Methylglyoxal and the brain microcirculation

Thesis summary

The brain is a highly metabolically active organ and is dependent on optimal delivery of oxygen and nutrients, which is reflected by its high vascularisation. The brain also requires optimal defence against pathogens, and therefore the vasculature is specialised to maintain this fine balance between demand and defence. Damage or disease of the small vessels of the brain, also referred to as cerebral small vessel disease (CSVD), will have detrimental consequences for brain functioning. This thesis focused on one of the most prevalent CSVD forms, the age and vascular risk factors associated type. These forms of CSVD are progressive, with no curing treatment available to date.

Age and diabetes are risk factors for developing CSVD. Methylglyoxal (MGO), a highly reactive by-product of glycolysis and a major precursor in the formation of advanced glycation endproducts (AGEs), is increased in the circulation of people with diabetes and further increases with age. MGO is known to play a role in microvascular damage in diabetes peripherally. However, the impact of MGO on the cerebral microvasculature, and its possible role in the aetiology of CSVD are unknown. Therefore, the overall aim of this thesis was to investigate the effect of MGO on the cerebral microvasculature.

A review of existing literature provided strong indications that MGO is toxic to brain microvascular endothelial cells and mural cells (**chapter 2**). The majority of published literature on this topic, however, has studied the effects of MGO in vitro with suboptimal experimental conditions.

To investigate the effect of MGO on the brain microcirculation in vivo, we firstly used young and healthy mice in which we increased plasma MGO concentrations two-fold to similar levels as what is observed in people with diabetes. We did not find any negative effects of increased plasma MGO on the brain microcirculation or cognitive function (**chapter 3**). We next increased endogenous formation of MGO by inducing hyperglycaemia in mice with or without an overexpression of glyoxalase 1, the major detoxifying enzyme of MGO. Here we found that increased MGO formation negatively affected visuospatial memory and reduced the neurovascular coupling response speed, which was attenuated by glyoxalase 1 overexpression (**chapter 4**). This shows that there are differential effects of MGO depending on whether the source is from endogenous formation, or exogenous application.

To determine whether the effects of MGO observed in mice translate to the human population, we investigated whether MGO levels measured in plasma were associated with imaging markers for CSVD and cognitive function in humans (**chapter 5**). For this, data from The Maastricht Study, a cross-sectional observational cohort study with oversampling of individuals with type 2 diabetes, was used. Here we found that people with intermediate, but not high, concentrations of MGO had significantly more lacunar infarcts compared to

people with low plasma MGO concentrations when adjusting for all appropriate confounding factors. We did not find any significant associations with other markers for CSVD (i.e., white matter hyperintensities larger than 3.0 mL and cerebral microbleeds), nor with cognitive function. We speculate the association between MGO and lacunar infarction to be due to the hypercoagulation effects of MGO.

As we found differential effects of MGO depending on the source, and we did not find any associations between plasma MGO and cognitive function, we investigated whether MGO from plasma is able to cross the blood-brain barrier. We firstly injected mice intravenously with MGO and measured MGO in the cerebral cortex several minutes to hours afterwards, however, we were unable to detect increases of MGO. Due to the highly reactive nature of MGO and the fact that the microcirculation only makes up a relatively small fraction of the brain, we investigated the passage of MGO in an in vitro model. Using physiologically relevant concentrations of pure MGO to mimic a post-prandial MGO spike, we found that MGO is not toxic. We found that MGO does in fact cross the brain endothelial monolayer, to a small extent (**chapter 6**). We therefore speculate that MGO may cross the blood-brain barrier, but because of its limited passage, it is unlikely to have any effect on brain function.

We conclude that MGO has differential effects on the brain microvasculature depending on the source of MGO, and that MGO from plasma by itself is unlikely to have a detrimental effect on the brain microcirculation or cognitive function. Furthermore, there is increasing evidence that MGO is not solely toxic, but may also confer beneficial effects on cellular metabolism by regulating glucose metabolism intracellularly. Dietary MGO intake also has been associated with improved insulin sensitivity and reduced inflammation. The effects of low MGO exposure and dietary MGO on the brain needs to be further investigated.

In this thesis, we emphasise the finding that the source matters: whether MGO is increased in plasma or formed in endothelial cells, each have different effects and consequences are very different, yet each of which has its own therapeutic interest. These findings are important to take into consideration for the design of future research. Critically, these findings reveal the potential of MGO as a therapeutic target in CSVD.