Chapter 1

General introduction
Neuroinflammation and nutrition

Neuroinflammation is a natural activation of the innate immune system of the brain aimed to restore disturbances of homeostasis, such as pathogen invasion and tissue damage. However, chronic and excessive neuroinflammation is believed to negatively affect brain functions, such as cognition, memory and mood. It is hypothesized that neuroinflammation plays a role in the pathology of several psychiatric and neurodegenerative brain diseases, such as depression, Alzheimer’s disease, Parkinson’s disease and cognitive decline [1–5].

Epidemiological studies demonstrate beneficial effects of some specific diets on the incidence of brain diseases in which neuroinflammation plays a role [6–9]. In recent years, there has been increasing interest in investigating nutrients that could be responsible for these protective effects [10–12]. These nutrients could be further investigated as dietary intervention for prophylaxis or treatment of brain diseases in which neuroinflammation is involved.

A short overview of anti-inflammatory vitamins, fatty acids and indigestible oligosaccharides

Recent studies have shown that food components, such as vitamins, omega-3 fatty acids, fibers and specific plant components, have anti-inflammatory properties [13,14]. Here we briefly discuss the most frequently investigated food components: fatty acids, vitamins and fibers (in particular indigestible oligosaccharides).

1. Fatty acids

1.1 Omega-3 fatty acids

The anti-inflammatory effects of omega-3 fatty acids, docosapentaenoic acid (DHA) and eicosahexaenoic acid (EPA), and the role of these fatty acids in modulation of microglia activation have been extensively studied in animal models of e.g. sepsis, brain ischemia, and aging [14–19]. The anti-inflammatory effects of omega-3 fatty acids observed in animals are often accompanied by beneficial effects on behavior, such as amelioration of memory impairment [20,21]. Omega-3 fatty acids are considered as promising candidates that may help to prevent or reduce
cognitive decline and depression [22–25]. The beneficial effects of omega-3 fatty acids could be mediated via an anti-inflammatory action of these nutrients, but may also be the result of their positive effects on neuronal signaling and plasticity. DHA is an indispensable component of the neuronal membrane, able to affect the lipid rafts and consequently to affect cell signaling [26].

Despite promising results in pre-clinical investigations, clinical studies did not provide compelling evidence for the beneficial effects of omega-3 fatty acids yet. Some studies have demonstrated that omega-3 fatty acids lower inflammation biomarkers in plasma and tissue in patients affected by inflammatory disease, but other studies did not show any effect of omega-3 fatty acids on inflammation [27,28]. This discrepancy could be due to differences in study design and statistical approach. Moreover, the clinical studies aimed to reverse the effects of already established inflammation, whereas animal studies usually aimed to prevent the onset of inflammation [28].

1.2 Phospholipids

Phospholipids are esters of fatty acids and the components of cell membranes. They play an important role in the function of membranes and intracellular proteins, receptors, ion channels and enzymes. Phospholipids are abundant in brain tissue and alterations in the phospholipid profile have been associated with diverse pathological processes. For example, altered phospholipid content is observed in the affected brain regions in patients with Alzheimer’s disease [29]. Some evidence from studies in humans suggests that phospholipids may have a positive effect on brain functions, such as memory and cognition [30–32]. In animals, phospholipids have also been shown to exhibit anti-inflammatory properties. The phospholipid phosphatidylcholine (lecithin) was shown to inhibit the production of tumor necrosis factor alpha (TNFα), nitric oxide (NO) and reactive oxygen species (ROS) by microglia that were stimulated with interferon gamma (IFN-γ) or β-amyloid [33]. The protective role of lecithin in inflammatory conditions was confirmed in lipopolysaccharide (LPS)-challenged rats, in which lecithin was able to reduce TNFα levels in plasma and NO levels in the brain [34]. Although a direct effect of lecithin and other phospholipids on neuroinflammation has not been demonstrated in patients yet, preclinical data indicate that these compounds are promising to be tested in humans [35].
2. Vitamins

2.1 Vitamin A

Vitamin A is essential for the normal functioning and maintenance of the immune system. Vitamin A deficiency is one of the most common nutritional deficiencies, which can induce inflammation or exacerbate existing inflammation [36]. Vitamin A is metabolized in the cell to its active form, retinoic acid (RA). RA acts as a transcription factor through binding to Retinoic Acid Receptors (RARs) [37]. In vitro studies have demonstrated the ability of RA to inhibit the release of proinflammatory markers from activated microglia [38] and astrocytes [39] via interactions with RARs and inhibition of the production of transcription factor, Nuclear Factor Kappa B (NF-κB). These effects were confirmed in animal models, in which strong systemic and/or central inflammation occurs. For example, in animal models for stroke [40], Alzheimer’s disease [41] and colitis [42] attenuation of disease progression due to inhibition of NF-κB by vitamin A was observed. Moreover, vitamin A is indispensable for normal brain functioning and has been shown to positively affect several neurogenesis pathways [43]. The potential role of RA in human diseases that are characterized by peripheral inflammation has been discussed in several reviews [44–46]. However, the beneficial effect of vitamin A on neuroinflammation has not been demonstrated in humans yet.

2.2 Vitamin D

The anti-inflammatory effect of vitamin D has been extensively discussed in recent reviews [47–50]. Vitamin D acts via vitamin D receptors (VDR). The anti-inflammatory and neuroprotective function of vitamin D has been demonstrated in vitro in microglia [51] and astrocytes [52], and in vivo in animal models with strong systemic and/or central inflammation such as colitis and stroke. Vitamin D supplementation in a rat model for colitis ameliorated disease progression and decreased the expression of proinflammatory cytokines by inhibition of apoptosis of intestinal epithelial cells [53]. In a rat model for stroke, a multi-nutrient supplementation containing elevated amounts of vitamin D decreased the brain damage and astrocyte proliferation [54], while vitamin D deficiency has been shown to increase brain injury and to deregulate the inflammatory response in a similar rat model of stroke [55]. There is also evidence for a positive impact of vitamin D on brain functions, such as cognition [56]. In humans, lower serum levels of vitamin D are associated with a higher incidence of mild cognitive
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impairment, multiple sclerosis, Parkinson’s disease and Alzheimer’s disease [57,58]. Therefore it has been suggested that vitamin D may play a role in the prevention or treatment of neurodegenerative diseases, because of, among others, its neuroprotective and anti-inflammatory properties [59].

2.3 B vitamins

Deficiencies in vitamins B$_6$, B$_9$ (folic acid) and B$_{12}$ have been reported to occur more frequently in patients with inflammatory disorders and neurodegenerative diseases [60,61]. Although these vitamins have never been shown to directly inhibit the activation of immune cells, they are involved in the metabolism of homocysteine. Depletion of vitamins B$_6$, B$_9$ and B$_{12}$ leads to increased levels of homocysteine [62]. High levels of homocysteine can lead to vascular dysfunction and consequently to vascular dementia [63]. In addition, homocysteine can trigger inflammatory processes in for example endothelial cells [64], microglia [65,66] and astrocytes [67] in vitro. In vivo, deficiency of vitamins B$_6$, B$_9$ and B$_{12}$, combined with excessive methionine consumption, leads to the development of hyperhomocysteinemia and vascular dementia with micro-hemorrhages and neuroinflammation [68]. Therefore, vitamins B$_6$, B$_9$ and B$_{12}$ appear to be indirectly involved in proper functioning of the immune system. Moreover, B vitamins are crucial for normal neuronal functioning, as a strong deficiency of vitamin B$_1$ can for example cause memory disorders (Korsakov syndrome).

3. Indigestible oligosaccharides

Dietary fibers are indigestible food components derived from plants. Components from dietary fibers like the indigestible galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) can act as prebiotics, which means that they are not digested, but stimulate the growth of gut microbiota. Gut microbiota are involved in maintaining the integrity of the intestinal barrier and regulation of the immune system. Disruption of the normal microbiota community in the gut can change gut permeability and thus increase the susceptibility for pathogen infection [69]. FOS and GOS were investigated in vitro and in vivo primarily with the aim to demonstrate beneficial effects on the intestinal barrier [70,71]. However, it has also been proposed that the beneficial effects of GOS and FOS are not only due to stimulation of gut microbiota and protection of the intestinal barrier, but could also be due to direct interaction with immune cells [72]. For example, GOS alone or in combination with FOS were shown to inhibit TNF$\alpha$ release from LPS-stimulated
peripheral blood mononuclear cells in vitro. However, the mechanism behind the direct modulation of immune cells by oligosaccharides remains unclear.

There is increasing evidence suggesting a connection between gut and brain inflammation. Therefore, it is conceivable that indigestible oligosaccharides may be able to affect brain physiology and behavior. FOS and GOS supplementation could elevate the levels of brain derived neurotrophic factor in the hippocampus of rats [73]. Indigestible oligosaccharide can also affect behavior, as demonstrated in LPS-treated mice. In this study, GOS not only inhibited the inflammatory response and normalized cortical 5-HT2A receptor and IL1-β concentration, but also reduced LPS-induced anxiety behavior [74]. A study in patients with minimal hepatic encephalopathy showed that FOS supplementation improved the score in the neuropsychological tests [75,76]. Supplementation of GOS (but not FOS) suppressed the neuroendocrine stress response (cortisol) in healthy volunteers [77].

There are promising preclinical studies indicating that indigestible oligosaccharides may exert anti-inflammatory properties and modulate brain physiology and behavior, however some other studies did not show any effects and consequently it is difficult to conclude whether indigestible oligosaccharides could have any therapeutic efficacy in humans. So far only a few clinical studies with indigestible oligosaccharides have been conducted and they do not provide sufficient information on anti-inflammatory effects of indigestible oligosaccharides in human diseases.

**In vivo investigation of the effects of nutrition on neuroinflammation**

The in vivo effect of a diet on neuroinflammation can be investigated in animal models. This allows preclinical evaluation of the efficacy of a nutritional intervention and assessment of the suitability of the intervention for clinical trials. The ability of the dietary intervention to affect neuroinflammation can be assessed by measuring the markers of neuroinflammation (activated astrocytes and microglia, proinflammatory signaling molecules) after the application of a diet. This can be investigated with diverse methods, like for example:
• post-mortem tissue analysis: immunohistochemistry, western blot, flow cytometry, enzyme-linked immunosorbent assay (ELISA).

• non-invasive imaging methods, e. g. positron emission tomography (PET), single photon emission computed tomography (SPECT), computed tomography (CT) and magnetic resonance imaging (MRI). The main advantages of non-invasive imaging are the possibilities (I) to investigate the inflammatory process in the intact organism and (II) to translate the results from animal studies to human studies, because the same imaging method can be applied in both species.

The principle of PET imaging

Positron emission tomography (PET) is a highly sensitive, non-invasive imaging technique to visualize, characterize and measure biological processes at the molecular and cellular level. PET imaging is used for research and diagnostic purposes [78]. This method is based on the detection of positrons emitted from radioactive nuclei.

Probes used in PET imaging (tracers) are biologically active substances, in which a radioactive isotope is incorporated. The most widely used isotopes for PET imaging are $^{11}$C, $^{18}$F, $^{89}$Zr, $^{13}$N, $^{15}$O, $^{64}$Cu, $^{62}$Cu, $^{124}$I, $^{76}$Br, $^{82}$Rb, $^{68}$Ga. All isotopes used in PET imaging are emitting $\beta^+$ radiation and have relatively short half-life of radioactive decay (the time in which half of the radioactivity is decayed), ranging from 1.27 min for $^{82}$Rb to 4.2 days for $^{124}$I. During $\beta^+$ decay, a proton (positively charged) is converted into a neutron (no charge), while emitting a positron and a neutrino. A positron is a particle with the same mass as an electron, but with an opposite charge. Positrons undergo annihilation with electrons in the tissue. Annihilation leads to the transformation of the mass of the electron and the positron into energy (1022 keV), according to Einstein’s formula $E=mc^2$. The energy is generated as two photons with an energy of 511 keV that travel in opposite directions (figure 1). The PET camera consists of a ring of detectors which register the emission of the two photons by coincidence detection (i.e. detection of the photons must be within the short time window by two approximately opposite detectors). Based on this principle, the origin of the radioactive decay can be reconstructed and thus the distribution of the tracer in the body can be assessed.
Due to their short half-life, the positron-emitting isotopes are produced with a generator or a cyclotron shortly before their use. For example, $^{18}$F can be produced from a natural, stable isotope of oxygen, $^{18}$O, by bombardment with high energy protons. $^{18}$F is released in a water solution and used for further synthesis of radiotracer. $^{11}$C is obtained from $^{14}$N atoms bombarded with high energy protons and is released in form of carbon dioxide ($^{11}$CO$_2$) or $^{11}$C-methane ([$^{11}$C]CH$_4$), which have low chemical reactivity, and are converted to more reactive species for the tracer synthesis.

For image acquisition, tracers are injected intravenously and their distribution through the body is detected by a PET camera.

**Figure 1** The principle of PET.

The information from the coincidence detection is used to create a 3D image in which the distribution of radioactivity (therefore, the tracer) across the body is visualized. PET images can be used for (I) visual assessment of the tracer distribution, e.g. for tumor detection; (II) semi-quantitative tracer uptake measurement (standardized uptake value), e.g. for therapy evaluation; (III) quantification of a biochemical of physiological parameter with kinetic modelling (binding potential and volume of distribution), e.g. for assessment of receptor expression or enzyme activity.

**PET imaging of neuroinflammation**

$^{[18F]}$FDG (2'-$^{[18F]}$fluoro-2'-deoxyglucose) is a $^{18}$F-labelled glucose analogue used for PET imaging of glucose metabolism. It is the most commonly used tracer in
clinical PET imaging, usually for detection of tumors or inflammation. However, glucose is also utilized as energy source by the brain, therefore FDG is not a specific probe for imaging of inflammation in the brain. For brain imaging, increased $[^{18}\text{F}]$FDG uptake can indicate for example inflammation, or increased neuronal activity, while decreased $[^{18}\text{F}]$FDG uptake can indicate brain damage (e.g. following ischemic stroke) or decreased neuronal activity (e.g. it can reflect decreased activity or cognitive decline). Therefore, $[^{18}\text{F}]$FDG uptake in the brain could be affected by several factors, including inflammation, changes in behavior and brain damage.

There are also more specific probes for detecting inflammation in the brain. For example, $[^{11}\text{C}]$PK11195 and $[^{11}\text{C}]$PBR28 are $^{11}\text{C}$-labelled probes, in which one of the carbon molecule was replaced by its radioactive $^{11}\text{C}$ isotope (figure 2).

**Figure 2** The structure of $[^{11}\text{C}]$PK11195 and $[^{11}\text{C}]$PBR28.

$[^{11}\text{C}]$PK11195 and $[^{11}\text{C}]$PBR28 are antagonists of translocator protein 18 kDa (TSPO) receptor. TSPO receptors are expressed on the outer membrane of mitochondria. Increased TSPO expression has been shown to be upregulated in activated immune cells, such as macrophages, microglia and astrocytes. It is therefore used as a marker of neuroinflammation.

In this study, we used PET imaging of glucose metabolism and TSPO receptors to investigate the effects of dietary intervention on brain glucose metabolism and neuroinflammation.
Aim and outline of this thesis

The objective of this thesis is to evaluate the potential therapeutic effect of multi-nutritional dietary intervention on inflammation in animal models of brain diseases in which neuroinflammation is involved. In the first part of the thesis, we focus on specific food components and investigate their anti-inflammatory properties in vitro. The main objective is to identify possible combinations of nutrients which could have stronger anti-inflammatory effects than the individual components. In the second part of the thesis, we investigate the efficacy of dietary intervention in animal studies.

Outline of the thesis

Chapter 2 describes results of an in vitro study on the effects of well-known vitamins and fatty acids on the cytokine release by activated microglia cells. We also compare the efficacy of individual nutrients with different combinations of sub therapeutic concentrations of these components. In chapter 3 we review the anti-inflammatory properties of rice bran components, as these substances are emerging as promising anti-inflammatory compounds that may warrant further investigation. In chapter 4, an in vitro study on the ability of diverse combinations of rice components to inhibit cytokine release by LPS-stimulated microglia is discussed. The results of these in vitro experiments form the basis for the composition of the anti-inflammatory dietary intervention that was tested in the second part of this thesis.

In chapter 5, we present the results of in vivo experiments aiming to evaluate the feasibility of \( [1^{11}C] \)PBR28 PET for imaging of peripheral and central inflammation in chemically-induced colitis in rats. In this study, we investigate the suitability of the animal model and the imaging technique for dietary intervention studies. By demonstrating the presence of neuroinflammation during colitis we aim to support the hypothesis that central inflammation can be responsible for the comorbidities between inflammatory diseases and brain disorders.

In the last two experimental studies in this thesis, we investigate the main goal of this thesis: the therapeutic effect of a dietary intervention aimed to modulate neuroinflammation. We used animal models of diseases for which
neuroinflammation is considered a promising therapeutic target. In chapter 6, we discuss the effects of the dietary intervention in a rat model of postoperative cognitive decline. Postoperative cognitive decline is a brain disorder associated with major surgery that causes peripheral inflammation, which can subsequently induce neuroinflammation. In chapter 7, we discuss the effects of dietary intervention in rat model of stroke. The role of neuroinflammation in the disease progression after the onset of stroke is well documented in preclinical investigations and some human studies. It is believed that the neuroinflammatory response is beneficial at the early stage following stroke, but persistent, chronic inflammation at later stages following stroke has a negative impact on neuronal survival, especially in the area at risk surrounding the lesion.

References


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