New avenues in PET imaging of multiple sclerosis

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Chapter

1

General Introduction

Multiple Sclerosis

Multiple sclerosis (MS) is an inflammatory and demyelinating disease of the central nervous system (CNS). MS is one of the most common neurological disorders causing disability in young adults. The estimated prevalence of MS in the world is 30 per 100,000. The highest prevalence is in Europe (80 per 100,000), followed by the Eastern Mediterranean (14.9), the Americas (8.3), the Western Pacific (5), South-East Asia (2.8) and Africa (0.3). MS is more common among women than men (WHO, 2008).

Typically, symptoms appear when patients are around 30 years of age. The most common symptoms are paresthesia; numbness or weakness in one or more limbs; bladder, bowel and sexual dysfunction; dysarthria, ataxia and tremor; optic neuritis, trigemal neuralgia, heat intolerance, fatigue, dizziness, lack of sleep, pain, cognitive difficulties and depression (Miljkovic & Spasojevic, 2013). Clinically, MS is classified based on the clinical course of the disease (Figure 1). About 85% of the patients present a relapsing-remitting type (RRMS), which is characterized by recurrent episodes of neurological disabilities, caused by inflammation and demyelination. These relapses are followed by periods of partial or complete recovery. In about half of the RRMS patients, the remission is not complete and the disease gradually takes a secondary progressive course (SPMS), in which neurological disabilities accumulate without proper recovery. In 15% of the patients, the disease course is progressive from disease onset, without remission or relapse phases. This type of MS is defined as primary progressive MS (PPMS). Progressive relapsing MS (PRMS) is a rare type of MS that initially presents as PPMS, but develops neurological exacerbations during the disease progression. There is still a fifth type of MS that - contrary to the other disease courses - show little or no disease progression and only minor neurological disabilities, even

decades after disease onset (Miljkovic & Spasojevic, 2013; Pittock & Lucchinetti, 2007; Luessi et al, 2012; Ramsaransing & De Keyser, 2006).

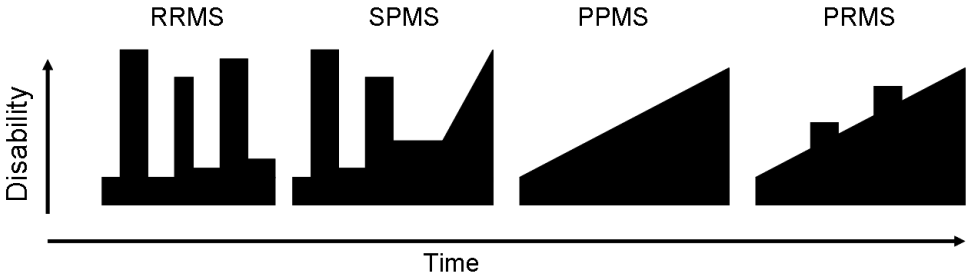

Figure 1: Clinical course of the 4 main types of MS: relapsing-remitting (RRMS), secondary progressive (SPMS), primary progressive (PPMS), and progressive relapsing (PRMS).

The pathological hallmark in MS is the formation of demyelinated lesions, also called sclerotic plaques, in the white and grey matter of the central nervous system. These lesions are the result of a complicated process involving inflammation, demyelination and remyelination, oligodendrocyte depletion, astrocytosis, and neuronal and axonal degeneration. The lesions can occur anywhere of the CNS, but sites as the optic nerve, brainstem, cerebellum and spinal cord are more related to clinical deficits (Lassman, 2013; Compston & Coles, 2008; MacDonald & Ron, 1999).

MS has long been considered a mere autoimmune disease caused by the invasion of myelin-autoreactive lymphocytes across the blood-brain barrier (BBB) and choroid plexus. These lymphocytes attack myelin and oligodendrocytes and induce an inflammatory cascade that involves macrophage recruitment and microglia activation and leads to demyelination, axonal damage and lesions

(Compston & Coles, 2008; Chen et al, 2012). However, new insights have led to a different view on the disease. Nowadays, MS is more considered as a chronic, gradually aggravating neurodegenerative disease with oligodendrocytes and axons as primary affected targets. In this view, primary progressive MS is thought to be the "purest" form of MS. In the relapsing-remitting type of MS, an aberrant, excessive immune response to antigenic debris (myelin, neurofilaments), caused by the ongoing neurodegeneration process, is convoluted on top of the underlying neurodegeneration. The continuous neurodegenerative nature of MS becomes apparent again in the secondary progressive stage, when aging tones down the undulating vigorous immune reactions. The observations that myelin sheath degradation in MS starts from within (not by cells attacking from the outside) and that all current anti-immune and anti-infiltration drugs for MS can only reduce or delay relapses, but cannot stop ongoing degeneration, seem to be in line with these new ideas about MS (Stys et al, 2012).

Animal models for multiple sclerosis

Multiple sclerosis is a disease that spontaneously occurs only in humans. From a practical and ethical point of view, it is often not possible to perform invasive studies in patients. The possibilities for noninvasive investigation of the actual pathological processes in the CNS are confined to analysis of postmortem brain tissue or imaging techniques that allow detection of lesions, but are not very specific for the actual disease processes involved. Therefore, most research still relies on the use of animal models that mimic MS. Due to the complexity of MS, none of the current animal models for MS is able to mimic all disease aspects, but the availability of different animal models allows studying of different relevant

features of the disease. The most commonly used animal models for multiple sclerosis are classified in 3 categories: (1) Experimental Autoimmune (or Allergic) Encephalomyelitis (EAE); (2) Viral induced demyelination models and (3) Toxin induced demyelination models.

Experimental autoimmune encephalomyelitis (EAE)

EAE is the most frequently used animal model for studying the inflammatory aspects of MS. EAE has been induced in a wide variety of species, such as mice, rats, guinea pigs, rabbits, goats, dogs, sheep and primates (Baxter, 2007).

EAE is induced by immunizing the animals with CNS antigens emulsified in an adjuvant. In general, these antigens are purified myelin, recombinant proteins or encephalitogenic peptides of myelin proteins. They cause the activation of peripheral antigen-specific T-cells that enter the CNS and induce lesion formation (Denic et al, 2011; van der Star et al, 2012).

The clinical course of the disease depends on the immunization protocol, the antigen used and the animal strain. In most cases, symptoms start with weight loss and loss of activity, and proceed into motor disability (limb paralysis). In some cases, EAE progresses very aggressively and can lead to the death of the animal.

Although, EAE is a valuable model for research in MS, it is recognized to cause severe animal discomfort and, because of that, its use is only justified when other, less harmful animal models are not suitable for answering a specific research question (Wolfensohn et al, 2013).

Viral induced model

The hypothesis that a viral infection early in life can trigger the aberrant immune response to myelin in the CNS and therefore could be a potential cause of MS has led to the use of virus-induced demyelination animal models. So far, a specific type of virus has not been unambiguously identified as the potential cause for MS (Denic et al, 2010).

In the most frequently used viral animal model for multiple sclerosis, demyelination is induced by the Theiler´s murine encephalomyelitis virus, which is a natural pathogen of mice. Infection of mice by the intracerebral injection of the virus causes paralysis and encephalomyelitis. The resulting disease can be characterized by, in general, 2 phases: (1) the acute phase, which is mild and usually subclinical and predominately confined to neurons and (2) the chronic phase, exhibiting slow progressive disability, as a consequence of demyelination, impaired remyelination, inflammation and axonal damage (Denic et al, 2011; Pachner, 2011).

The disadvantages of this model in comparison to the EAE model is that it can be induced only in mice and that the disease manifestations occur months after the initial infection (Pachner, 2011).

Toxin induced model

The toxin-induced models are predominately used for studying the demyelination and subsequent spontaneous endogenous remyelination aspects of MS.

Demyelination is induced by focal application or by systemic administration of the toxin, depending on the toxin applied.

Systemic toxin

Cuprizone [oxalic acid bis(cyclohexylidene hydrazide)] is a copper chelating agent and the most frequently used toxin to induce demyelination in the CNS by systemic administration. The most common demyelination protocol comprises the feeding of C57Bl/6 mice with 0.2% cuprizone for 5-6 weeks. The model is species, strain and age dependent. Cuprizone has failed to induce demyelination in several adult rat strains (Carlton, 1969; Love, 1988). Only a single report has been published, in which cuprizone-induced demyelination in young Wistar rats was observed (Adamo et al, 2006). The administration of cuprizone in mice induces oligodendrocyte death without significant damage to other neural cell types. This activity of cuprizone requires microglia activation and their secretion of pro-inflammatory cytokines (Pasquini et al, 2007). Short exposure to the cuprizone diet induces acute demyelination, which is most prominent in the corpus callosum and the cerebellar peduncle. Demyelination is followed by spontaneous remyelination when normal chow diet is restored. Chronic demyelination occurs when animals are kept on a cuprizone diet for a long period of time (12 weeks or more) (Kipp et al, 2009; Torkildsen et al, 2008; Matsushima & Morell, 2001).
The highly reproducible, the simplicity of the protocol, the fast demyelination induction, the spontaneous remyelination after restoration of normal food, as well as the lack of peripheral cell infiltration, make the cuprizone-induced model a valuable model for studying demyelination and remyelination processes.

Focal toxin

The most commonly used toxins to induce focal demyelinated lesions are ethidium bromide and lysolecithin. Because these toxins are injected directly into the CNS, demyelination can be induced in all species and in different CNS regions of preference.

Lysolecithin (lysophosphatidylcholine, LPC) is an activator of phospholipase A2 that disrupts membranes, especially damaging myelin. This induces an acute inflammatory response, including microglia activation and monocyte infiltration at the site of the lesion. Lysolecithin has a short half-life (5 hours) and remyelination occurs spontaneously, already evident 14 days after injection and almost completed by 6 weeks (Denic et al, 2011; van der Star, 2012).

Ethidium bromide is a DNA intercalating agent that has a cytotoxic effect on all nucleated cells. Therefore, the damage induced by ethidium bromide is not specific for myelin producing cells alone (van der Star, 2012). Demyelination reaches a peak at 2 weeks and the remyelination predominates at 4 weeks after injection (Blakemore, 1982).

Imaging

Animal models are indispensable for studying ongoing *in vivo* processes and to better understand disease mechanisms. Eventually, the results of animal studies need to be translated to and validated in patients. Imaging techniques are valuable tools for monitoring the *in vivo* processes and can be applied both in animals and in humans, which makes them attractive tools for translational

research. In MS research, the development of new imaging techniques and/or the validation of new imaging applications are still needed, as the existing methods do not suffice.

"The capacity to image cellular and molecular targets *in vivo"* defines the term molecular imaging. Positron emission tomography (PET) is probably the best example of a molecular imaging device. PET allows the quantification of tracer distribution and redistribution, providing biochemical and physiological information of the living organism. The advance in PET instrumentation has made the technology also available for preclinical research, which allows the translation of preclinical results to human applications (Grenier & Brader, 2011; Hutchins et al, 2008).

Positron emission tomography

Positron emission tomography (PET) is a molecular imaging technique that quantitatively and non-invasively measures biochemical and physiological processes *in vivo* by using specific targeting molecules labeled with positron emitting isotopes. These labeled molecules are called PET tracers or PET radiopharmaceuticals (Paans et al, 2002; van den Hoff, 2005).

The positron emitters, such as ^{15}O, ^{11}C, ^{13}N, ^{18}F, are neutron deficient isotopes that decay by the emission of a positively charged electron: a positron. This positron travels through the surrounding tissue losing energy due to collision with electrons. When the energy of the positron is sufficiently low, it can combine with an electron and annihilation can occur. In this process, the mass of the positron and electron is converted into two gamma rays with an energy of 511 keV, according to Einstein famous formula $E=mc^2$. Because of the law of conservation

of momentum, these gamma rays travel in opposite directions (at an angle of 180° from each other) (Townsend, 2004; Basu et al, 2011).

The emitted gamma rays are detected by a circular (360°) detector structure, using the principle of coincidence detection. Coincidence detection means that the detection is only accepted as a true event if the two gamma rays are almost simultaneously (typically within 100 ns) detected by opposite detectors. Each coincidence event represents a straight line in space between the two detectors, on which the point of origin of the emission of the positron is located. This line is called a line-of-response (LOR). A combination of LORs is used for reconstruction of a computerized image, representing the 3D distribution of the injected radiopharmaceutical in the tissue (Andreyev & Celler, 2011; Townsend, 2004).

Besides physical properties of PET tracers, the chemical and biological characteristics of these molecules determine whether the radiopharmaceutical can be successfully used for brain imaging. These characteristics include binding affinity and specificity, metabolism and elimination rates, and brain penetration (Wadsak & Mitterhauser, 2010). Only complete knowledge about the injected radiopharmaceutical, gathered by preclinical and clinical research, can guarantee the correct interpretation of the resulting image.

Aim and outline of the thesis

The progressive characteristic and the lack of an effective therapy, make constant monitoring a crucial factor in the evaluation of disease progression and treatment effects in multiple sclerosis. Non-invasive imaging techniques are the best option for monitoring the disease progression and evaluating new drugs, since they can be applied multiple times and induce low discomfort to the patient. However,

imaging techniques for disease-specific processes in MS still need to be evaluated and validated in models of the disease, before they can be implemented in clinical practice.

Imaging techniques can also be extremely important in preclinical research, enabling longitudinal studies of ongoing processes that could help to understand mechanisms involved in disease progression. PET imaging could become a tool to specifically monitor different disease-related aspects of MS in a non-invasive manner. Therefore, the aim of the work described in this thesis was to evaluate and compare different PET tracers with potential for imaging multiple sclerosis hallmarks. For this purpose, different approaches were used: *i.* The cuprizone-induced mouse model was used to evaluate [^{11}C]CIC and [^{11}C]MeDAS as candidate PET tracers for imaging demyelination and remyelination processes. *ii.* The lysolecithin rat model was applied to assess the feasibility of monitoring glucose metabolism, neuroinflammation, demyelination and remyelination in focal lesions over time. *iii.* The lysolecithin rat model was also used for evaluating the pharmacokinetic proprieties of [^{11}C]CIC, [^{11}C]MeDAS and [^{11}C]PIB and for testing the ability of these tracers to image focal demyelination and remyelination. *iv.* Finally, the feasibility of PET imaging to evaluate treatment efficacy was investigated by monitoring longitudinal changes in neuroinflammation and demyelination in EAE rats treated with dexamethasone or placebo. A brief outline of this thesis is given below.

Chapter 2 reviews the available PET tracers for application in MS and discusses potential other targets that could be imaged by this technique in the future.

Chapter 3 describes the comparison of two PET tracers for imaging demyelination and remyelination processes in the cuprizone-induced mouse model for multiple sclerosis.

Chapter 4 shows the potential of PET imaging for monitoring transient changes in different characteristics of focal demyelinated lesions in the lysolecithin rat model: glucose metabolism, neuroinflammation, demyelination and remyelination.

Chapter 5 describes the results of the kinetic modeling studies of 3 PET tracers for myelin imaging and presents a comparison of the ability of these tracers to detect demyelination and remyelination processes in a focal lesion in the lysolecithin rat model.

Chapter 6 describes the monitoring of disease progression and the therapeutic effects of dexamethasone in the EAE rat model by PET imaging of neuroinflammation, demyelination and T cell infiltration.

Chapter 7 gives some future perspectives for *in vivo* monitoring of disease progression in MS with PET.

Chapter 8 summarizes the major findings of this thesis.

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