.
If the hypothesis holds true, drugs that are able to elevate intracellular cAMP might be beneficial for patients with MS. These drugs might reduce both inflammation as axonal degeneration in MS. Since fluoxetine is able to increase cAMP signalling in astrocytes this drug was chosen to study in patients with MS.

The aim of this thesis was to study effects of fluoxetine in patients with MS. Since symptomatic disease activity in MS is infrequent and unpredictable, effects of medication are difficult to measure on clinical grounds alone. With MRI different pathophysiological features of MS can be visualized noninvasively, and disease activity can be easily monitored. We evaluated the use of MRI to monitor treatment and used a number of MRI techniques to study effects of fluoxetine in patients with MS.

In the first part of this thesis we reviewed the effect of fluoxetine on neurological disorders (Chapter 2). Fluoxetine elevates neurotrophic factors, increases glycogenolysis in astrocytes, and blocks voltage gated calcium and sodium channels, which all might prevent axonal loss. Although we found many studies evaluating the use of fluoxetine for depression in neurological disorders, no well-designed studies were available that looked at effects of fluoxetine on neurological function.

In the second part of this thesis we performed several studies using conventional MRI. In chapter 3 we showed in a double-blind, placebo-controlled study that MS patients using fluoxetine have a tendency to have less enhancing lesions on MRI, which is a surrogate marker for inflammation. Effects became more apparent after 8 weeks of treatment, which is in line with the time it takes for fluoxetine to reach stable plasma levels and to have antidepressant effects. Patients using fluoxetine also had less frequent MRI scans with enhancing lesions. These results must be confirmed in larger studies before fluoxetine can get an application in clinical practice. In chapter 4 different techniques to measure brain

atrophy were compared. Except for corpus callosum area all atrophy measurements were moderately to strongly correlated. Brain parenchymal fraction might be best to use in future MS studies. Next, we wondered what the long term relevance is of measuring T2 lesions and atrophy in patients with MS. To answer this question we re-evaluated all available cerebral MRI scans of MS patients that were performed before 1997 at our clinic. The clinical follow-up data of all patients are registered in the Groningen MS-database. In chapter 5 we showed that more T2 lesions are an independent predictor for the onset of secondary progression. This is supported by our findings in chapter 6, which showed that T2 lesions are predictive for earlier progression of disability in relapsing remitting MS. However, in primary and secondary progressive MS the number of T2 lesions doesn't have an influence on the progression of disability.

In the third part of our thesis we performed several studies with ^{1}H-MRS. We studied cerebral lactate formation in healthy persons after performing a motor task (chapter 7). Lactate is produced by astrocytes and transported to axons as fuel during intense neural activity. According to our hypothesis, the astrocytic beta-2 adrenergic receptor defect in MS would impair the production of lactate in astrocytes. However, we could not detect cerebral lactate in healthy persons and we concluded that this activation study is not appropriate to investigate the lactate metabolism in MS patients. In chapter 8 we looked at the reproducibility of N-acetylaspartate/Creatine (NAA/Cr) after 4 weeks in clinically stable MS patients. NAA/Cr is regarded as a marker of axonal function and may be a suitable marker for axonal degeneration. Good surrogate markers are valid and reproducible. We showed that NAA/Cr is well reproducible after 4 weeks. The use of NAA/Cr in MS trials can thus be recommended. In chapter 9, we described an increase in NAA/Cr after two weeks of treatment with fluoxetine in an open study of 11 patients with relapsing remitting and secondary progressive MS. This supports possible beneficial effects of fluoxetine on axonal function.

Future directions

Fluoxetine is cheap, world-wide available, taken orally, and safe to use long-term. This thesis provides a theoretical basis for the use of fluoxetine as disease modifying therapy in patients with MS. It contains two studies showing positive effects of fluoxetine on cerebral MRI measurements in patients with MS. Since these studies were small, had a maximum follow-up time of six months and did not use clinical endpoints, the prescription of fluoxetine as disease modifying therapy can yet not be advised. However, this thesis justifies and encourages further studies with fluoxetine in patients with MS.
In patients with relapses a new phase 2 study could show whether the trend towards improvement we observed is reproducible. It should have the cumulative number of enhancing lesions as primary outcome measure and focus on effects of fluoxetine after 8

weeks of treatment. The launch of a large, multi-center trial, evaluating the effect of fluoxetine on clinical exacerbations in MS, can also be considered. Since many patients with exacerbations are treated with interferon-beta or glatiramer acetate and it is unethically to withhold patients of treatment for several years, a study using fluoxetine as add-on treatment to current immunomodulating therapy would be the way to go.
The effect of fluoxetine on clinical progression in primary and secondary progressive MS is currently investigated in a small randomized, placebo-controlled study at the University Medical Center Groningen. Taking into account the lack of effective treatment in the progressive phase of the disease, the start of a larger trial in patients with progressive forms of MS is an interesting option.
The effects of fluoxetine on disease activity support the hypothesis that enhancing cAMP in astrocytes might be beneficial for patients with MS. Studies with drugs, other than fluoxetine, that are able to enhance cAMP in astrocytes, can also be encouraged in patients with MS.

The cumulative number of new enhancing lesions and number of new T2 lesions on cerebral MRI scans is frequently used as surrogate marker for inflammatory disease activity in MS studies. However, the clinical relevance of suppressing these lesions is unclear. The finding that more T2 lesions on cerebral MRI scans increase the risk of becoming secondary progressive in patients with relapsing remitting MS, indicates a relationship between the number of T2 lesions and secondary progression. Further studies must show whether suppression of new T2 lesions by immunomodulating therapies delays the onset of secondary progression. In patients with progressive forms of MS the number of T2 lesions does not predict the time to progression of disability, suggesting that T2 lesions have no influence on progression of disability in progressive forms of MS. Monitoring new T2 lesions in follow-up studies can show whether increases in T2 lesions are associated with progression of disability. Currently measurement of T2 lesions in clinical trials of progressive forms of MS seems not useful.
Brain atrophy is a surrogate marker for axonal degeneration and is increasingly used in MS trials. Although the different MRI derived atrophy measurements are strongly correlated, BPF might be best to use in new studies.
New MRI techniques show abnormalities in grey and white matter that looks normal on conventional MRI. These techniques might provide better surrogate markers for disease activity than conventional MRI measurements. Of these, the use of MR Spectroscopic derived NAA/Cr can be recommended as marker for axonal function. With diffusion tensor imaging and magnetization transfer imaging it is possible to quantify cerebral white and grey matter structures. The importance of grey matter pathology is increasingly recognized and is visualized with three-dimensional (3D) double inversion recovery and T1-weighted 3D spoiled gradient-recalled echo sequences. Although technically challenging, the use of these techniques can also be considered.

We were unable to measure lactate formation with proton MR Spectroscopy. Phosphorus MR Spectroscopy is another technique that provides information on energy compounds like phosphocreatine (PCr) and adenosine triphosphate (ATP) in the CNS, and might be a suitable method to measure cerebral energy metabolism in MS.
All techniques discussed should be further investigated for their suitability as surrogate marker for current and past disease activity in MS. Reproducibility and validity studies are warranted. All can be considered in new trials evaluating the use of fluoxetine in multiple sclerosis.

Chapter 11

Nederlandse Samenvatting

Multiple Sclerose (MS) is een chronisch aandoening van het centraal zenuwstelsel, welke gekenmerkt wordt door inflammatoire demyelinisatie en axonale degeneratie. Alle huidige medicijnen werken op het perifere immuunsysteem. Voor de progressieve fase van de ziekte, waarin axonale degeneratie op de voorgrond staat, zijn er geen bewezen effectieve medicijnen. Veel MS onderzoekers geloven dat vroege en agressieve onderdrukking van de ontstekingen axonale degeneratie voorkomt. Tot nu toe heeft agressieve immunosuppressieve therapie echter de ziekte nog niet tot stilstand gebracht.
In 2004 hebben we een hypothese gepresenteerd die gebaseerd is op het ontbreken van de beta-2 adrenerge receptoren op astrocyten bij patienten met MS. Het verlies van de beta-2 adrenerge receptoren zorgt voor een verminderde cAMP productie in de astrocyt wat ervoor zorgt dat:
1) astrocyten door interferon-gamma geinduceerd MHC klasse 2 tot expressie kunnen brengen, waardoor de astrocyt een antigeen presenterende cel wordt, en dus een cruciale rol gaat spelen in het ontstekingsproces
2) in de astrocyt de productie van lactaat, wat naar het axon wordt getransporteerd wordt als energiebron, is afgenomen. Hierdoor ontstaat een energietekort in het axon waardoor deze degenereert.
Als de hypothese klopt, dan kunnen medicijnen die cAMP doen stijgen in astrocyten zowel de ontsteking als de axonale degeneratie in MS doen afnemen. Aangezien het antidepressivum fluoxetine hiertoe in staat is, is besloten om de effecten van dit medicijn te onderzoeken bij patienten met MS.
De ziekte activiteit in MS is onregelmatig, onvoorspelbaar en moeilijk op klinische gronden vast te leggen en te meten. Met behulp van MRI is het mogelijk om verschillende pathofysiologische kenmerken van de ziekte noninvasief te bekijken en te vervolgen. Ziekteactiviteit op de MRI kan gevonden worden zonder dat patienten klachten hebben.

In het eerste deel van dit proefschrift bekijken we de effecten van fluoxetine op neurologische aandoeningen (hoofdstuk 2). Fluoxetine verhoogt neurotrofe factoren, geeft een toename van de glycogenolyse in astrocyten, en blokkeert calcium en natrium kanalen. Dit kan axonale schade voorkomen. Alhoewel er veel studies zijn waarin het gebruik van fluoxetine voor de behandeling van depressie bij neurologische aandoeningen is onderzocht, ontbreken er goed opgezette studies naar de effecten van fluoxetine op neurologische klachten.

In het volgende deel van dit proefschrift voeren we enkele studies met behulp van conventionele MRI uit.
In hoofdstuk 3 laten we in een dubbel-blind, placebo-gecontroleerd onderzoek zien dat het gebruik van fluoxetine door niet depressieve MS patienten leidt tot een trend tot afname van het aantal aankleurende lesies op de MRI van het cerebrum. Het aantal aankleurende lesies is een surrogaatmarker voor de ontstekingsactiviteit bij patienten met MS. Deze

trend werd duidelijker na 8 weken behandeling, wat overeenkomt met de tijd dat het duurt om stabiele plasmalevels van fluoxetine te bereiken en om antidepressieve werking van fluoxetine te kunnen zien. Patienten die fluoxetine gebruikten, hadden ook minder MRI scans waarop ontstekingsactiviteit werd gevonden. Grotere studies zijn nodig voordat fluoxetine een plaats kan krijgen in de behandeling van MS. Hoofdstuk 4 beschrijft de vergelijking van verschillende technieken om hersenatrofie te meten. Op corpus callosum area na, waren alle atrofiemetingen redelijk goed tot sterk aan elkaar gecorreleerd. Brain parenchymal fraction leek de meest geschikte methode om te gebruiken in toekomstige MS studies.
Vervolgens vroegen wij ons af wat de klinische relevantie is van het meten van T2 lesies en hersenatrofie bij mensen met MS. Om hier meer inzicht in te verkrijgen, hebben we alle cerebrale MRI scans van MS patienten, die verricht waren voor 1997, proberen te verkrijgen. Deze hebben we geanalyseerd met betrekking tot het aantal T2 lesies en een aantal hersenatrofiematen. Alle klinische patientengegevens werden bijgehouden in de Groningen MS-database. In hoofdstuk 5 laten we zien dat meer T2 lesies op de oude MRI scan een onafhankelijke voorspeller is voor het eerder optreden van secundaire progressie. Dit wordt ondersteund door onze bevindingen dat meer T2 lesies een voorspellende factor zijn voor het eerder optreden van toename van beperkingen (hoofdstuk 6). In de primaire en secundair progressieve beloopsvormen van MS hebben het aantal T2 lesies geen invloed op het ontstaan van nieuwe beperkingen.

In het laatste deel van dit proefschrift hebben we verschillende studies uitgevoerd met ^{1}H-MRS. We hebben geprobeerd om lactaat te meten bij gezonde proefpersonen die knijpbewegingen uitvoerden (hoofdstuk 7). Lactaat wordt geproduceerd door astrocyten en wordt getransporteerd naar axonen als brandstof tijdens intensieve neuronale activiteit. Volgens onze hypothese is de productie van lactaat in astrocyten verstoord door het ontbreken van de beta-2 adrenerge receptoren. Als we lactaat hadden kunnen detecteren, wilden we hetzelfde onderzoek doen bij mensen met MS. We konden echter geen lactaat vinden bij gezonde personen en moeten concluderen dat onze methode niet geschikt is om het lactaat metabolisme te meten.
In hoofdstuk 8 hebben we gekeken naar de reproduceerbaarheid van N-acetylaspartate/Creatine (NAA/Cr) na 4 weken bij klinisch stabiele MS patienten. NAA/Cr is een surrogaatmarker voor axonale functie en mogelijk ook voor axonale degeneratie. Goede surrogaatmarkers zijn valide en reproduceerbaar. We laten zien dat NAA/Cr goed reproduceerbaar is na 4 weken en het gebruik van NAA/Cr kan dus worden aanbevolen in MS trials. Als laatste beschrijven we in een open studie bij 11 MS patienten een toename van NAA/Cr nadat ze 2 weken fluoxetine gebruikt hebben (hoofdstuk 9). Dit ondersteunt een mogelijk gunstig effect van fluoxetine op axonale functie.

Toekomstige richtingen

Fluoxetine is goedkoop, wereldwijd beschikbaar, beschikbaar in tabletvorm en veilig bij langdurig gebruik. Dit proefschrift geeft een theoretische basis voor het gebruik van fluoxetine als ziektemodulerend medicijn bij patienten met MS. Het bevat 2 studies met positieve effecten van fluoxetine op cerebrale MRI metingen bij mensen met MS. Aangezien deze studies klein zijn en slechts een maximale follow-up hebben van 6 maanden zonder dat er gebruik gemaakt is van klinische eindpunten, is het te vroeg om fluoxetine aan patienten met MS voor te schrijven. Wel moedigen de resultaten in dit proefschrift nader onderzoek naar de effecten van fluoxetine bij mensen met MS aan.
Bij patienten met relapsing-remitting MS kan een nieuwe fase 2 studie aantonen of de trend tot verbetering met fluoxetine reproduceerbaar is. Als primaire uitkomstmaat zou dan ook het cumulatieve aantal aankleurende T1 lesies gebruikt kunnen worden waarbij dan de nadruk moet liggen op de effecten van fluoxetine na 8 weken. Het starten van een grote, multi-center trial naar de effecten van fluoxetine op klinische exacerbaties bij MS kan ook overwogen worden. Aangezien veel patienten met exacerbaties behandeld worden met interferon-beta of glatirameer-acetaat en het onethisch is om mensen meerdere jaren behandeling te ontzeggen, is een studie waarbij fluoxetine als toevoeging gegeven wordt aan huidige immunomodulerende therapie een goede optie. Het effect van fluoxetine op toename van beperkingen bij mensen met primair en secundair progressieve MS wordt momenteel onderzocht in een kleine gerandomiseerde, placebo-gecontroleerde studie in het Universitair Medisch Centrum Groningen. Een grotere studie naar de effecten van fluoxetine op progressieve beloopsvormen van MS valt te overwegen, zeker omdat effectieve behandelingen in de progressieve fase van de ziekte ontbreken.
De effecten van fluoxetine op ziekte activiteit ondersteunen de hypothese dat een toename van cAMP in astrocyten voordelig kan zijn voor patienten met MS. Studies met andere medicijnen, die de hoeveelheid cAMP doen toenemen in astrocyten, kunnen aangemoedigd worden.

Het cumulatieve aantal aankleurende lesies en aantal nieuwe T2 lesies op cerebrale MRI-scans is vaak gebruikt als surrogaat marker voor ontstekingsactiviteit in MS onderzoeken. De klinische relevantie van het onderdrukken van deze lesies is onduidelijk. Het feit dat meer T2 lesies op cerebrale MRI scans een voorspellende waarde hebben voor het optreden van secundaire progressie, geeft aan dat er een verband kan bestaan tussen het aantal T2 lesies en secundaire progressie. Nieuwe studies moeten laten zien of het onderdrukken van nieuwe T2 lesies met immunomodulerende therapie het begin van de secundair progressieve fase uitstelt. Bij patienten met progressieve beloopsvormen van MS voorspelt het aantal T2 lesies niet de tijd tot het optreden van toename van beperkingen. Dit suggereert dat T2 lesies geen invloed hebben op het optreden van toename van beperkingen in progressieve vormen van MS. Vervolgstudies kunnen laten zien of een

toename van het aantal T2 lesies geassocieerd is met een toename van beperkingen. Momenteel lijkt het meten van T2 lesies in studies met progressieve beloopsvormen van MS niet zinvol.

Hersenatrofie is een surrogaat marker voor axonale degeneratie en wordt in toenemende mate gebuikt in MS studies. Alhoewel de verschillende manieren om atrofie te bepalen sterk met elkaar correleren, lijkt de Brain Parenchymal Fraction (BPF) het meest geschikt om te gebruiken in nieuwe studies.

Nieuwere MRI technieken laten afwijkingen zien in de grijze en witte stof die er normaal uitziet op de conventionele MRI. Deze technieken zouden betere surrogaatmarkers kunnen zijn voor ziekte activiteit dan de metingen bepaald met conventionele MRI. Het gebruik van NAA/Cr, bepaald met MR spectroscopie, kan aanbevolen worden als marker voor axonale functie. Met diffusion tensor imaging en magnetization transfer imaging is het mogelijk om de cerebrale witte en grijze stof te kwantificeren. Het belang van grijze stof pathologie wordt in toenemende mate ingezien en kan aangetoond worden met three dimensional (3D) double inversion recovery en T1-weighted 3D spoiled gradient-recalled echo sequences. Alhoewel deze technieken technisch ingewikkeld zijn, is het gebruik hiervan het overwegen waard.

We konden geen lactaat meten met proton MR Spectroscopie. Phosphorus MR spectroscopie is een andere techniek waarmee stoffen die een rol spelen in de energiehuishouding, zoals phophscreatine (PCr) en adenosine triphosphate (ATP), in het CZS gemeten kunnen worden. Deze techniek zou dus een betere methode kunnen zijn om het cerebrale energie metabolisme bij MS te meten.

Alle besproken technieken zouden verder onderzocht moeten worden om hun geschiktheid te bepalen als surrogaatmarker voor actieve en eerdere ziekteactiviteit. Studies naar reproduceerbaarheid en validiteit zijn nodig. Bij nieuwe studies naar effecten van fluoxetine zouden deze technieken gebruikt kunnen worden.

Dankwoord

Dit proefschrift was nooit tot stand gekomen zonder de hulp van velen. De meest belangrijke bijdragers wil ik bij deze dan ook heel erg bedanken.

Beste prof de Keyser, beste Jacques, hartelijk dank voor de kans die je mij 6 jaar geleden gaf om dit onderzoek te gaan doen. Het was een genoegen om met je samen te werken. Met je goede toegankelijkheid, snelle correcties en reacties, en je nimmer aflatende enthousiasme en optimisme ben je voor mij de ideale promotor.

Beste prof van Buchem, beste Mark, je geloof om met behulp van MRI cerebrale ziekteprocessen te kunnen meten en monitoren is er een die ik deel. Het ter beschikking stellen van jouw mensen met je sturing vanuit de achtergrond was voor mij heel erg belangrijk.

Beste dr Heersema, beste Thea, je groningse nuchterheid heeft ervoor gezorgd dat ik de onderzoeksbevindingen beter kon nuanceren. Je menselijkheid herinnerde me er continu aan dat er meer is op deze wereld dan onderzoek doen.

De leden van de beoordelingscommissie, prof Barkhof, prof Kuks en prof Snoek, wil ik bedanken voor het grondig doorlezen van het manuscript en voor hun nuttige opmerkingen.

Het neuroimaging centrum in Groningen wil ik bedanken voor de mogelijkheid om de MRI-scans daar te kunnen verrichten. Anita, de vele uren die wij bij de scanner hebben doorgebracht zal ik niet snel vergeten. Je directe manier van spreken en aanpak was voor mij wennen maar klikte heel erg goed. Hans, bedankt voor je hulp bij het analyseren van de MRI's.

Van de afdeling beeldverwerking van de afdeling radiologie van het LUMC wil ik Faiza, Roald, Hans en Jasper bedanken voor hun hulp bij het analyseren van de MRI-scans. Ik vind het jammer dat jullie grotendeels je carriere voortzetten in het bedrijfsleven.

De afdeling radiologie in het UMCG wil ik bedanken voor de mogelijkheid om daar MRI scans te kunnen verrichten. Paul, je kennis en analyse van de spectroscopie scans waren voor mij onmisbaar. Jan-Cees, ik heb veel geleerd van je kennis van statistiek en je rustige, maar overzichtelijke en begrijpelijke manier van aanpak bij het opzetten van onderzoek en het analyseren van data.

De afdeling klinische neurofysiologie, en dan met name Han, Natasha en de laboranten, wil ik bedanken voor hun tijd en interesse in het onderzoek met de 128-kanaals VEP. Er komt vast een geschikte analyse methode beschikbaar!

Marcus, onze discussies over MS en onderzoek in het algemeen waren leuk en zetten mij altijd aan tot extra nadenken. Maarten, Wilma en Geeta, bedankt voor de onderzoeksdiscussies en de gezelligheid.

De studenten geneeskunde Marieke, Yde en Iris, die hun afstudeeronderzoek bij de afdeling neurologie gedaan hebben, wil ik bedanken voor hun hulp bij de analyse van de data. Ik hoop dat jullie net zoveel van mij geleerd hebben als ik van jullie.

Alle (voormalig) arts-assistenten, stafleden en (secretariele) medewerkers (met name Tineke!) van de afdeling neurologie van het UMCG wil ik bedanken voor hun ondersteuning voor het onderzoek, en voor de goede werksfeer.

Marco en Cobi, de MS nurse practitioners, bedankt voor het samenwerken en voor de goede zorg die jullie leveren aan MS patienten.

Alle deelnemers aan mijn onderzoeken ben ik veel dank verschuldigd.

Marc en Harold, bedankt dat jullie mijn paranimfen wilden zijn. Goede vrienden zijn onmisbaar en zeldzaam.

Beste papa, mama, Maarten, Pim, opa, oma en de hele familie Sleyfer, bedankt voor jullie continue ondersteuning en interesse in het onderzoek.

Lieve Claartje, het onderzoek heeft veel beslag gelegd op ‘onze’ tijd. Zonder jouw onvoorwaardelijke steun en geloof in mij was het nooit gelukt!

List of Publications

Sijens PE, Mostert JP, Irwan R, Potze JH, Oudkerk M, De Keyser J.
Impact of fluoxetine on the human brain in multiple sclerosis as quantified by proton magnetic resonance spectroscopy and diffusion tensor imaging.
Psychiatry Res. 2008 Dec 30;164(3):274-82.

De Keyser J, Steen C, Mostert JP, Koch MW.
Hypoperfusion of the cerebral white matter in multiple sclerosis: possible mechanisms and pathophysiological significance.
J Cereb Blood Flow Metab. 2008 Oct;28(10):1645-51.

Mostert JP, Koch MW, Heerings M, Heersema DJ, De Keyser J.
Therapeutic potential of fluoxetine in neurological disorders.
CNS Neurosci Ther. 2008 Summer;14(2):153-64.

Mostert JP, Admiraal-Behloul F, Hoogduin JM, Luyendijk J, Heersema DJ, van Buchem MA, De Keyser J.
Effects of fluoxetine on disease activity in relapsing multiple sclerosis: a double-blind, placebo-controlled, exploratory study.
J Neurol Neurosurg Psychiatry. 2008 Sep;79(9):1027-31.

Mostert JP, Blaauw Y, Koch MW, Kuiper AJ, Hoogduin JM, De Keyser J.
Reproducibility over a 1-month period of 1H-MR spectroscopic imaging NAA/Cr ratios in clinically stable multiple sclerosis patients.
Eur Radiol. 2008 Aug;18(8):1736-40.

Mostert JP, Huits RM.
Diagnostic image (360). A woman with lowered consciousness.
Ned Tijdschr Geneeskd. 2008 Feb 9;152(6):319-20.

Koch M, Uyttenboogaart M, Heerings M, Heersema D, Mostert J, De Keyser J.
Progression in familial and nonfamilial MS.
Mult Scler. 2008 Apr;14(3):300-6.

Koch M, Mostert J, De Keyser J, Tremlett H, Filippini G.
Interferon-beta treatment and the natural history of relapsing-remitting multiple sclerosis.
Ann Neurol. 2008 Jan;63(1):125-6; author reply 126-7.

Mostert JP, de Groot JC, Ramsaransing GS, Koch MW, De Keyser J.
Relationship between the extent of T2 lesions and the onset of secondary progression in multiple sclerosis
Eur J Neurol. 2007 Nov;14(11):1210-5.

Koch MW, Mostert JP, de Vries JJ, De Keyser J.
Treatment with interferon beta-1b delays conversion to clinically definite and McDonald MS in patients with clinically isolated syndromes.
Neurology. 2007 Apr 3;68(14):1163; author reply 1163-4.

Koch M, Heersema D, Mostert J, Teelken A, De Keyser J.
Cerebrospinal fluid oligoclonal bands and progression of disability in multiple sclerosis.
Eur J Neurol. 2007 Jul;14(7):797-800.

Koch M, Mostert J, Arutjunyan AV, Stepanov M, Teelken A, Heersema D, De Keyser J.
Plasma lipid peroxidation and progression of disability in multiple sclerosis.
Eur J Neurol. 2007 May;14(5):529-33.

Koch M, Mostert J, Heersema D, De Keyser J.
Progression in multiple sclerosis: further evidence of an age dependent process.
J Neurol Sci. 2007 Apr 15;255(1-2):35-41.

Koch M, Mostert J, Heersema D, De Keyser J.
Tremor in multiple sclerosis.
J Neurol. 2007 Feb;254(2):133-45.

Koch M, Mostert J, Heersema D, Teelken A, De Keyser J.
Plasma S100beta and NSE levels and progression in multiple sclerosis
J. Neurol. Sci. 2007 Jan 31;252(2):154-8.

Mostert JP, Sijens PE, Oudkerk M, De Keyser J.
Fluoxetine increases cerebral white matter NAA/Cr ratio in patients with multiple sclerosis.
Neurosci Lett. 2006 Jul 10; 402(1-2):22-4.

Sijens PE, Irwan R, Potze JH, Mostert JP, De Keyser J, Oudkerk M.
Relationships between brain water content and diffusion tensor imaging parameters (apparent diffusion coefficient and fractional anisotropy) in multiple sclerosis.
Eur Radiol. 2006 Apr;16(4):898-904.

Sijens PE, Mostert JP, De Keyser J, Oudkerk M.
(1)H MR spectroscopy of the brain in multiple sclerosis subtypes with analysis of the metabolite concentrations in gray and white matter: initial findings.
Eur Radiol. 2006 Feb;16(2):489-95.

Wesselius T, Heersema DJ, Mostert JP, Heerings M, Admiraal-Behloul F, Talebian A, van Buchem MA, De Keyser J
A randomized crossover study of bee sting therapy for multiple sclerosis.
Neurology 2005 Dec 13;65(11):1764-8.

Sijens PE, Irwan R, Potze JH, Mostert JP, De Keyser J, Oudkerk M.
Analysis of the human brain in primary progressive multiple sclerosis with mapping of the spatial distributions using 1H MR spectroscopy and diffusion tensor imaging.
Eur Radiol. 2005 Aug;15(8):1686-93.

Mostert JP, Ramsaransing GS, Heersema DJ, Heerings M, Wilczak N, De Keyser J.
Serum uric acid levels and leukocyte nitric oxide production in multiple sclerosis patients outside relapses.
J Neurol Sci. 2005 Apr 15;231(1-2):41-4.

Wilczak N, Ramsaransing GS, Mostert J, Chesik D, De Keyser J.
Serum levels of insulin-like growth factor-1 and insulin-like growth factor binding protein-3 in relapsing and primary progressive multiple sclerosis.
Mult Scler. 2005 Feb;11(1):13-5.

Mostert JP, Sijens PE, Oudkerk M, De Keyser J.
(1)H Magnetic resonance spectroscopy of the internal capsule in human brain: a feasibility study to detect lactate following contralateral motor activity.
Eur Radiol. 2005 Jul;15(7):1349-52.

Boucard CC, Mostert JP, Cornelissen FW, De Keyser J, Oudkerk M, Sijens PE.
Visual stimulation, (1)H MR spectroscopy and fMRI of the human visual pathways.
Eur Radiol. 2005 Jan;15(1):47-52.

De Keyser J, Zeinstra E, Mostert J, Wilczak N.
Response to Cosentino et al.: β2-Adrenoceptors in multiple sclerosis
Trends Pharmacol Sci. 2004 Jul;25(7): 351-2.

De Keyser J, Zeinstra E, Mostert J, Wilczak N.
β2-adrenoceptor involvement in inflammatory demyelination and axonal degeneration in multiple sclerosis.
Trends Pharmacol Sci. 2004 Feb;25(2):67-71.

Curriculum Vitae

Jop Mostert is geboren op 19 januari 1977 in Leiderdorp. In 1995 heeft hij eindexamen gedaan aan het Stedelijk Gymnasium te Leiden. Aansluitend is hij begonnen met de studie Geneeskunde aan de Rijksuniversiteit in Groningen. In 2002 behaalde hij het arts examen waarna hij begon met de opleiding tot neuroloog in het Universitair Medisch Centrum Groningen. Vanaf 2003 combineerde Jop zijn opleiding met onderzoek. Begin oktober 2009 zal Jop zijn opleiding tot neuroloog afronden. Hierna zal hij gaan werken als neuroloog in het Rijnstate Ziekenhuis in Arnhem en in het Ziekenhuis Zevenaar met als aandachtsgebied Multiple Sclerose.
Jop woont al geruime tijd samen met zijn vriendin Claartje Sleyfer.