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Breaking the vicious cycle of epileptogenesis

Focus on brain inflammation and matrix metalloproteinases

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CHAPTER

9

Summary

Summary

Epilepsy is a brain disease, currently affecting 65 million people worldwide, characterized by an enduring predisposition to generate epileptic seizures. Epilepsy is an umbrella term including a heterogeneous group of brain diseases and can be caused by various factors, including genetic mutations and brain injury caused by trauma, stroke or status epilepticus (SE). The process that leads to the development of epilepsy and the progression of epilepsy after spontaneous seizures appear is called epileptogenesis and during that period multiple neurobiological changes take place resulting in a lower threshold for seizure activity. Patients mostly rely on antiepileptic drugs to reduce seizure frequency, but unfortunately, over 30% of people with epilepsy do not benefit from the available antiepileptic drugs. Moreover, treatments that prevent or cure epilepsy do not exist. Therefore, there is a need for novel therapies that are aimed at targets that can reduce or even halt the development of epilepsy. One of the main pathological characteristics that can be found in the epileptogenic brain is the activation of the inflammatory system. Moreover, damage to the blood-brain barrier (BBB) can lead to aberrant activity of inflammatory processes as well as increased neuronal excitability. Regulators of the extracellular matrix (ECM), such as matrix metalloproteinases (MMPs), are involved in the regulation of the inflammatory response as well as the integrity of the BBB. In this thesis, we aimed to investigate the involvement of the inflammatory system, BBB disruption and ECM dysregulation during epileptogenesis in order to find novel therapeutic targets. Additionally, we aimed to examine several therapeutic strategies using *in vitro* and *in vivo* models of epilepsy.

In **chapter 2**, we aimed to examine the involvement of the innate and the adaptive immune system in temporal lobe epilepsy (TLE) as well as during epileptogenesis. We observed high expression of several markers of the innate immune response, indicating that it is persistently activated in human TLE. Additionally, the increased expression of several of these markers were related to the number of spontaneous seizures in the post-SE rat model. This suggests that the innate immune system can contribute to epileptogenesis possibly via increased BBB permeability, while the adaptive immune response seems to play a less prominent role.

In **chapter 3** and **4**, we studied the (immuno)proteasome, which is responsible for intracellular protein degradation and is also involved in brain inflammation. We observed higher expression of both constitutive and inducible subunits of the (immuno)proteasome in TLE as well as in malformations of cortical development (MCD), including focal cortical dysplasia (FCD) and tuberous sclerosis complex (TSC). Moreover, increased subunit expression was positively correlated with seizure frequency in MCDs. As the activation of the mammalian target of rapamycin (mTOR) pathway is evident in these pathologies, we investigated whether reduction of mTOR activity with rapamycin could affect the expression of the (immuno)proteasome. We observed that rapamycin could attenuate inflammation-induced increase of (immuno)proteasome subunits *in vitro* and could reduce both (immuno)

proteasome expression and the number of spontaneous seizures in post-SE rats. This indicates that the mTOR pathway may represent an interesting drug target in genetic as well as acquired epilepsy.

In **chapter 5**, **6** and **7**, we investigated the expression of several MMPs, which are ECM regulatory proteins that are involved in inflammation and BBB regulation and are suggested to play an important role in the development of epilepsy. We observed increased RNA and protein expression of MMP2, MMP3, MMP9 and MMP14 along with their tissue inhibitors, TIMPs, in resected hippocampi of drug-resistant TLE patients and in cortical tubers of TSC patients, where it was associated with disruption of the BBB. Moreover, higher MMP expression was also seen in the post-SE rat model both before and after the occurrence of spontaneous seizures, indicating that the dysregulation of the MMP/TIMP proteolytic system is not only clinically relevant, but that there is also an interesting window of opportunity for treatment. In **chapter 5** and **6**, the therapeutic potential of inflammation-associated microRNAs (miRNAs) 155, 146a and 147b were investigated. In TSC-derived astrocyte cultures, overexpression of the anti-inflammatory miR146a or miR147b could attenuate inflammation-induced MMP3 upregulation and downregulation of TIMPs. In human foetal astrocytes, inhibition of miR155, which is highly expressed in the epileptogenic brain, could reduce MMP3 expression under inflammatory conditions. This suggest that these miRNAs deserve further investigation as potential therapeutic strategies for pathologies in which brain inflammation and ECM dysregulation is apparent, such as epilepsy.

The effects of MMP inhibition were further explored in **chapter 7**, in which the novel MMP2/9 inhibitor IPR-179 was tested in two *in vivo* models of epilepsy. In the rapid kindling rat model, treatment with IPR-179 resulted in less severe seizures during the kindling phase. In the absence of the drug, animals previously treated with IPR-179 had a persistent decrease of seizure severity following kindling, suggesting an antiepileptogenic effect of this MMP inhibitor. In the intrahippocampal kainic acid mouse model, IPR-179 treatment led to a reduction in seizure duration and number of seizures. Furthermore, these effects were persistent for at least 7 weeks after discontinuation of IPR-179. Moreover, seizure-induced memory impairment was attenuated in IPR-179-treated mice and this treatment did not lead to adverse effects in either rodent model. This indicates that IPR-179 has both anti-ictogenic as well as antiepileptogenic effects, which is very promising. Further drug development for clinical trials is ongoing.

The findings of these studies are discussed in **chapter 8**. Taken together, this thesis shows that neuroinflammatory processes as well as ECM regulation play important roles in the development and progression of epilepsy. We identified several potential novel targets, such as the innate immune system, the (immuno)proteasome and several MMPs. Furthermore, several therapeutic targets were evaluated using both *in vitro* and *in vivo* models and the mTOR inhibitor rapamycin, microRNA 155, 146a and 147b, and the MMP inhibitor IPR-179 are promising novel therapeutic strategies to be further explored in preclinical and clinical settings.