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## The impact of nutrition on neuroinflammation in vitro and in vivo

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# *Chapter 8*

## **English summary**

## **Neuroinflammation and nutrition**

Neuroinflammation is an activation of the innate immune system of the brain that is necessary to restore disturbances of homeostasis. However, chronic and excessive neuroinflammation can have detrimental effects on brain function and plays an important role in several psychiatric and neurodegenerative disorders. Therefore, neuroinflammation is believed to be an attractive target for future treatment strategies against these brain diseases.

Nutrition can have impact on the inflammatory process. Epidemiological studies point towards a beneficial effect of specific diets on the incidence of brain diseases in which neuroinflammation plays a role. For this reason, there has been increasing interest in recent years in investigating nutrients that could be responsible for these protective effects.

Food components can exert anti-inflammatory properties through several mechanisms, such as direct interaction within the brain or via indirect pathways mediated by peripheral immune cells or gut microflora. Given the diversity in biological effects and mechanisms of action of nutrients, we hypothesized that combining diverse food components acting on convergent anti-inflammatory pathways might be a suitable approach to design an effective nutrition-based anti-inflammatory intervention. Based on a literature survey, a nutritional concept has been formulated. The nutritional concept investigated in this study consists of several food components known to have anti-inflammatory properties, including vitamins, omega-3 fatty acids and rice bran components. The components of this concept are known to act either directly on the immune cells or interact indirectly, for example via gut microbiota.

### **The aim of this thesis**

- The objective was to evaluate potential therapeutic effect of multi-nutritional dietary intervention on inflammation in animal models of brain diseases in which neuroinflammation is involved.

## ***In vitro* experiments on activated BV2 cells**

In chapter 2 we describe *in vitro* experiments with diverse combinations of nutrients, which have been described to exert anti-inflammatory properties, such as vitamins A, B<sub>6</sub>, B<sub>9</sub>, B<sub>12</sub> and D, the fatty acids docosahexaenoic (DHA) and eicosapentaenoic (EPA) and the amino acids L-tryptophan and L-cysteine. The objective of this study was to evaluate possible additive anti-inflammatory effects on activated microglia. BV-2 immortalized mouse microglia cells activated with lipopolysaccharide (LPS) were used as an *in vitro* model of neuroinflammation. *In vitro* testing of individual nutrients demonstrated a direct anti-inflammatory effect of vitamins A and D, and fatty acids DHA and EPA on LPS-stimulated microglia. These anti-inflammatory effects were manifested by a reduction of the LPS-stimulated release of the pro-inflammatory markers, nitric oxide (NO) and interleukin-6 (IL-6). Mechanistic studies on the anti-inflammatory effect of vitamin A were performed on activated BV-2 cells in order to complement the available literature data. Inhibition studies demonstrated the involvement of RAR $\alpha$  in the anti-inflammatory action of vitamin A. The main objective of this *in vitro* study was to evaluate possible additive effects of the investigated nutrients. For this purpose, anti-inflammatory properties of the combination of vitamins A and D, and fatty acids DHA and EPA were compared to the effectiveness of the single nutrients. All the nutrients were used in a concentration that does not cause a significant anti-inflammatory effect when used as a single nutrient (sub-effective concentration). The combination of substances, however, caused a significant decrease in LPS-stimulated NO release from BV-2 cells. These results demonstrate that *combining* sub-effective concentrations of vitamins and fatty acids can enhance their activity and cause a significant overall anti-inflammatory effect.

### Rice bran components as anti-inflammatory nutrients

In chapter 3, rice bran components are reviewed as emerging anti-inflammatory nutrients. Indigestible oligosaccharides from rice bran, such as galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) have been demonstrated to modulate gut microbiota, to protect the intestinal barrier and to exert anti-inflammatory properties. Recent clinical studies have demonstrated that these oligosaccharides also have central effects, as is evident from the observed changes in behavior and cognition. Phytosteryl pterulates (gamma-oryzanol,  $\gamma$ -OZ) and ferulic acid are rice bran components which have also been shown to modulate

inflammatory response of the immune cells *in vitro* and in animals. Isoprenoids, such as geranylgeraniol (GGOH), farnesol (FOH) and geraniol (GOH), present in rice and in other plants, have been shown to exert anti-inflammatory properties, mainly in *in vitro* studies. Taken together, rice bran components seem to be promising anti-inflammatory nutrients, but their mechanism of action is still poorly understood.

In chapter 4 the results from the *in vitro* investigation of rice bran components ( $\gamma$ -OZ, GGPP, FA and GGOH) alone or in combination with vitamins A and D and fatty acids DHA and EPA are presented. In a similar approach as described in chapter 2, the nutrients were investigated for their ability to reduce LPS-induced release of NO and IL-6 by BV-2 cells. This study has demonstrated that high concentrations of single rice bran components are able to modulate the release of these pro-inflammatory markers (especially GGPP and FA). Combination  $\gamma$ -OZ, GGPP, FA and GGOH did not significantly enhance their anti-inflammatory effect. However, combining these substances with vitamin D, at concentrations at which they individually had little effect (sub-effective), resulted in a trend towards enhanced anti-inflammatory efficacy. Thus, combining different nutrients acting on the convergent anti-inflammatory pathways may lead to an increased anti-inflammatory effect.

In conclusion, the *in vitro* studies discussed in chapter 2 and 4 have demonstrated anti-inflammatory effects of several individual nutrients. These anti-inflammatory effects could be enhanced when combinations of nutrients were used, resulting in efficacy at concentrations where individual nutrients were ineffective.

In the second part of the project we aimed to investigate the impact of nutrition on neuroinflammation and diseases progression in animal models. The suitability of an animal model for nutritional intervention is determined by the severity of the inflammatory response: i.e. the model should present sufficient neuroinflammation to allow detection of subtle immune-modulating effects, but should not have excessive neuroinflammation that would prevent any efficacy of the intervention. In chapter 5, we investigated the suitability of an animal model of colitis for this purpose.

## **Crosstalk between the gut and the brain: neuroinflammation in animal models of peripheral diseases.**

Neuroinflammation is believed to be involved in pathology of brain diseases, such as depression and neurodegenerative disorders. Since a high rate of comorbidity of some peripheral immune-related diseases (e.g. colitis) with depression is observed, it has been hypothesized that peripheral inflammation can lead to inflammation in the brain via the gut-immuno-brain axis. This hypothesis suggests that brain associated comorbidity of peripheral inflammatory diseases could be mediated via induction of neuroinflammation.

In the study described in chapter 5, we investigated whether gut inflammation in colitis can cause neuroinflammation in a rat model of TNBS-induced colitis. The main goal of this experiment was to assess the feasibility of PET imaging to detect peripheral and central inflammation in this model and, if successful, to use this model to assess the efficacy of dietary intervention in the gut and the brain. The reason for applying non-invasive PET imaging in colitis was the possibility to design longitudinal studies aimed to monitor dynamic changes in the inflammatory process and the effects of intervention thereon in the same subject over time. The method would also allow easy translation to human studies.

Intrarectal administration of TNBS in rats caused the induction of characteristic symptoms for colitis (loss of bodyweight, diarrhea, rectal bleeding). Monitoring of peripheral and central inflammation was performed using PET with the TSPO tracer [ $^{11}\text{C}$ ]PBR28. This imaging method was chosen because colitis is accompanied by infiltration of activated macrophages; TSPO is overexpressed by activated macrophages, microglia and astrocytes in response to inflammatory stimuli. PET imaging did not demonstrate any significant increase in the tracer uptake in the brain or in the gut. However, ex-vivo biodistribution studies demonstrated an increase in [ $^{11}\text{C}$ ]PBR28 uptake in cecum and descending colon of animals with colitis, suggesting that [ $^{11}\text{C}$ ]PBR28 could detect subtle inflammation in the gut ex-vivo, but not in-vivo. The possible reasons for this apparent discrepancy might be spill-over effects that blurred the imaging signal due to insufficient resolution of the PET camera. Moreover, ex-vivo biodistribution studies of the brain showed significantly enhanced tracer uptake in cerebellum, but not in

other brain regions, indicating that colitis in this animal model was indeed accompanied by a mild immune response in the brain.

The subtle inflammation detected in this study, however, makes it difficult to robustly measure any beneficial effects of intervention and therefore we considered TNBS-induced colitis a not suitable model for investigation of dietary intervention aimed to modulate inflammatory processes. Therefore, we investigated another model of peripheral inflammation for dietary intervention: postoperative cognitive decline (POCD). In this model, the association between peripheral inflammation with neuroinflammation has recently been described.

## **Dietary interventions targeting neuroinflammation**

Postoperative cognitive dysfunction (POCD) is a common complication after surgery that can have long-lasting negative impact on the patient's quality of life. Although the underlying mechanisms are still unknown, evidence suggests that neuroinflammation may mediate cognitive impairment following surgery. The rat model of POCD used in this study aimed to mimic major surgery in humans. The goal of the study described in the chapter 6 was to confirm the presence of neuroinflammation in a rat model of POCD by PET imaging and to investigate the anti-inflammatory effects of a specific multi-nutrient supplementation diet containing anti-inflammatory ingredients (i.e. investigational diet, formulated based on the nutritional concept described in chapters 2 – 4). In addition, we investigated whether this diet was also able to prevent or moderate the symptoms of POCD and the concomitant alterations in brain metabolism. Experimental animals were subjected to one of three dietary regimens: control diet for the whole experiment, or investigational diet starting either 2 weeks before (pre-operative treatment) or immediately after the surgical intervention (post-operative treatment).

In accordance with the previous studies abdominal surgery caused significant bodyweight loss, reduced mobility, increased anxiety (measured with the open field test) and a trend towards decreased spatial memory (measured with the novel location recognition test). [ $^{11}\text{C}$ ]PK11195 PET imaging and immunohistochemistry confirmed the presence of neuroinflammation in several brain regions after surgery. [ $^{18}\text{F}$ ]FDG PET imaging revealed increased brain metabolism in part of the cortex,

pons and amygdala, whereas reduced metabolism was observed in the motor cortex, somatosensory cortex and striatum.

Dietary intervention started after surgery reversed astrocyte activation in cerebellum and the periventricular zone and decreased brain metabolism in the piriform cortex, but it had no beneficial effect on anxiety and spatial memory. Dietary intervention started prior to surgery had a positive impact on recovery, resulting in faster gain in body weight and normalization of exploratory behavior and spatial memory. This improvement was accompanied by reversal of astrocyte activation in the periventricular zone – but not in other brain regions – and normalization of brain metabolism in part of the motor cortex.

This study shows that major surgery can be accompanied by neuroinflammation and changes in glucose metabolism in several brain regions. Preventive intervention with a diet containing elevated amounts of anti-inflammatory nutrients can affect neuroinflammation and brain metabolism and has a positive effect on the recovery from abdominal surgery in rats.

Finally, we also investigated the effectiveness of the dietary intervention in stroke, a central nervous system disease in which the role of neuroinflammation in disease progression is well described. Stroke is a leading cause of death and disability worldwide. Since effective treatment options are limited, there is a lot of interest in developing new therapeutic strategies against stroke. One of the possible targets for stroke management is neuroinflammation. As discussed in the chapter 7, the photothrombotic model of stroke in rats was used to monitor the possible therapeutic effects of dietary intervention. Rats were subjected to one of three diet regiments: control diet for the whole experiment, or investigational diet starting either 2 weeks before (pre-ischemic treatment) or immediately after the stroke induction (post-ischemic treatment).

The induction of ischemia caused transient lateral movement impairment on day 3, which was normalized on day 6 and 20. Photothrombotic stroke induced focal brain damage, surrounded by strong and persistent astrocyte activation. Stroke was accompanied by decreased glucose metabolism in the contralateral hemisphere on day 7, but not on day 21. The investigational diet applied two weeks before the induction of ischemia did not affect astrocyte activation on day 7, but it increased brain glucose metabolism in the ipsilateral hemisphere. When the investigational diet was started immediately after the induction of ischemia, astrocyte activation

was even further increased on day 7, while glucose metabolism was not affected. Both treatments with the investigational diet reduced astrocyte activation on day 21 after the induction of ischemia, but did not affect glucose metabolism. Lesion size was not significantly affected by the dietary intervention on day 7, but the post-ischemic dietary intervention prevented the lesion growth between day 7 and 21.

This study reveals a potential beneficial effect of an investigational diet containing elevated amounts of specific anti-inflammatory nutrients on the recovery from ischemic brain damage, when the dietary intervention was started immediately after stroke.

## **Concluding remarks**

The main conclusion of this study is that the investigated nutritional concept can modulate inflammation *in vitro* and *in vivo*.

- *In vitro* studies have demonstrated additive anti-inflammatory effects between some of the diet components like vitamins A, D and fatty acids, DHA and EPA and trends towards enhanced anti-inflammatory properties between rice bran components and vitamin D. Therefore, dietary intervention should focus on diets with specific combinations of anti-inflammatory components.
- As demonstrated in animal studies in the POCD and stroke models in rats, the investigational diet can not only modulate neuroinflammation *in vivo*, but can also normalize other disease parameters (e.g. anxiety behavior in POCD and lesion growth in stroke).