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The impact of nutrition on neuroinflammation in vitro and in vivo

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Chapter 10

Future perspectives

The studies described in this thesis aimed to provide preclinical evidence for the hypothesis that nutritional intervention can be applied for prevention or treatment of brain diseases in which inflammation is involved. The main findings from these studies are that:

(I) combining different food components acting on convergent anti-inflammatory pathways can enhance their anti-inflammatory effects (chapter 2 and 4);

(II) multi-nutrient dietary intervention can modulate inflammation in the brain in animal models (chapter 6 and 7).

In the *in vivo* part of this study we used an investigational diet, consisting of a combination of specific nutrients that were selected based on the literature data and our own *in vitro* experiments. The investigational diet was not only able to modulate neuroinflammation, but also to affect the symptoms associated with the investigated disease models. However, this concept requires further preclinical investigation aimed to further explore its beneficial effects on brain function and disease symptoms, such as memory, anxiety or depressive-like behavior. The ultimate goal of this research would be to apply the nutritional concept in a clinical trial. Since all nutrients investigated in this thesis are part of our regular diet, the nutritional intervention could be readily applied in clinical trials without the necessity of toxicological studies.

1. *In vitro* investigation of combinations of anti-inflammatory nutrients

Epidemiological studies demonstrated positive effects of certain diets on the incidence of several inflammation-related diseases [1–3]. Consequently, there is increasing interest in identifying the specific food components that are responsible for these effects.

Interaction of individual nutrients with different signaling pathways can increase the anti-inflammatory effect of a dietary supplementation, as was discussed in chapter 2, 3 and 4. Individual components can complement each other, resulting in additive or even synergistic effects. As a result, lower concentrations of the active nutrients can be used to achieve a beneficial effect. Some food components, like the fat-soluble vitamins A, D, E and K, can be toxic when used in high concentrations.

Multi-nutrient supplementation with low concentrations of individual nutrients will be a much safer approach, than administration of high doses of a single component.

The approach of *in vitro* screening of nutrients presented in chapter 2 and 4 can also be applied for new compounds with possible anti-inflammatory properties. Evaluation of nutrients on immortalized cell lines allows testing of a large number of compounds and combinations thereof. The use of immortalized microglia cell lines, rather than isolated primary microglia, in the early steps of evaluation of new components is recommended for practical (faster screening) and ethical reasons (no animals have to be sacrificed for the isolation of primary microglia).

For the characterization of possible additive effects of different combinations of compounds, we proposed to test mixtures of substances at concentrations where they do not exert significant effects on their own (sub-effective). This approach increases the detection window of possible additive or synergistic anti-inflammatory effects by the combination of nutrients. The ingredients can be chosen based on their individual mechanism of action.

2. Elucidation of mechanisms of action of food components

In order to design an effective nutritional intervention, evaluation of the mechanism of action of nutrients is an indispensable step to optimally benefit from the complementary effects of different components.

From the nutrients investigated in this study, the mechanism of action of vitamins A, B, D and fatty acids have been extensively investigated. On the other hand, rice bran components have been scarcely studied so far and consequently little is known about their anti-inflammatory mode of action. A study can be designed to gain more insight in the mechanisms of action of these and other nutrients. This can be achieved *in vitro* or *in vivo* by:

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2.1. Analysis of the metabolites and metabolic pathways of the nutrients.

The anti-inflammatory effect of a nutrient can be due to a direct effect of the nutrient itself, but can also be due to the effect of an active metabolite. Therefore, it is important to characterize the metabolism of the nutrient. The type of analysis

will depend on the chemical characteristics of the nutrient and its metabolites. Since many anti-inflammatory food components are lipids (e.g. fatty acids, rice components), a lipidomic analysis can be applied in which lipid components can be separated and analyzed. Separation of lipid components from cell, tissue or blood samples can be done with chromatographic techniques like (I) gas chromatography (GC), which can be used for the analysis of small molecules, (II) high performance liquid chromatography (HPLC) [4], or (III) thin layer chromatography (TLC). Separated lipid components can be further analyzed with mass spectrometry (MS). Combination of chromatography and MS is widely used for lipid characterization in plasma, tissue (e.g. brain) or cerebrospinal fluids. Moreover, since the transport, biosynthesis and modification of lipids involves proteins, combining lipidomics with proteomics allows better understanding of metabolic pathways of the lipids [5]. Proteomic analysis can be performed with SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) followed by MS analysis of separated proteins [6].

Another method to analyze metabolism is to label the compound of interest (in this case, nutrient) with a radioactive isotope, such as ^{3}H or ^{14}C [7–9]. This allows tracking the nutrient and its metabolites in the tissues, blood or urine.

2.2. Analysis of the interaction of the nutrient or their active metabolites with receptors and signaling pathways

After obtaining information about the metabolism of the nutrient, the anti-inflammatory properties of the main metabolites can be screened to determine if metabolites might have a therapeutic effect. Next, the interaction of the nutrient or its active metabolite with specific receptors and signaling pathways can be investigated.

2.2.1. Binding studies

Binding studies with selective competitors may be applied in order to test the interaction of nutrients with specific receptors involved in relevant anti-inflammatory pathways. Alternatively, the pharmacological response of a nutrient-receptor interaction can be monitored.

2.2.2. Analysis of expression/concentrations of signaling molecules

Analysis of gene expression or proteomics allows detection of the increased expression of specific receptors or signaling molecules following the treatment with a particular nutrient. These receptors can be further investigated in cells and tissues by western blot, immunohistochemistry or flow cytometry.

2.2.3. Inhibition of key mediators in specific signaling pathway

The interaction of the nutrient with a receptor and the resulting physiological effects can be explored *in vitro* and *in vivo* by inhibition of receptor signaling by chemical blocking with a specific antagonist, or inhibition of receptor expression by gene knockout or treatment with antisense RNA. Chemical inhibitors provide a very effective approach, as was demonstrated for the interaction of vitamin A with retinoic acid receptors (RAR) in this project (chapter 2). Chemical inhibitors, however, may not be specific to only one target. Therefore, this data may need to be confirmed with more specific approaches, such as gene knockout or antisense RNA targeting specific receptors.

In vitro findings on receptor interactions need to be validated in animal models, because the effects observed *in vitro* may be compensated by alternative mechanisms *in vivo*. For example, previous studies with chemical inhibitors have demonstrated that partial inhibition of RARα can be compensated for *in vivo* by increasing RARβ and RARγ expression [10]. In contrast, knockout of RARα did not induce any compensation mechanisms. Therefore, a similar approach as for *in vitro* studies (chemical blocking, gene knockout or antisense RNA) could be applied *in vivo* to confirm the data from cell studies.

3. Animal studies with dietary interventions

The anti-inflammatory properties of nutrients observed *in vitro* can be validated in animal models. Some *in vitro* results may be false positive for nutrients forinstand as they might not sufficiently penetrate brain tissue *in vivo*. On the other hand, some *in vitro* data may also be false negative for nutrients which do not act directly on microglia (the target cell type we investigate), but exert their effect via an indirect pathway.

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3.1. Timing

Nutrition can be considered as a prophylactic tool for prevention of brain diseases, as suggested by the outcomes from epidemiological studies on the effect of diets on the incidence of brain diseases [1-3]. However, preclinical investigation show that dietary intervention can also be applied after the onset of disease and still have a therapeutic effect on disease progression. Therefore, in this project we investigated both prophylactic and therapeutic treatment designs with the investigational diet (as described in chapter 6 and 7). Both strategies resulted in beneficial effects and therefore both approaches should also be included in the future preclinical studies.

3.2 Type of intervention

The two main approaches to investigate the effects of the diet *in vivo* are: (I) supplementation or (II) depletion/elimination of the specific nutrients. Both approaches may provide important information about the role of specific nutrients in healthy animals and during disease pathology. Depletion/elimination studies may provide information about the role of essential nutrients during inflammation. On the other hand, supplementation studies can provide information on the possible use of increased concentrations of nutrients as a prophylactic or therapeutic tool.

3.3. Toxicity

Although normal concentrations of nutrients can generally be used safely in humans, for some nutrients, in particular those that have been poorly investigated (such as rice bran components or novel plant components), are applied in high doses or can potentially accumulate in the organism (such as fat-soluble vitamins), toxicity studies might still be required. Toxicity can be determined in animal studies in order to identify possible side effects of the substances and to determine the dosage window that can be used safely.

3.4. The choice of the disease model

The primary criteria for choosing of disease model for preclinical investigations is its suitability for translation to the human pathology in which neuroinflammation is involved. Therefore, we have chosen models mimicking the human pathology. Photothrombotic stroke model in rats mimics the mechanism of the onset of this disease in humans, the formation of thrombi which blocks the blood supply in part

of the brain. In postoperative cognitive decline model in rats we mimic the general surgery in humans which may lead to the cognitive decline.

3.4.1. Severity of inflammation

The animal models used for anti-inflammatory diet intervention must be validated for the presence of inflammation and there should be evidence that neuroinflammation is instrumental in disease progression. Moreover, the animal models used for dietary intervention should be characterized by relatively low severity. We expect that the effect of a nutritional intervention might be subtle and therefore may not be able to significantly suppress excessive neuroinflammation due to a possible ceiling effect. Choosing animal models with low severity increases the chances of successful modulation of inflammatory process with a dietary intervention (Weber's law). Although it seems to be an optimal approach for proof-of-concept study, the results need to be confirmed in a model that more closely mimics the extent of inflammation in patients.

3.4.2. Behavior

To complement our data on the modulation of neuroinflammation, more emphasis on behavioral improvement could be put in future experiments. The ultimate goal of anti-inflammatory dietary intervention is to improve *brain function*, ameliorate disease symptoms and stop disease progression via modulation of neuroinflammation. This can be achieved by designing adequate behavioral studies. Behavioral tests are in general more sensitive to confounding factors (and therefore the data contain more noise) than imaging or *in vitro* tests and therefore larger sample sizes may be required. For example, in chapter 6 we investigated spatial memory impairment after abdominal surgery in rats, but did not find statistically significant abnormalities with a sample size of n=8, whereas PET imaging and immunohistochemistry did reveal significant effects. The sample size in this study was calculated based on the imaging data, not on behavioral tests. Therefore, in the future studies, the sample size should not only be estimated for imaging, but also for the behavior tests. The exact sample size could be estimated based on the results of the abdominal surgery obtained in this study.

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Another parameter that is important for model selection is the temporal progression of symptoms. Behavioral symptoms that persist over a longer period of time will help to investigate the effect of the diet, especially if the dietary

intervention is applied after the disease onset. For example, in chapter 7, we observed that the severity of our photothrombotic stroke model was relatively low and that lateral motor impairment was only present up to three days following ischemia. More persistent lateral motor impairment (as observed in a slightly more severe stroke model) may increase the possibility to detect the modulation of this symptom by dietary intervention in the future studies. Since the severity of photothrombotic model can be modulated by the time of irradiation, we could consider increasing the irradiation time and therefore, aggravating the resulting brain damage in this model in order to obtain longer lasting behavior effects.

3.4.3. Disease models for psychiatric disorders

In this thesis, we investigated the dietary intervention in a POCD and a stroke model. Since neuroinflammation is believed to be involved in many psychiatric and neurodegenerative disorders, the investigational diet could also be tested in other animal models in which the presence of neuroinflammation has been established. Several animal models of mood disorders or cognitive decline can be considered for future dietary intervention. Some examples are: lipopolysaccharide (LPS) induced anxiety- and depressive-like behavior in mice or rats [11,12], repeated social defeat-induced depressive-like behavior [13], unpredictable chronic mild stress (UCMS) [14], cognitive decline in aged rats (naïve or subjected to the surgical procedures exacerbating the cognitive decline) [15]. From these models, LPS-induced anxiety- and depressive-like behavior might be too severe, since LPS is endotoxin which causes a very strong pro-inflammatory reaction. Moreover, it leads to sickness behavior for which the symptoms overlap with the depressive symptoms. Social defeat and unpredictable chronic stress may better reflect the mechanism of anxiety- and depressive-like behavior in humans which is not related to sickness behavior. However, a complication for a dietary intervention in these models is that they are characterized by anhedonia, substantial decrease in food intake and body weight. It is therefore difficult to maintain the same level of food intake between experimental groups and to ensure sufficient intake of the active components from the diet. This can be overcome with intravenous injections of nutrients. Cognitive decline in aged animals seem to be the best model to study the effect of diet on neuroinflammation because (I) ageing is related to chronic *mild* neuroinflammation (II) maintaining sufficient food intake may be more feasible in these models, since even after the abdominal surgery, the weight loss in aged animals does not exceed 10% [16].

Psychiatric disorders and ageing in humans are often associated with depletion of substantial nutrients. Therefore, nutritional supplementation in animals receiving complete control diet differs from the clinical situation in which patients may be depleted from some important dietary components. An option to overcome these differences and to increase the likelihood of finding beneficial effects could be to apply nutrient deprivation before dietary intervention in the animal model, analogous to a likely clinical situation.

3.4.4. Disease models of peripheral inflammation

Epidemiological studies suggest an association between psychiatric disorders and diseases with strong peripheral inflammation [17]. For example, a higher incidence of depression is observed in patients with colitis. It is believed that neuroinflammation can be induced by peripheral triggers and thus could be the key player that connects the comorbidity between peripheral inflammation and brain diseases. However, further studies need to be performed in order to confirm the presence of neuroinfammation in response to for example colitis. This can be done by implementing animal models for these peripheral inflammatory diseases (e.g. DSS-induced chronic colitis) and investigating the brain with sensitive methods to characterize the markers of inflammation (immunohistochemistry, flow cytometry, western blot, qPCR or imaging methods). If neuroinflammation can be confirmed, animal models of for example colitis could be applied for anti-inflammatory dietary intervention. This however will need discrimination between the direct effects of the anti-inflammatory diet on neuroinflammation and the indirect effect (ameliorating peripheral inflammation and therefore induction of less severe neuroinflammation). The first step for this discrimination would be to monitor disease progression in peripheral tissues. In case the diet will affect disease progression and peripheral inflammation, further discrimination can be applied. For example, a positive control with an anti-inflammatory treatment injected into the brain or transgenic models with impaired microglia response can help to assess selectively the effect of inhibition of neuroinflammation.

3.4.5. Disease models for neurodegenerative disorders

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Neuroinflammation is not only believed to play a role psychiatric disorders and peripheral inflammatory diseases, but is also involved in the development of neurodegenerative diseases, like Alzheimer's disease (AD) and Parkinson's disease (PD). Animal models for neurodegenerative diseases could therefore be considered

for future dietary intervention studies. Several models of neurodegenerative diseases with proven neuroinflammation have been described. Some examples are: the colchicine induced rat model of AD [18], amyloid-induced AD [19], transgenic AD models [20], rotenone-induced PD [21], alpha-synuclein-induced PD [22], and 6-hydroxydopamine-induced PD [23]. Modulation of the severity of pharmacologically-induced models is feasible by changing the dose of a toxin. Therefore these models seem to be more appropriate for dietary intervention which requires low severity.

Many preclinical studies on neurodegenerative disorders are performed in transgenic models. In patients, however, genetics are not the only cause of neurodegenerative diseases, as most of these disorders are triggered by multiple factors. Familial AD, for example, accounts for only approximately 5% of the AD cases [24]. Similarly, only approximately 10% of the PD patients have a family history of this disorder [25]. This suggests an important role of environmental factors in the development of AD in PD in the majority of patients. It is believed that neurodegenerative diseases are caused by complex interactions between environmental and genetic factors. Therefore, using pharmacologically-induced models, or a combination of a transgenic model with a pharmacological trigger, may be more appropriate [24]. Intracerebral injection of a drug may cause by itself strong inflammation and sickness behavior. Therefore, models in which toxin can be delivered intravenously or intraperitoneally seem to be a better approach (e.g. systemic rotenone-induced PD) [26]. However, given the complexity of the neurodegenerative diseases, it is unlikely that one model will resemble all the diseases characteristics [27]. The optimal approach would be to choose a model which most closely resembles the pathology observed in patients in order to ensure highest changes of success when translating the preclinical results to clinical studies. However, as discussed above, models usually do not ideally represent the clinical situation and only mimic some specific aspects of the disease. To compensate for this, multiple complementary models can be used that together cover all characteristics of the disease.

4. Human studies on the impact of nutrition

4.1. Observational human studies: Epidemiological studies

Lifestyle, which includes a person's diet, has been recognized as an important modifiable factor affecting the incidence of diseases in which neuroinflammation may play a role. Prospective studies, in which the dietary patterns and disease incidence of a cohort of subjects are assessed for a long period of time, provide large datasets on the effect of diet on the incidence of brain diseases in which (neuro)inflammation may be involved [1–3]. There are two main methods for assessment of food intake in the epidemiological studies: (I) objective observation and (II) subjective reporting by the participant [28]. Objective observation can be performed by collection of food samples for analysis or by direct observation by trained staff. These methods are difficult to apply for large cohort studies. Subjective reporting methods include 24-hour dietary recall, dietary recording with a questionnaire and reporting of dietary history. These methods provide detailed data about food intake, but they are time-consuming and subject to bias, as they depend on the memory of the respondent. As another method for subjective reporting, food frequency questionnaires allow simple assessment of regular daily food intake in large cohorts in a cost-effective and time-effective way. This method might be less accurate as compared to others and requires validation of the questionnaire used for the study, but it seems to be a practical approach for the large epidemiological studies [28].

Epidemiological studies serve to identify dietary factors that have an impact on disease incidence and disease progression. The factors identified in epidemiological studies should be further tested in preclinical investigations and clinical trials [29], in order to confirm a causal relationship.

4.2. Experimental human studies: Randomized clinical trials (RCT)

Randomized clinical trials (RTC) allow testing of the efficacy of nutritional factors in similar groups of subjects. RCT should be used to confirm the hypotheses derived from epidemiological studies and/or preclinical investigations in cells and animal models. There are challenges in clinical trials on dietary interventions that differ from clinical trials on pharmaceutical drugs. For example, dietary intervention is:

- more sensitive to external variables
- formed by heterogeneous mixtures (e.g. food or supplemented food products, in contrast to a single pharmaceutical compound in pharmacological trials)
- multi-targeting (since the dietary intervention consists of multiple active components)

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- consumed throughout the day, resulting in difficulties to standardize the intake. Moreover, participants in dietary intervention trials are continuously exposed to easily accessible food, which may contain the same nutrients as those included in the intervention and therefore change the dosage of the intervention.

During dietary interventions, it is important to take the background food intake (before and during the intervention) into account and to closely monitor food intake during the experiment, which can be done prospectively by direct observation, food records, or food diary; or retrospectively by 24h diet recall, or questionnaires. The retrospective methods highly depend on the accurate memory and reliability of the respondent and on the ability of the respondent to estimate portion size [30]. For hospitalized patients, food intake might be easier to control and record.

Placebo-controlled, double-blind studies are more difficult to design for dietary interventions than for pharmaceutical drugs, especially in case of interventions which aim to change the whole diet. Placebo-controlled studies are more feasible if a dietary supplement with a specific (combination of) nutrient(s) is tested.

4.3. Diet formulation

The formulation of a dietary intervention is highly dependent on the nature of the investigational nutrients. The diet investigated in this thesis was based on standard laboratory animal chow AIN-93G [31] and therefore adapted for the investigation in mice and rats. Applying the same dietary concept in clinical trials would require designing and preparation of a formulation appropriate for use in humans. Supplementation in humans can be applied in diverse formulations, including tablet, capsule and liquid. Since our dietary intervention is multi-nutrient and contains both water-soluble components (B vitamins, amino acids, carbohydrates) and fat-soluble components (DHA, EPA, vitamin A and D), the best approach would be to apply them in a form of emulsion.

Dosage of the active ingredients will need to be extrapolated to humans based on the daily intake recommendations and toxicity of some ingredients (e.g. fat-soluble vitamins). This should be accompanied with measurement of absorption of active ingredients from the formulation in humans.

4.4. Selection of a patient cohort

Neuroinflammation is believed to be involved in the development of several psychiatric and neurodegenerative disorders. It is also believed to mediate comorbidity between peripheral inflammation and brain diseases. Therefore, a

variety of patients with psychiatric or neurodegenerative diseases can be considered for the treatment with anti-inflammatory dietary intervention. As examples of disorders with neuroinflammation, we focus the discussion here on the two disorders, for which we described promising preclinical results in this thesis: postoperative cognitive decline and stroke.

4.4.1. Postoperative cognitive dysfunction

Cognitive dysfuction forms a category of brain disorders which are hypothesized to be triggered by neuroinflammation. In fact, there is substantial preclinical evidence that postoperative cognitive dysfuction is triggered by neuroinflammation [32]. Our study with dietary intervention for POCD in rats not only confirmed the presence of neuroinflammation in rats subjected to surgery, but also provided evidence for a therapeutic effect of the diet on neuroinflammation and other disease symptoms caused by the surgery. Although preclinical studies and indirect evidence in humans suggested an inflammatory component in the pathology of POCD, the presence of neuroinflammation has not been demonstrated directly in the brain of patients with cognitive decline yet.

Clinical studies to detect neuroinflammation or to investigate the effectiveness of an anti-inflammatory diet intervention for POCD will be challenged by the high variability in the condition of the patients before and after surgery, in the nature of the surgical intervention and in concomitant diseases. Therefore, proper inclusion criteria need to be established (for example, the same type of intervention, age range, no concomitant diseases). Moreover, this type of studies would require a big sample size, since POCD does not develop in all patients subjected to the surgical procedure. Persisting POCD (longer than 3 months post-surgery) is seen in approximately 10% of patients over 60 years of age [32,33]. Since the occurrence of POCD is the highest in this group of patients (>60), this group might be optimal for future intervention studies. However, in elderly patients also the occurrence of concomitant diseases is highest and this may complicate the selection of homogenous group for the study.

Since surgery can be planned, the designing of both preventive and postoperative dietary intervention is feasible in this patient group.

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4.4.2. Stroke

The presence of neuroinflammation has been well described in animal models of stroke. Human studies have shown increased levels of activated leukocyte cell adhesion molecule (ALCAM), a marker of neuroinflammation, in stroke patients. High levels of ALCAM were correlated to increased long-term mortality in stroke patients [34]. [^{11}C]PK11195 PET studies revealed the presence and spatiotemporal changes of neuroinflammation following cerebral ischemia [35]. Animal studies have shown not only the presence of neuroinflammation following cerebral ischemia, but also the therapeutic effects of anti-inflammatory treatment on disease progression after stroke.   It is believed that chronic and persistent neuroinflammation in the area surrounding the lesion (penumbra) can lead to further neuronal damage and therefore increase the lesion size. Therefore, anti-inflammatory treatment should aims to ameliorate or prevent further neuronal damage following ischemia. Therefore, stroke patients are a good target group for the new anti-inflammatory treatment strategies. Some clinical studies have demonstrated a positive impact of anti-inflammatory treatment on the patients recovering from stroke [36–38], while epidemiological studies demonstrated a promising effect of anti-inflammatory diet in the prevention of stroke [39]. Therefore, a clinical study to investigate preventive and therapeutic effects of anti-inflammatory diet in humans may be a promising approach.

Testing the possible prevention of stroke in a clinical trial would require a long-term follow-up. This implies that the dietary intervention would have to be applied daily for a long period of time. Therefore, it seems to be more feasible to perform observational study on the preventive impact of the diet on stroke incidence, as it was done before [39].
Clinical trials could more easily be applied to test the efficacy of anti-inflammatory diet during the recovery phase after the onset of ischemia in patients who suffered from cerebral ischemia. In this case, it will also be challenging to achieve a homogeneous patient cohort, because of the possible concomitant diseases and big variability in brain damage between the subjects. It is therefore necessary to correlate the outcome of the dietary intervention to the progression of brain damage. A solution could be to apply a within-subject design in which patients after stroke would be scanned twice, before and after dietary intervention. Furthermore, it could be assessed whether the brain regions with neuroinflammation (detected on the baseline scan with for example [^{11}C]PK11195PET) could be salvaged. Brain

damage could be assessed with for example [18F]FDG PET, [11C]flumazenil PET or MRI.

In conclusion, dietary intervention in POCD seems to be more feasible to design and plan. Both pre-surgical and post-surgical treatment can be applied. However, a big sample size is necessary for this study, because only a small fraction of the patients will develop chronic cognitive decline.

Diet intervention in stroke patients can be applied only after the onset of ischemia. However, the possibility of applying smaller sample size can facilitate the study, since all patients will develop neuroinflammation.

5. PET imaging

5.1. The role of PET in nutritional research

As it has been reviewed by others [40], a large variety of PET tracers have been used in food science. These tracers include radiolabeled amino acids, peptides, proteins and antibodies, lipoproteins, fatty acids, lipids, nucleotides, trace elements, carbohydrates, plant secondary metabolites, vitamins and vitamin derivatives. For example, a vitamin D3 receptor (VDR) ligand tracer ([26,27-11C]dihydroxyvitamin D3) was developed. In a similar way, a potential tracer to investigate other relevant receptors could be designed. These tracers would allow determination of the receptor occupancy following diet intervention. For example, a tracer that binds to retinoic acid receptors could help to elucidate the anti-inflammatory mechanism of vitamin A *in vivo* and thus to confirm our *in vitro* results. A PET study with radiolabeled food components would help to elucidate how each of the components of the diet is interacting with its molecular target. PET imaging may also help to investigate the metabolism and accumulation in tissue of specific nutrients, as it was done with amino acids (e.g. 11C-labelled methionine was used for study of distribution and metabolism of methionine) or fatty acids and lipids (for example 11C and 18F-labelled fatty acids applied to investigate fatty acid kinetics and tissue uptake) [40,41]. PET imaging may be very helpful in the investigation of metabolism and uptake of nutrients in brain, allowing the investigation in human subjects. For example, a human study with 11C-labelled docosahexaenoic acid, allowed to characterize incorporation of DHA into brain tissue [40]. These studies

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could help to design an effective dietary intervention and to estimate the doses of nutrients required for achieving a sufficient concentration in human brain tissue.

In general, a disadvantage of this approach is that separate tracers need to be used for each individual nutrient.

PET imaging of neuroinflammation may be a suitable alternative to monitor the effectiveness of an anti-inflammatory diet in patients. To our knowledge, PET studies on effect of anti-inflammatory diets in humans have not been performed yet.

5.2. Peripheral inflammation

In clinical practice, [18F]FDG is successfully used to image peripheral inflammation. [18F]FDG PET imaging is not suitable for measurement of neuroinflammation because of the high physiological uptake of this tracer in the brain. Therefore, other tracers have to be used for brain imaging of inflammation, for example TSPO tracers.

5.3. Neuroinflammation

The most widely used TSPO tracer for the measurement of neuroinflammation, [11C]PK11195, has a low signal-to-noise ratio, which hinders the detection of subtle changes, especially in small animals. In fact, [11C]PK11195 PET imaging was able to detect neuroinflammation caused by abdominal surgery, but could not detect any effect of dietary intervention, as was described in chapter 6 of this thesis.

Therefore, new PET tracers with improved image properties have been developed, such as [11C]PBR28, [18F]PBR06, [18F]FEPPA, [11C]DAA1106, [11C]DPA713, [18F]DPA714 and [18F]PBR111. These tracers have a higher binding affinity for the TSPO. This facilitates the detection of subtle changes in TSPO expression in animals. However, in humans, the binding affinity of the second generation TSPO PET radioligands is strongly affected by TSPO receptor polymorphism in Ala147Thr. This results in a trimodal distribution in binding affinity of the tracer (low, medium and high affinity) [42,43]. Although testing for polymorphism allows exclusion of subjects with the low-binding polymorphism, this requires additional procedures and may introduce a significant bias, as the impact of the polymorphism on the process of interest is generally not known. For this reason, development of new tracers for new targets involved in neuroinflammation in humans might be the best approach. At the current stage, we still consider [11C]PK11195 as the best tracer to investigate neuroinflammation in humans, since

its uptake does not depend on TSPO receptor polymorphism. The sensitivity of [11C]PK11195 PET imaging can be increased by applying full quantification of receptor binding with pharmacokinetic modelling. At the moment, [11C]PK11195 PET imaging seems to be the best approach for clinical investigations, while in pre-clinical studies also the second generation TSPO PET radioligands can be used, since they are not affected by polymorphism in mice and rats.

5.4. New tracers for PET imaging of peripheral and central inflammation

Apart from TSPO receptors and glucose metabolism, also other targets are considered for the measurements of inflammation in central and/or peripheral tissues. These other targets include membrane markers on inflammatory cells (e.g. cannabinoid receptors), inflammatory cytokines (e.g. cyclooxygenase, matrix metalloproteinases, IL-2, TNFα) and inflammation-related targets on blood vessels (e.g. integrin receptor, vascular adhesion protein-1, vascular cell adhesion molecule-1, vessel permeability). This has been reviewed in more detail elsewhere [42].

In general, a disadvantage of TSPO receptor imaging is that these methods do not allow distinction between microglia, astrocytes and infiltrating macrophages. This could be solved with tracers targeting specifically one cell type. For example [11C]deprenyl, a tracer that targets monoamine oxidase B (MAO-B) which is specifically overexpressed by activated astrocytes, was used to detect astrogliosis in human patients with AD [44]. Furthermore, it is not known whether TSPO is overexpressed by both M1 and M2 type microglia/macrophages. M1 activation is the *classical* response of immune cells to infection or tissue injury, while the M2 phenotype is characteristic for the *alternative* activation of immune immune cells in response to parasitic infections or allergens. Therefore, TSPO receptors measurement does not allow discrimination between these microglia phenotypes. More specific tracers, targeting one cell type or characteristic only to M1 or M2-type inflammation, could allow more specific assessment of neuroinflammation by PET imaging. However, which markers can be considered characteristic for M1 or M2 activation is still debatable. Includible nitric oxide synthase (iNOS) is commonly used as a marker for M1 activation, while M2 activation seems to be more difficult to detect. Several markers of M2 activation are considered, for example C-Type Mannose Receptor 1 (CD206) and Hemoglobin-Haptoglobin Scavenger Receptor (CD163), early growth response protein 2 (Egr2) and arginase-1 [45]. *In vitro* studies have shown that protein expression of many M2 markers is

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low (e.g. arginase-1, CD206) or markers are non-selective (e.g. arginase-1). CD163 has been proposed as a good M2 marker, but was shown to be unreliable when used alone. Therefore it is recommended for immunohistochemistry staining to analyze multiple markers for the M2 phenotype [46]. Investigation of new M1/M2 markers is still in progress. For example, recent studies investigating expression patterns in different phenotypes of macrophages proposed CD38 and Egr2 as promising markers of M1 and M2 activation, respectively [47]. Molecules interacting with these markers may be considered as lead compounds for more specific tracers for inflammation. This, however, would require further validations.

Moreover, immunohistochemistry markers are not always applicable for brain PET imaging. Recently, M2 activated macrophages were detected in tumors with PET, using [^{18}F]Camelid Single-Domain Antibody Fragments in peripheral tissue tumors [48]. This method may be difficult to apply in brain imaging, since the passage of antibodies through blood brain barrier (BBB) is very limited. However, recent research has demonstrated a possibility to use antibody-based tracers in brain imaging [49].

In conclusion, the perfect tracer for imaging of neuroinflammation might be different for brain and peripheral imaging. In both cases, a high specificity, high signal to noise ratio and slow metabolism is desirable. For brain imaging, additionally a good passage of BBB is necessary. The TSPO receptor is not the optimal target and therefore other targets need to be found. Until then, TSPO seems to be the best-known target for brain imaging of neuroinflammation, although more sensitive tracers than PK11195 that are not sensitive to the TSPO polymorphism, are urgently awaited.

References

[1]   Rienks J, Dobson AJ, Mishra GD. Mediterranean dietary pattern and prevalence and incidence of depressive symptoms in mid-aged women: results from a large community-based prospective study. Eur J Clin Nutr 2013;67:75–82. doi:10.1038/ejcn.2012.193.

[2]   Koyama A, Houston DK, Simonsick EM, Lee JS, Ayonayon HN, Shahar DR, et al. Association between the Mediterranean diet and cognitive decline in a biracial population. J Gerontol A Biol Sci Med Sci 2015;70:354–9. doi:10.1093/gerona/glu097.

[3]   Sánchez-Villegas A, Delgado-Rodríguez M, Alonso A, Schlatter J, Lahortiga F, Serra Majem L, et al. Association of the Mediterranean dietary pattern with the incidence of depression: the Seguimiento Universidad de Navarra/University of Navarra follow-up (SUN) cohort. Arch Gen Psychiatry 2009;66:1090–8.

doi:10.1001/archgenpsychiatry.2009.129.

[4]   Chan RB, Oliveira TG, Cortes EP, Honig LS, Duff KE, Small SA, et al. Comparative lipidomic analysis of mouse and human brain with Alzheimer disease. J Biol Chem 2012;287:2678–88. doi:10.1074/jbc.M111.274142.

[5]   Fonteh AN, Fisher RD. Combining lipidomics and proteomics of human cerebrospinal fluids. Methods Mol Biol 2009;579:71–86. doi:10.1007/978-1-60761-322-0_4.

[6]   Aebersold R. Quantitative Proteome Analysis: Methods and Applications. J Infect Dis 2003;187:S315–20. doi:10.1086/374756.

[7]   Juan H, Sametz W. Uptake, stimulated release and metabolism of (1-14C)-eicosapentaenoic acid in a perfused organ of the rabbit. Naunyn Schmiedebergs Arch Pharmacol 1983;324:207–11.

[8]   Tocher DR, Bell JG, Sargent JR. Incorporation of [3H]arachidonic and [14C]eicosapentaenoic acids into glycerophospholipids and their metabolism via lipoxygenases in isolated brain cells from rainbow trout Oncorhynchus mykiss. J Neurochem 1991;57:2078–85.

[9]   Santos FF, de Turco EB, Gordon WC, Peyman GA, Bazan NG. Alterations in rabbit retina lipid metabolism induced by detachment. Decreased incorporation of [3H]DHA into phospholipids. Int Ophthalmol n.d.;19:149–59.

[10]   Manshouri T, Yang Y, Lin H, Stass SA, Glassman AB, Keating MJ, et al. Downregulation of RAR alpha in mice by antisense transgene leads to a compensatory increase in RAR beta and RAR gamma and development of lymphoma. Blood 1997;89:2507–15.

[11]   Tang M, Lin W, Pan Y, Guan X, Li Y. Hippocampal neurogenesis dysfunction linked to depressive-like behaviors in a neuroinflammation induced model of depression. Physiol Behav 2016;161:166–73. doi:10.1016/j.physbeh.2016.04.034.

[12]   Zheng X, Liang Y, Kang A, Ma S-J, Xing L, Zhou Y-Y, et al. Peripheral immunomodulation with ginsenoside Rg1 ameliorates neuroinflammation-induced behavioral deficits in rats. Neuroscience 2014;256:210–22. doi:10.1016/j.neuroscience.2013.10.023.

[13]   Niraula A, McKim DB, Tarr AJ, Wohleb ES, Shea DT, Atchenson KM, et al. Repeated social defeat stress induces neuroinflammation and impairs hippocampal neurogenesis that differentially regulate mood and cognition. Brain Behav Immun 2015;49. doi:10.1016/j.bbi.2015.06.058.

[14]   Farooq RK, Isingrini E, Tanti A, Le Guisquet A-M, Arlicot N, Minier F, et al. Is unpredictable chronic mild stress (UCMS) a reliable model to study depression-induced neuroinflammation? Behav Brain Res 2012;231:130–7. doi:10.1016/j.bbr.2012.03.020.

[15]   Hovens IB, Schoemaker RG, van der Zee E a., Absalom AR, Heineman E, van Leeuwen BL. Postoperative cognitive dysfunction: Involvement of neuroinflammation and neuronal functioning. Brain Behav Immun 2014;38:202–10. doi:10.1016/j.bbi.2014.02.002.

[16]   Hovens IB, Schoemaker RG, van der Zee E a, Heineman E, Nyakas C, van Leeuwen BL. Surgery-induced behavioral changes in aged rats. Exp Gerontol 2013;48:1204–11. doi:10.1016/j.exger.2013.07.011.

[17]   Maes M, Yirmyia R, Noraberg J, Brene S, Hibbeln J, Perini G, et al. The

10

inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. Metab Brain Dis 2009;24:27–53. doi:10.1007/s11011-008-9118-1.

[18] Sil S, Ghosh T, Aloisi F, Anand R, Kumar A, Chidambaranathan N, et al. Role of cox-2 mediated neuroinflammation on the neurodegeneration and cognitive impairments in colchicine induced rat model of Alzheimer's Disease. J Neuroimmunol 2016;291:115–24. doi:10.1016/j.jneuroim.2015.12.003.

[19] Chen J-H, Ke K-F, Lu J-H, Qiu Y-H, Peng Y-P. Protection of TGF-β 1 against neuroinflammation and neurodegeneration in Aβ 1-42-induced Alzheimer's disease model rats. PLoS One 2015;10:e0116549. doi:10.1371/journal.pone.0116549.

[20] Liu B, Le KX, Park M-A, Wang S, Belanger AP, Dubey S, et al. In Vivo Detection of Age- and Disease-Related Increases in Neuroinflammation by 18F-GE180 TSPO MicroPET Imaging in Wild-Type and Alzheimer's Transgenic Mice. J Neurosci 2015;35:15716–30. doi:10.1523/JNEUROSCI.0996-15.2015.

[21] Ojha S, Javed H, Azimullah S, Haque ME. β -Caryophyllene, a phytocannabinoid attenuates oxidative stress, neuroinflammation, glial activation, and salvages dopaminergic neurons in a rat model of Parkinson disease. Mol Cell Biochem 2016;418:59–70. doi:10.1007/s11010-016-2733-y.

[22] Qin H, Buckley JA, Li X, Liu Y, Fox TH, Meares GP, et al. Inhibition of the JAK/STAT Pathway Protects Against α -Synuclein-Induced Neuroinflammation and Dopaminergic Neurodegeneration. J Neurosci 2016;36:5144–59. doi:10.1523/JNEUROSCI.4658-15.2016.

[23] Nadella R, Voutilainen MH, Saarma M, Gonzalez-Barrios JA, Leon-Chavez BA, Jiménez JMD, et al. Transient transfection of human CDNF gene reduces the 6-hydroxydopamine-induced neuroinflammation in the rat substantia nigra. J Neuroinflammation 2014;11:209. doi:10.1186/s12974-014-0209-0.

[24] Lecanu L, Papadopoulos V. Modeling Alzheimer's disease with non-transgenic rat models. Alzheimers Res Ther 2013;5:17. doi:10.1186/alzrt171.

[25] Klein C, Westenberger A. Genetics of Parkinson's disease. Cold Spring Harb Perspect Med 2012;2:a008888. doi:10.1101/cshperspect.a008888.

[26] Cannon JR, Tapias V, Na HM, Honick AS, Drolet RE, Greenamyre JT. A highly reproducible rotenone model of Parkinson's disease. Neurobiol Dis 2009;34:279–90.

[27] Blesa J, Phani S, Jackson-Lewis V, Przedborski S, Blesa J, Phani S, et al. Classic and New Animal Models of Parkinson's Disease. J Biomed Biotechnol 2012;2012:1–10. doi:10.1155/2012/845618.

[28] Shim J-S, Oh K, Kim HC. Dietary assessment methods in epidemiologic studies. Epidemiol Health 2014;36:e2014009. doi:10.4178/epih/e2014009.

[29] Gibson TM, Ferrucci LM, Tangrea JA, Schatzkin A. Epidemiological and clinical studies of nutrition. Semin Oncol 2010;37:282–96. doi:10.1053/j.seminoncol.2010.05.011.

[30] Yao CK, Gibson PR, Shepherd SJ. Design of clinical trials evaluating dietary interventions in patients with functional gastrointestinal disorders. Am J Gastroenterol 2013;108:748–58. doi:10.1038/ajg.2013.77.

[31] Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the

reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939–51.

[32]   Hovens IB, Schoemaker RG, van der Zee EA, Heineman E, Izaks GJ, van Leeuwen BL. Thinking through postoperative cognitive dysfunction: How to bridge the gap between clinical and pre-clinical perspectives. Brain Behav Immun 2012;26:1169–79. doi:10.1016/j.bbi.2012.06.004 [doi].

[33]   Price, Catherine C. U of F, Garvan, Cynthia W. U of F, Monk, Terri G. , Duke University Medical Center, Durham NC. Type and severity of cognitive decline in older adults after noncardiac surgery. Anesthesiology 2008;108:8–17. doi:10.1097/01.anes.0000296072.02527.18.Type.

[34]   Smedbakken L, Jensen JK, Hallén J, Atar D, Januzzi JL, Halvorsen B, et al. Activated leukocyte cell adhesion molecule and prognosis in acute ischemic stroke. Stroke 2011;42:2453–8. doi:10.1161/STROKEAHA.110.612440.

[35]   Gerhard A, Schwarz J, Myers R, Wise R, Banati RB. Evolution of microglial activation in patients after ischemic stroke: a [11C](R)-PK11195 PET study. vol. 24. 2005. doi:10.1016/j.neuroimage.2004.09.034.

[36]   Lampl Y, Boaz M, Gilad R, Lorberboym M, Dabby R, Rapoport A, et al. Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. Neurology 2007;69:1404–10. doi:10.1212/01.wnl.0000277487.04281.db.

[37]   Tobinick E, Kim NM, Reyzin G, Rodriguez-Romanacce H, DePuy V. Selective TNF inhibition for chronic stroke and traumatic brain injury: an observational study involving 629 consecutive patients treated with perispinal etanercept. CNS Drugs 2012;26:1051–70. doi:10.1007/s40263-012-0013-2.

[38]   Shaikh AK, Mohammad QD, Ullah MA, Ahsan MM, Rahman A, Shakoor MA. Effect of dexamethasone on brain oedema following acute ischemic stroke. Mymensingh Med J 2011;20:450–8.

[39]   Tektonidis TG, Åkesson A, Gigante B, Wolk A, Larsson SC, Murphy SL, et al. A Mediterranean diet and risk of myocardial infarction, heart failure and stroke: A population-based cohort study. Atherosclerosis 2015;243:93–8. doi:10.1016/j.atherosclerosis.2015.08.039.

[40]   Bergmann R, Pietzsch J. Small animal positron emission tomography in food sciences. Amino Acids 2005;29:355–76. doi:10.1007/s00726-005-0237-2.

[41]   Nagata T, Tsuyuguchi N, Uda T, Terakawa Y, Takami T, Ohata K. Examination of 11C-methionine metabolism by the standardized uptake value in the normal brain of children. J Nucl Med 2011;52:201–5. doi:10.2967/jnumed.110.082875.

[42]   Wu C, Li F, Niu G, Chen X. PET Imaging of Inflammation Biomarkers. Theranostics 2013;3:448–66. doi:10.7150/thno.6592.

[43]   Lavisse S, Garcia-Lorenzo D, Peyronneau M-A, Bodini B, Thiriez C, Kuhnast B, et al. Optimized Quantification of Translocator Protein Radioligand 18F-DPA-714 Uptake in the Brain of Genotyped Healthy Volunteers. J Nucl Med 2015;56:1048–54. doi:10.2967/jnumed.115.156083.

[44]   Choo ILH, Carter SF, Schöll ML, Nordberg A. Astrocytosis measured by ^{11}C-deprenyl PET correlates with decrease in gray matter density in the parahippocampus of prodromal Alzheimer's patients. Eur J Nucl Med Mol Imaging 2014;41:2120–6. doi:10.1007/s00259-014-2859-7.

[45]   Rőszer T. Understanding the Mysterious M2 Macrophage through Activation Markers and Effector Mechanisms. Mediators Inflamm 2015;2015:816460.

10

doi:10.1155/2015/816460.

[46]   Barros MHM, Hauck F, Dreyer JH, Kempkes B, Niedobitek G. Macrophage polarisation: an immunohistochemical approach for identifying M1 and M2 macrophages. PLoS One 2013;8:e80908. doi:10.1371/journal.pone.0080908.

[47]   Jablonski KA, Amici SA, Webb LM, Ruiz-Rosado J de D, Popovich PG, Partida-Sanchez S, et al. Novel Markers to Delineate Murine M1 and M2 Macrophages. PLoS One 2015;10:e0145342. doi:10.1371/journal.pone.0145342.

[48]   Blykers A, Schoonooghe S, Xavier C, D'hoe K, Laoui D, D'Huyvetter M, et al. PET Imaging of Macrophage Mannose Receptor-Expressing Macrophages in Tumor Stroma Using 18F-Radiolabeled Camelid Single-Domain Antibody Fragments. J Nucl Med 2015;56:1265–71. doi:10.2967/jnumed.115.156828.

[49]   Sehlin D, Fang XT, Cato L, Antoni G, Lannfelt L, Syvänen S. Antibody-based PET imaging of amyloid beta in mouse models of Alzheimer's disease. Nat Commun 2016;7:10759. doi:10.1038/ncomms10759.