

Advanced glycation endproducts in multiple sclerosis

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Valorisation

Valorisation

Multiple sclerosis (MS) is the main cause of disability in young adults [1]. According to the National MS Society, it is estimated that 2.3 million individuals worldwide are diagnosed with MS [2]. Over the years, the incidence of MS has been increasing in the Netherlands, from 4/100.000 in 1996-2004 to 9/100.000 in 2007/2008 [3]. To this date, the prevalence of MS in the Netherlands is estimated to be 100/100.000 [4]. In Belgium, there are no recent studies examining the prevalence of MS. However, it was documented to be 87.9/100.00 in 1994 [5], which is similar to the prevalence in the Netherlands. These results indicate that 17.000 individuals in the Netherlands and 13.500 individuals in Belgium [6], are diagnosed with MS. MS patients experience a variety of symptoms which affect their daily life. As the disease progresses, the symptoms worsen, leading to an increase in disability and a reduction in mobility of MS patients [7]. A survey conducted with 200 MS patients from Spain, Germany, Norway and Italy revealed that 86% of the MS patients was unable to maintain the same level of study/work as before the diagnosis [7]. In addition to work and study, their disease also impacted their social life. Besides the impact of MS on the quality of life, the socio-economic burden of MS is also significant. Uitdehaag et al. investigated the annual health care costs per MS patient in the Netherlands. These costs include inpatient care, consultations, medication and disease modifying therapies (DMTs) but also costs related to service and informal care costs [4]. For patients with a mild disease (EDSS 0-3), the mean annual health care costs are €10.626, with increasing costs reaching €30.716 as the disease worsens to EDSS 7-9 [4]. Moreover, they showed that with increasing EDSS, the indirect costs such as short-term and long-term absence, invalidity and early retirement also increase, resulting in a total annual cost of €50.500. Apart from the annual costs of health care and medication, MS patients are also more vulnerable for additional health treats. Research has shown that MS patients have a higher risk of infection, which are even associated with relapses [8]. Wijnands et al. revealed that MS patients are twice as likely to be hospitalized due to infections such as pneumonia, urinary system infections, intestinal infections and skin infections compared to people without MS [9]. This also contributes to high health care costs.

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Although a lot of research has been performed in the last decades, the cause of MS remains to be identified. Subsequently, this means that there is no cure for the disease yet. Current therapies only aim to reduce the inflammatory component of the disease. These DMTs are therefore prescribed to patients with relapsing-remitting MS and are less functional for secondary progressive MS patients. The drawback of these therapies are the side effects such as nausea, headache, increased risk of infections and diarrhoea depending on the therapy [10].

Our research has focussed on the role of advanced glycation endproducts (AGEs) in MS. AGEs were previously described to be increased in inflammatory diseases

[11-14], which could potentially contribute to the disease or their complications. The aim of our study was to elucidate whether AGEs are formed in the CNS during the development of MS and their potential effects on disease pathology. This fundamental research was performed using human post-mortem specimens from MS lesions and a mouse model for MS, the experimental autoimmune encephalomyelitis (EAE) model. Moreover, we aimed to target AGEs in the EAE model to determine their therapeutic value.

Valorisation of the key findings of this thesis

This thesis revealed that MGO-derived MG-H1 is increased in post-mortem lesions of MS patients. Moreover, we discovered that AGEs accumulate in the CNS of mice subjected to EAE. Although we were unable to reduce AGE levels with generally accepted AGE lowering strategies in the animal model of MS, we believe that further research is needed to investigate the therapeutic potential of AGE lowering in MS patients. If proven to be beneficial, AGE lowering therapies could be considered as an add-on treatment, improving the quality of life for MS patients.

First, we evaluated the potential of AGE levels in the cerebrospinal fluid (CSF) as a biomarker for disease progression of MS. AGE levels in the CSF were determined and correlated to markers for disease progression such as number of relapses, EDSS and disease duration. These analyses were performed to determine whether AGE levels in the CSF could be used as a biomarker for MS disease progression. Our human data revealed that we could not detect positive correlations between AGE levels in the CSF and markers for disease progression. There may be several reasons why we could not identify a clear link between CSF AGE levels and disease severity. Use of anti-inflammatory drugs, common treatments for MS, at the time that sample were collected could directly impact the AGE formation in the CNS. Moreover, whether or not patients are experiencing a relapse during sample collection can also influence the AGE levels in the CSF. Since we are not aware whether these patients experienced a relapse, it is difficult to discover the exact link between AGE levels in the CSF and disease progression in MS. The potential to use AGE levels in the CSF as a biomarker for MS should be investigated further in detail, for example include patients with and without anti-inflammatory drug use and collect samples of the same patient during a relapse and in the remitting phase following that relapse. Moreover, as we have revealed that free AGE and α -dicarbonyl levels in the CSF correlate with their respective levels in the CSF, it should be investigated whether plasma AGE levels could be used as a biomarker for disease progression. Blood is obtained more easily and with less discomfort for the MS patients compared to CSF. The potential use of AGE levels as a biomarker for disease progression will also increase the knowledge about AGE formation in MS patients, contributing to the realization of the therapeutic potential of AGE lowering in MS. However, one must take into account that levels of AGEs in the CSF or blood may not reflect the AGE levels in the brain parenchyma which

could be important drivers of inflammation and thus disease progression. Taken all together, we were able to detect AGEs in CSF and plasma of MS patients, but their use as biomarkers for disease progression may be limited. Nonetheless, we cannot conclude whether or not AGEs play a role in the etiology of the disease and thus we cannot exclude the therapeutic potential of targeting AGEs in MS patients.

Lifestyle interventions as a therapy for MS

The increase in prevalence of MS, as mentioned above, is attributed to the revision of the diagnostic criteria and the possibility to diagnose patients after one clinical incident [15], which makes diagnosis more specific. However, a study on the prevalence and incidence of MS in Norway suggested that the change in lifestyle factors, such as decrease of vitamin D and fatty fish intake, could also contribute to the increase prevalence of MS [16]. Therefore, one could suggest that the dietary intake of AGEs could affect the prevalence of MS. Using young healthy mice, our study revealed that high intake of dietary AGEs for 5 weeks results in increased levels of AGEs in the CNS, which was accompanied by elevated expression patterns of pro-inflammatory cytokines. Therefore, lifelong accumulation of AGEs in the CNS of MS patients may contribute to the neuroinflammatory environment of the CNS. Our research suggests that dietary AGEs are degraded in the intestine into single amino acids and absorbed, leading to increased levels of free AGEs in the plasma. Moreover, we have shown that free AGEs are able to cross the blood-brain barrier, as we observed an accumulation of AGEs in the CNS. This together suggests that reducing the intake of dietary AGEs could be beneficial, for MS patients but also for healthy individuals or individuals who suffer from other inflammatory diseases. Preliminary data from our laboratory revealed that switching mice from a high AGE to a low AGE diet reduced the amount of free AGEs in the plasma drastically within one week. This data is promising and implies that diet could help prevent and reduce AGE accumulation in tissues such as the CNS.

First, it would be important to investigate whether an AGE-rich diet results in a worse disease progression or higher relapse rate. This can be easily monitored by food diaries. MS patients can keep track of what they eat and based on this information the dietary AGE intake can be determined. This can be done by using already available databases which contain the AGE content of commonly consumed food items [17]. This data will be used to analyse the correlations between dietary AGE intake and disease progression markers such as number of relapses and EDSS. If these results show an increased disease progression with AGE-rich diets, the next step would be to investigate whether a reduction in dietary AGE intake is beneficial for MS patients. To evaluate the effect of lowering dietary AGE intake on disease progression or EDSS, the study has to be performed over a long period of time in which patients will follow a personalized diet low in AGEs. To date it is still unknown how fast a low AGE diet is able to decrease AGE levels in the tissues. We are currently investigating in our

laboratory whether a switch from high AGE to low AGE diet can reduce AGE levels in various tissues including the CNS. Based on our mouse data, it can be speculated that free plasma AGE levels are a reflection of dietary intake, which result in a fast decline in AGE levels since the intake is lower. However, in the tissue, the decrease of AGE levels is expected to be slower as this needs to be cleared from the tissue. The results of our current animal study in which animals fed a high AGE diet for 5 weeks and then switch to a low AGE diet for 5 weeks should give insight in the potential of dietary AGE restriction and their respective levels in tissue. These data can be used to translate into the human situation.

During the clinical dietary AGE restriction study, disease progression, number of relapses and/or wellbeing of MS patients should be monitored closely to detect any signs of relapses. Previous research has shown that supplementation of vitamin D, in patients with low plasma vitamin D levels, in addition to natalizumab treatment, led to reduce rate of the annual relapses within one year [18]. This shows that with an additional therapy, differences in relapse rate can be achieved. In addition to the close monitoring of the disease progression, MS patients enrolled in the clinical trial are coached by dieticians. Personalized diets can be set up to decrease dietary AGE intake. This can be done by replacing high AGE content food such as croissants, biscuits, peanut butter and black pudding with food that is low in AGEs such as fresh vegetables, fruit, jam, milk and white bread [17]. A reduction in dietary AGE intake can also be achieved by changing cooking methods. It is known that cooking methods such as grilling, frying, baking and toasting increase the amount of AGEs in food [19]. Therefore, the stimulating alternative cooking methods such as steaming and poaching, and cooking on lower heat, will reduce the formation of AGEs during cooking [20]. This could be interesting for manufactures of steam ovens, high pressure cookers or airfryers.

When these trials reveal that a low AGE diet has a positive effect on MS disease progression and wellbeing of MS patients, it could be implemented for all MS patients, and even for patients with other (neuro)inflammatory diseases as an add-on therapy. Moreover, it could be speculated that lowering dietary AGE intake might be beneficial for all people to reduce the accumulation of AGEs in the body. It is expected that a change in lifestyle will have a low impact on MS patients as it concerns a switch in low AGE food products and other cooking methods which are not expected to give any side effects. Clinicians and dieticians should help the MS patients to adapt to their new life style.

Potential AGE lowering therapeutics for MS

Lowering of AGEs can also be done by therapeutics. In this thesis, we investigated the effect of pyridoxamine during EAE. Pyridoxamine is a natural vitamin B6 analogue that is known to scavenge MGO thereby preventing AGE formation [21]. Twice daily oral supplementation of pyridoxamine was unfortunately unable to reduce the disease score in the EAE model. However,

examination of plasma and CNS of pyridoxamine treated mice revealed that pyridoxamine was unsuccessful at reducing AGE levels in the plasma and CNS. One explanation for the lack in AGE lowering in the EAE model could be the severity of the model. Pyridoxamine has been used previously in a small clinical trial (n = 10) to reduce pentosidine levels in 80% of schizophrenia patients [22]. It has been proven that 24 weeks of pyridoxamine supplementation is able to reduce AGE levels in clinical trials studying osteoarthritis and diabetic nephropathy [23, 24]. Moreover, Maessen et al. have previously showed that pyridoxamine treatment in mice was able to inhibit adipose tissue inflammation during obesity, despite the absence of a strong reduction in AGE levels [25]. Therefore, we cannot exclude the possibility that pyridoxamine treatments might be beneficial for MS patients. To evaluate the disease progression of MS patients, the clinical study should be conducted over a long period of time to assess the disease progression of MS patients. To ensure that pyridoxamine enters the circulation without converting to its other isoform pyridoxal-5'-phosphate [26, 27], pyridoxamine levels should be monitored in MS patients during the clinical trial. Whether pyridoxamine levels can be increased *in vivo* might limit the potential of pyridoxamine as a AGE lowering intervention. Therefore, other AGE lowering therapeutics should also be investigated.

Therapeutics such as aminoguanidine [28], alagebrium [29] and metformin [30], are previously used investigating diabetes type 1, type 2 and cardiovascular diseases. These substances are known to reduce AGEs, but when used in clinical trials, the AGE lowering potential of aminoguanidine and alagebrium was not examined [28, 31]. Metformin, a known therapeutic for diabetes, was able to reduce the levels of plasma AGEs in women with polycystic ovary syndrome after 6 months of treatment [32]. Therefore, it is valuable to investigate the AGE lowering effect of these therapies in MS patients. Eventually, results from these clinical trials could lead to add-on therapies for MS patients.

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Translational capacity of the animal model

The research conducted in this thesis used the EAE model. This model is induced by the sensitization to myelin peptides, such as myelin oligodendrocyte glycoprotein (MOG). To boost the immune system, mice are injected with pertussis toxin following sensitization. This results in paralysis of the tail which gradually extends towards the front legs [33, 34]. In the EAE model we see infiltration of immune cells, demyelination and remyelination, which is comparable to the processes seen in MS patients [35]. However, MS is a complex heterogeneous disease in which genetic and environmental factors play an important role. It is therefore difficult to capture all of these aspects in one animal model [36]. Moreover, an important difference between the EAE model and MS is that in the EAE model the trigger for the disease is known. The EAE model is commonly used to investigate the effects of various therapies on the disease progression but this does not imply that therapies successful in the EAE

model are successful in MS patients [37-39]. Therefore, one must be careful in the translation from animal model to the human situation.

Conclusion

The societal utilization of the findings presented in this thesis are for the distant future as clinical trials are needed to elucidate whether lowering AGEs is beneficial for MS patients. This will require time to set-up clinical trials including a long follow-up time and analysis of the data. The results of these clinical trials could be easily implemented in the life of MS patients as it requires lifestyle changes or the addition of a therapeutic. Dieticians could coach and help MS patients to make the dietary changes. Beneficial outcome of AGE lowering on disease progression, number of relapses and patient wellbeing could eventually result in less hospitalizations and lower health care costs as a result. Ultimately, this will contribute to a better quality of life for MS patients.

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