CHAPTER 8

Summary

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Chapter 1 provides a general introduction for this thesis entitled “Activated Microglia After Traumatic Brain Injury, The Neuroinflammatory Mastermind”. According to the World Health Organization (WHO), traumatic brain injury (TBI) will surpass many diseases as the major cause of death and disability by the year 2020 (1). Besides emotional, financial and social burden to the patients, their families and their social network, the burden of mortality and morbidity that TBI imposes on society, makes this heterogeneous disease with respect to cause, pathology, severity and prognosis, a pressing public health and medical problem.

Over the last decades, substantial progress has been made in a better understanding of the pathophysiological processes after TBI. It is nowadays widely accepted that TBI is not just a simple event but a complex entity with ongoing pathophysiological mechanisms (106, 152, 153). Posttraumatic neuroinflammatory sequelae involves two components: a cellular component, including microglia activation, and a second component regarding secretion of immune mediators (161). Microglia express a dual and opposing role after activation under pathological conditions. Probably depending on the type of signal emitted by the neuron in distress, activated microglia are the initiators of an endogenous auto-protective mechanism in order to rescue the neuronal cell (18), or turn out to be the key role player in initiating and continuing the acute posttraumatic neuroinflammatory response, characterized by a massive release of inflammatory cytokines, including interleukin (IL)–1β and IL-6, chemokines and complement activation proteins (17).

Because of the heterogeneity of the disease, animal models of TBI, classified in focal and diffuse brain injury, provide the opportunity to study posttraumatic pathophysiological mechanisms under stable and controlled environmental conditions, minimizing variability with regard to the mechanism of injury. We used the radionuclide *(R)*-[^{11}C]PK11195, that binds to the microglial translocator protein (TSPO), and positron emission tomography (PET) to measure microglia activation. As a reflection of the posttraumatic neuroinflammatory response, we monitored the release of IL-1β and IL-6 and excitatory amino acids (EAA) using cerebral microdialysis, a neuromonitoring

technique based on diffusion of substances from the extracellular fluid (ECF) through a semipermeable membrane into a perfusion fluid.

In **chapter 2** we established, as a proof of principle, extraction efficiency of IL-1ß and IL-6 by an *in vitro* microdialysis-perfusion system, using a polyether sulfon (PES) microdialysis probe, especially developed for recovery of macromolecules such as cytokines. To explore the feasibility of cytokine extraction from the ECF using cerebral microdialysis and to elucidate the temporal profile of extracellular IL-1ß and IL-6, rats were exposed to focal brain injury or sham procedure. It appeared that cytokine extraction was feasible *in vitro* after adding a carrier protein and that both IL-1ß and IL-6 was detectable using cerebral microdialysis. Our findings suggest that IL-1ß release precedes a gradual increase in expression of IL-6.

In **chapter 3** we investigated the posttraumatic microglia activation over time related to the release of glutamate. Rats underwent three sequential dynamic *(R)*-[^{11}C]PK11195 PET scans, at baseline and one and ten days after TBI. Glutamate in the ECF was measured using cerebral microdialysis in the acute posttraumatic phase and brains were processed for histopathology and (immuno)-histochemistry. We found that ten days after TBI, *(R)*-[^{11}C]PK11195 binding was significantly increased in TBI rats compared with both baseline values and sham controls. This increased cerebral uptake of *(R)*-[^{11}C]PK11195 followed a significant acute posttraumatic increase in ECF glutamate levels. Post mortal, significant differences were found between TBI and sham in representative examples of brain tissue stained for ED-1 (positive cells are highly activated microglia and/or macrophages), OX-6 (marker of activated microglia), GFAP (expressed in astrocyte cells), Perl's (indicative of haemorrhages), and Fluoro-Jade B (indicative of degenerating neurons).

In most studies *(R)*-[^{11}C]PK11195 binding has been quantified using reference tissue approaches. The purpose of the study described in **chapter 4** was to assess the validity of the simplified reference tissue model (SRTM) for analyzing *(R)*-[^{11}C]PK11195 studies in TBI, where blood-brain barrier (BBB) disruptions are likely. Dynamic *(R)*-[^{11}C]PK11195 scans were acquired at three time points after TBI. Plasma input derived binding potential

(BP_{ND}^{PI}), volume of distribution (V_T) and K_1/k_2, and SRTM derived BP_{ND}^{SRTM} were obtained. Simulations were performed to assess effects of varying K_1/k_2. We conclude that early after TBI, *(R)*-[^{11}C]PK11195 studies should be analysed using plasma input models.

The objective of the study described in **chapter 5** was to measure *(R)*-[^{11}C]PK11195 binding as an indirect marker of neuronal damage after TBI. We therefore acquired dynamic *(R)*-[^{11}C]PK11195 PET scans in TBI patients, six months after trauma. Supervised cluster analysis (SVCA4) was used to generate an appropriate reference tissue input. We found an increased whole brain binding of *(R)*-[^{11}C]PK11195 in TBI patients compared to healthy controls and regional analysis indicated that increased *(R)*-[^{11}C]PK11195 binding was widespread over the brain, indicative of diffuse neuronal damage.

In **chapter 6** we assessed longitudinal changes in microglia activation in traumatic brain lesions and surrounding perilesional zones. Therefore, we performed fully quantitative *(R)*-[^{11}C]PK11195 PET-scans with arterial sampling were performed in TBI patients, at three different time points: 7-10 days, one and six months after trauma. *(R)*-[^{11}C]PK11195 BP_{ND}, K_1/k_2 and V_T of traumatic brain lesions and surrounding perilesional zones were measured using a validated tracer kinetic model with metabolite-corrected plasma input function. Traumatic brain lesions were defined on magnetic resonance imaging scans. In the acute stage (7-10 days) in traumatic brain lesions and six months after TBI in perilesional zones, significantly higher *(R)*-[^{11}C]PK11195 BP_{ND} values were found compared to healthy controls. Increased *(R)*-[^{11}C]PK11195 BP_{ND} in perilesional zones after TBI indicates microglia activation and/or macrophage influx in these regions. Prolonged increase in *(R)*-[^{11}C]PK11195 binding in perilesional zones points to long-term posttraumatic microglia activation, which may be a therapeutic target for future neuroprotective studies.

Finally, the results of this thesis are discussed in **chapter 7**. *(R)*-[^{11}C]PK11195 PET imaging in TBI patients has some logistical and safety matters and methodological limitations, though, this neuroimaging technique provides a unique opportunity to monitor neuroinflammation *in vivo*, in time.

Promising novel TSPO radioligands in TSPO imaging are suitable as new biomarkers in evaluating microglia activation after TBI in the very near future. Future research in the field of TBI should focus on the dual and opposing role of activated microglia to mastermind the complex neuroinflammatory cascades in this, in many ways, devastating disease.