PET Imaging of Mild Traumatic Brain Injury and Whiplash Associated Disorder

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Concluding remarks and future perspectives

Functional imaging using SPECT and PET techniques provide researchers and clinicians the possibility to perform target-specific studies, facilitating the investigation of multiple health conditions at different stages and the evaluation of therapeutic interventions. Nuclear medicine imaging techniques are especially relevant in those situations where conventional structural imaging (i.e. CT and MRI) fail to detect alterations, as they are naturally focused on morphological features and therefore cannot easily predict changes in neurocognitive functions or functional outcomes. This seems to be the case in mild traumatic brain injury (mTBI) and whiplash associated disorder (WAD). Even when there is a theoretical framework and indirect evidence of the lesion-based models, tissue damage is frequently undetected by conventional imaging, which cannot be used to predict the neurocognitive outcome of the patient.

Herein, this thesis presents the feasibility of PET imaging to detect neurocognitive functional changes in the specific conditions of interest: mTBI and WAD.

Methodological aspects

Currently, scientific studies utilize rodent models for various human brain diseases. PET and SPECT have the ability to provide functional insight into physiological processes and biochemical pathways *in vivo*. This ability allows for longitudinal follow-up within a single animal and greatly facilitates the investigation of chronic diseases and the evaluation of new pharmacological interventions. However, the spatial resolution that can be obtained in current animal scanners is a limiting factor in analysis. Therefore, optimal use of imaging data becomes crucial.

After reconstruction of the images, a relevant process is the 'normalization' of the data. Differences in animal weight, injected dose of the tracer or changes in brain size of the animals, due to aging or therapeutic interventions, must be considered prior to the analysis. There are two main normalization areas to consider. First, a powerful and widely-used approach is the adoption of a common reference space to which images from individual subjects and time points are spatially normalized. This procedure is frequently used in human studies, where individual MRI or CT images are also acquired. However, high-resolution anatomical image data in preclinical brain PET and SPECT studies is often not available. As such, the normalization of these functional images without its accompanying structural images becomes challenging. In **chapter 2** of this thesis it was automatized and tested a procedure for the construction of tracer-specific PET and SPECT templates. These templates allow accurate registration of the functional brain data, with registration errors below the spatial resolution of the camera. Although in this study the

methodology was validated under several conditions, it would be of interest to further evaluate the performance of the templates with different animal species, disease models and radiolabeled tracers in future studies. Also, it would be of great interest to compare the results obtained from the use of these functional templates with those obtained from the use of combined small animal PET/CT or small animal PET/MRI. This procedure is expected to provide a more robust normalization, since structural data makes the registration less dependent on the tracer-specific uptake pattern or disease state.

Additionally, **chapter 2** presents the software package called SAMIT ('Small Animal Molecular Imaging Toolbox'). The aim of this toolbox is to facilitate the construction of the templates and the subsequent voxel-based analysis of the small animal PET and SPECT brain images. Functional neuroimaging data is frequently analyzed in humans using a voxel-based approach, instead of the more 'classical' volume-of-interest approach (VOI). The voxel-based analysis could, in theory, identify subtle changes better than VOI-based analysis, as the voxel-based analysis is limited mainly by the spatial resolution of the scanner rather than by the size of the VOIs. Moreover, it is not restricted to hypothesis-based regions and rather allows the investigation of the whole brain. Further improvement of the toolbox will be needed, since its testing was limited to the conditions of ongoing experiments in our department. Hence, unexpected errors and improvements will arise with an extensive use of the toolbox by other researcher groups and institutions.

After acquisition of the PET and SPECT images, a second important aspect is the normalization of the uptake values. The most frequent approach is the use of standardized uptake values (SUV), where data are corrected for the injected dose and the body weight of the subject. However, SUV is a semi-quantitative measurement that can be affected by several biological and technological factors. Therefore, whenever possible, the use of quantitative measurements by pharmacokinetic modeling is advisable. This approach was used in the methodology of **chapter 3** where the novel imidazopyridine compound [^{11}C]CB184 was evaluated in a rat model of herpes encephalitis (HSE). Overexpression of the translocator protein (TSPO) is used for monitoring neuroinflammation in several neurological and psychiatric disorders. However, the widely used [^{11}C]PK11195 PET tracer suffers from several limitation, and therefore there has been an effort in recent years to develop more sensitive and selective PET ligands. Nevertheless, most of these new TSPO ligands are still in the early stages of investigation, and the differences in methodology of the studies make direct comparison of the results difficult. Our group has previously used the HSE model for the evaluation of some of these new TSPO ligands ([^{11}C]DAA1106, [^{11}C]DPA-713 or [^{18}F]DPA-714),

and it would be interesting to further evaluate other promising tracers such as [^{11}C]PBR28 in the same model. Despite the promising results obtained and presented in chapter 3, further clinical imaging studies with [^{11}C]CB184 must be performed to assess the added value of this new TSPO radioligand to determine its suitability as an alternative for [^{11}C]PK11195.

The optimal use of the data is especially crucial in neuroimaging studies, and even more important in preclinical studies. Future experiments must implement the most recent techniques in image data analysis. Specifically, the scientific community must change from the classical VOI-based analysis to the voxel-based analysis. This change must be accompanied with the use of more sophisticated statistical methods, such those including corrections for multiple measurements, the use of non-parametric analysis of the images, or the construction of parametric images based on pharmacokinetic modeling, among other possibilities.

Traumatic Brain Injury

Mild TBI is the most frequent cause of brain trauma in our society. It has long been underestimated due to the frequent absence of detectable pathophysiological alterations in conventional imaging. However, there is an increased awareness of mTBI due to the high rates of sport-related brain injuries during adolescence and young adulthood, and the growing evidence of a relationship between mTBI and an increased risk of depression, Alzheimer's disease, chronic encephalopathy, and other neurodegenerative diseases. Moreover, it is an emerging area of research in relation with the persistent symptoms experience by military personnel after blast mTBI.

As discussed in **chapter 4,** the use of PET and SPECT radioligands as markers of specific cellular processes could help in the detection of secondary neuronal injury mechanisms that are involved in the pathophysiology of mTBI, and in a better evaluation of the tissue damage and therapeutic approaches. Most of the studies have been focused on [^{18}F]FDG PET, which appears useful for explaining neurological states in acute and chronic phases of TBI. However, the cost-effectiveness of this technique over other neuroimaging techniques or the neuropsychological assessment remains to be demonstrated. A better understanding of the pathophysiology associated with TBI is needed, exploring not only blood perfusion and glucose metabolism, but other aspects as neuronal integrity, neuroinflammation, or cholinergic and dopaminergic systems.

More emphasis should be given to the mTBI patients in clinical research. Few studies address these patients, though they represent 80% of the total TBI population. In this respect two clinical studies were initiated in recent years in our department focusing in the existence of a neuroinflammatory process

as an underlying mechanism of mTBI symptoms. The first experiment includes patients presenting long-lasting symptoms within few months to one year after the accident. While the second study investigates athletes that suffer repetitive head traumas due to their activity. In both experiments, [^{11}C] PK11195 PET imaging will be combined with the acquisition of structural and functional MRI data. Unfortunately, these experiments were not completed in time to be included in the present thesis, and are now included project of another PhD student. We can only wait with interest to see the results that will come from these experiments, and hope that further research focused in the mTBI patients will come out in the near future.

There are a variety of experimental animal models to investigate the pathophysiology of TBI and, similar to human studies, most of these models are focused on moderate and severe TBI. Open head injuries are most widely used in rodents, and are mostly used for the research of a focal cortical lesion. However, these models do not reflect completely the pathological features seen in human mTBI where most of the patients do not show skull fracture, and an observable focal lesion in conventional structural imaging is minimal or non-existent. In **chapter 5** the weight-drop injury model was selected to induce a single mTBI, without observing skull fracture, respiratory depression, acute neurological symptoms or mortality in any of the animals. The presence of an acute neuroinflammatory process and a long lasting alteration of the glucose metabolism in several brain regions were observed in the absence of detected behavioral alterations. It can be consider that these results support the potential importance of nuclear medicine neuroimaging into assess and monitor brain function after mTBI. While a single trauma may not be sufficient to elicit behavioral alterations in a healthy brain, it is highly relevant to extend the research into the possible adverse effects of a second head injury to a brain that is undergoing the process of recovery and adaptation, as depicted by the long lasting alterations detected in the animals of our study. In addition, it would be of great interest to explore the pathophysiology of mTBI including other PET tracers. [^{11}C]flumazenil could be an useful tracer for differentiating the hypometabolism caused by selective neuronal loss from hypometabolism caused by other factors, and it is therefore an interesting tracer to be included in future experiments.

Whiplash Associated Disorder

There is increasing evidence of pathophysiological alterations in WAD patients, mostly reflected in fatty infiltration on neck muscles detected by MRI studies, and an ongoing process of central hyperexcitability. In addition, the PET and SPECT studies performed in these patients have shown hypoperfusion and hypometabolism mostly located in the posterior parietal

occipital cortex, hyperperfusion in regions such as thalamus or prefrontal gyrus, and attenuated NK1 availability in ventromedial prefrontal cortex, insular and periaqueductal gray, among other regions. However, there was not a clear hypothesis about the injury mechanism producing brain functional alterations in a process that appears to be purely a mechanical neck injury. In **chapter 6** a new hypothesis was presented in which WAD symptoms may be induced by a mismatch in the midbrain, periaqueductal gray and adjoining regions, between the ascending information from cervical structures via the upper cervical cord to the mesencephalon, and the information from the vestibular and visual systems. It would be of great interest to develop further studies to confirm or refute this hypothesis. There is a variety of animal models beginning to define the mechanism involved in whiplash, but similar to what happens in most of the clinical studies, most of the attention is focused on the pain associated with the injury. While this is clearly an important factor of the pathology, WAD is a more complex disorder and attention should be broader, covering other psychological aspects.

The **chapter 7** presents a clinical study comparing WAD female patients with healthy volunteers. Females were selected as they seem to be more prone to develop the condition, and to reduce the variability in our study. There are two important limitations in our study. The first is that the presented results are drawn from a relative small sample size. The experiment was finished without the expected number of participants due to difficulties encountered during the recruitment period, and as consequence of the replacement of the Siemens Ecat HR+ PET camera for a PET/CT system. However, the $[^{15}O]H_2O$ PET scans were performed three times per condition, which strengthens the control of the intra-subject variance in the statistical analysis. Secondly, the absence of individual MRI acquisition may have influenced the accuracy of the registration procedure. This effect is especially relevant in the brainstem structures, where alterations were expected. Nevertheless, differences in brain perfusion were observed in different brain regions in WAD patients, and correlated with regions previously reported. Strengthening these results will necessitate performing further evaluation of these patients for a better understanding of the pathophysiology and their symptoms. Without a better understanding of these mechanisms, it will be impossible to develop an appropriate therapy for afflicted patients, as it was stated in the letter published in *The Lancet* (**chapter 8**).

In conclusion, functional imaging using PET and SPECT techniques may greatly contribute to a better understanding of multiple brain conditions and disorders given their capacity to visualize neuro-metabolic alterations obscured in conventional imaging, as seems to be the case in mTBI and WAD. Future studies should aim to combine image acquisition strategies using

PET/MRI or PET/CT cameras, and including the combination of radiotracers that target different cellular processes. Finally, the analysis of these data must be performed with the newest statistical methods, always searching for an optimal handling of the image.