11 C Molecular Imaging in Focal Epilepsy

TORSTEN DANFORS
Abstract


Epilepsy is a common neurological disease affecting 6 million people in Europe. Early prevention and accurate diagnosis and treatment are of importance to obtain seizure freedom. In this thesis new applications of carbon-11-labelled tracers in PET and autoradiographic studies were explored in focal epilepsy.

Patients with low-grade gliomas often experience epileptic seizures. A retrospective PET-study assessing seizure activity, metabolic rate measured with 11C-methionine and other known prognostic factors was performed in patients with glioma. No correlation was found between seizure activity and uptake of methionine. The presence and termination of early seizures was a favourable prognostic factor.

Activation of the neurokinin-1 (NK1) receptor by substance P (SP) induces epileptic activity. PET with the NK1 receptor antagonist GR205171 was performed in patients with temporal lobe epilepsy (TLE) and healthy controls. In TLE patients an increased NK1 receptor availability was found in both hemispheres, most pronounced in anterior cingulate gyrus ipsilateral to seizure onset. A positive correlation between NK1 receptors and seizure frequency was observed in ipsilateral medial structures consistent with an intrinsic network using the NK1-SP receptor system for transmission of seizure activity.

The uptake of 18F-fluoro-deoxy-glucose (FDG) is related to cerebral blood flow (CBF). Previously, methods to estimate blood flow from dynamic PET data have been described. A retrospective study was conducted in 15 patients undergoing epilepsy surgery investigation, including PET with 11C-FDG and 11C-Flumazenil (FMZ). The dynamic FMZ dataset and pharmacokinetic modeling with a multilinear reference tissue model were used to determine images of relative CBF. Agreement between data of FDG and CBF was analyzed showing a close association between interictal brain metabolism and relative CBF.

Epilepsy often occurs after traumatic brain injuries. Changes in glia and inhibitory neuronal cells contribute to the chain of events leading to seizures. Autoradiography with 11C-PK11195, 11C-L-deprenyl and 11C-Flumazenil in an animal model of posttraumatic epilepsy studied the temporal and spatial distribution of microglia, astrocytes and GABAergic neurons. Results showed an instant increase in microglial activity that subsequently normalized, a late formation of astrogliosis and an instant and prolonged decease in GABA binding. The model can be used to visualize pathophysiological events during the epileptogenesis.

Keywords: positron emission tomography, methionine, NK1, substance p, flumazenil, deprenyl, pk11195, phosphoimager, autoradiography, TBI, traumatic brain injury, iron chloride, ferrous chloride

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<td>Analysis of covariance</td>
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<tr>
<td>BBB</td>
<td>Blood Brain Barrier</td>
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<td>Bmax</td>
<td>Maximal binding of tracer</td>
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<td>BP</td>
<td>Binding potential</td>
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<td>CT</td>
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<td>FWHM</td>
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<tr>
<td>GABA</td>
<td>Gamma aminobutyric acid</td>
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<td>IBE</td>
<td>The International Bureau for Epilepsy</td>
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<td>ILAE</td>
<td>The International League Against Epilepsy</td>
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<tr>
<td>Kd</td>
<td>Dissociation constant</td>
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<td>KPS</td>
<td>Karnofsky Performance Status scale</td>
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<td>LGG</td>
<td>Low grade glioma</td>
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<td>MET</td>
<td>Methionine</td>
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<tr>
<td>MNI</td>
<td>Montreal Neurological Institute</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>MRTM</td>
<td>Multilinear reference tissue model</td>
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<td>NK</td>
<td>Neurokinin</td>
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<td>Positron Emission Tomography</td>
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<td>Post traumatic epilepsy</td>
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<td>rCBF</td>
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<td>Region of Interest</td>
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<td>Traumatic brain injury</td>
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<td>Temporal lobe epilepsy</td>
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<td>VD</td>
<td>Volume of distribution</td>
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<td>VOI</td>
<td>Volume of Interest</td>
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Introduction

Epilepsy

Epilepsy is one of the most common disorders requiring long-term health contact. The prevalence of active epilepsy in Sweden is 5.5 in 1000 people\(^1\),\(^2\) and overall, around 50 million people in the world have epilepsy\(^3-6\).

Epilepsy is not a specific disease, but rather a broad category of symptoms arising from different disorders in brain functions. Most people will be cured from seizures with medical treatment, but one-third continues to have seizures with ensuing poor quality of life\(^7\). Epilepsy also carries the risk of premature death\(^8\). This thesis is an attempt to develop new investigative tools and treatment algorithms for severe epilepsy.

Definition of epilepsy

The International League Against Epilepsy (ILAE) and International Bureau for Epilepsy (IBE) have defined the terms epileptic seizure and epilepsy as follows: An epileptic seizure is a transient occurrence of signs and symptoms due to abnormal, excessive, or synchronous neuronal activity in the brain. Epilepsy is a disorder of the brain characterised by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological, and social consequences of this condition. The definition of epilepsy requires the occurrence of at least one epileptic seizure\(^9\).

Classification of seizures

Seizures are paroxysmal manifestations of the electrical properties of the cerebral cortex. A seizure results when a sudden imbalance occurs between the excitatory and inhibitory forces within the network of cortical neurons in favour of a sudden-onset net excitation. There is a distinction between focal and generalised seizures. Focal seizures are conceptualised as originating at some point within networks limited to one hemisphere. Generalised seizures originate at some point within and rapidly engaging bilaterally distributed networks\(^10\).
Etiology

Epilepsies can be categorised according to etiology. Three main causes have been suggested in the classification scheme from 2011 11:

- Genetic: Epilepsy as a direct result of a genetic cause.
- Structural/metabolic: Epilepsy as the result of a distinct structural or metabolic condition or disease. Structural lesions may for example include tumours such as gliomas and trauma. They may also be of genetic origin.
- Unknown: the nature of the underlying cause is as yet unknown. It is presently, by far the largest group.

Treatment

The management of people with epilepsy mainly relies on treatment with antiepileptic drugs (AEDs). New types of drugs are constantly being developed and evaluated 12. In the last 10 years, approximately one new drug, every 2 years, has appeared on the market. Also, other methods such as ketogenic diet 13, vagal nerve stimulation 14, and deep brain stimulation 15,16 has evolved and show promising results in different patient groups. All these newly developed treatment alternatives have greatly improved the quality of life of several patients in terms of less side effects and compliance to treatment, but the percentage of people who achieve seizure freedom remains approximately the same level 17-19.

Surgical treatment and preoperative evaluation

For the group of patients that continues to have seizures despite the best available treatment, a surgical evaluation should be considered 20-25. Epilepsy surgery is highly effective and the results are consistent over time and across regions world wide. The cornerstones of the investigation relies on long term video-EEG monitoring 26 which is aimed at recording the patient’s typical seizures, and a specifically designed high quality magnetic resonance imaging (MRI) 27. The latter demonstrates an abnormality within the epileptogenic lobe in the majority of cases. When MRI findings are absent (MRI-negative), or when EEG and MRI findings are discordant, other functional neuroimaging techniques are required 28. Depending on seizure semiology and EEG-findings, ictal and interictal single-photon emission computed tomography (SPECT) 29,30, Magnetencephalography (MEG) 31, positron emission tomography (PET) 32,33 can be used.
Low-grade glioma

Classification and epidemiology

Brain tumours are classified by the World health organisation (WHO). The greatest proportion is supratentorial, arising from the frontal, parietal and temporal lobes. The majority are gliomas (86%), and occur as either low-grade or high-grade tumours. Gliomas are named after their specific histological cell-type (astrocytoma, oligodendroglioma, oligoastrocytoma or ependymoma) and are further categorized by grade of malignancy. High-grade gliomas (grade III-IV) are undifferentiated. For the most common and malignant type, grade IV glioblastoma, the prognosis is poor with a median survival time of about one year.

Low-grade gliomas (LGG) are well-differentiated tumours and patients have a survival time of 5–10 years although the clinical course may vary a lot. For some patients the tumour may be more aggressive with fast transformation into a more malignant state, while others may have a more stable disease for many years. The majority, if not all, will develop a higher grade of malignancy. Fewer patients with astrocytoma than oligodendroglioma will survive for 5 years which is normally thought to be 50% and 70-80% respectively. The annual incidence of low-grade gliomas is 1.5-1.8 per 100000 inhabitants and are affecting otherwise healthy young people with an average age of approximately 40 years at the time of diagnosis. For this reason the search for reliable prognostic factors that can help patients to plan their life is important.

Clinical symptoms

Epilepsy

The clinical symptoms of brain tumour greatly depend on the site of origin. The most common presenting symptom are seizures, and they occur in 30–50% patients with brain tumours. Patients with slowly growing tumours, i.e. low-grade astrocytomas and oligodendrogliomas, are the ones with the highest incidence of epilepsy varying between 60–85%.

The reason why brain tumours and slow-growing gliomas in particular cause epileptic seizures is unclear. The tumours cause an imbalance between neuronal excitation and inhibition that causes seizures. It has been hypothesized that hyperexcitability may be an intrinsic feature of certain types of tumour cells, there is also evidence, however, that seizures in the setting of a brain tumour may be due to alterations in the brain tissue surrounding the tumour, rather than within the tumour. Slow-growing low-grade gliomas may induce epileptogenesis by changes in the synaptic vesi-
cles, enhanced intercellular communications through increased expression of gap-junction channels, persistent neurons in white matter, or changes in local concentration of GABA and glutamate. Other possible pathophysiological explanations may be impaired glial cell function and insufficient vascular supply or permeability. In rapidly growing progressive malignant tumours, seizures may be triggered by tissue damage with cell necrosis and deposition of hemosiderin.

Other symptoms and prognostic factors
Headache, mental disturbance, motor disturbance, or focal neurological deficits are present in 20–53% patients with LGG. Important prognostic factors are large tumour (diameter >6 cm), tumour crossing midline, higher age (>40 years), astrocytic subtype, and presence of neurological deficits. Low-risk patients with presence of 2 or less risk factors have an expected median survival of more than 7 years, while patients carrying 3 or more risk factors should be considered at high risk with significantly short expected median survival time. Seizure activity and metabolic rate may correlate to prognosis of survival.

Positron Emission Tomography
Molecular imaging can aid in visualising and quantifying various biochemical and physiological processes in vivo. There are different modalities such as MRI, optical imaging, single photon emitted tomography (SPECT), and PET that can be used for molecular imaging. SPECT and PET utilize radiopharmaceuticals for imaging. Each of them has their strengths and weaknesses. PET has the advantage of high sensitivity and can detect concentrations of 10^{-12} mol/L. Unlike most other imaging techniques, it is possible to make measurements in absolute terms. The disadvantage of poor spatial resolution has been solved by a combination of PET and computed tomography (PET/CT) or, just recently, with MRI (PET/MR).

The PET method is based on a synthesis of biomarkers. Biomarkers can be any substance such as amino acids, carbohydrates, neuropeptides, or drugs. They can be labelled with short-lived, positron emitting radionuclides produced with the aid of a cyclotron. The preparation is injected intravenously, after which its distribution and turnover in space and time are identified by the PET camera. Biomarkers interact with the surrounding tissue and in turn change the image according to the molecular changes that occur. The method provides regional, quantitative, and functional biochemical information on parameters such as blood perfusion, cellular metabolism, or receptor and enzyme kinetics.

Measurements of blood flow and glucose consumption are the 2 most common studies performed using PET. Together with measurements of oxy-
gen utilization, they are the reason why PET has become the golden standard of quantitative imaging in physiological experiments. However, these experiments only give an overview of the functional status of the tissues examined. With the PET technique, it is also possible to measure different aspects of receptor and transmitter systems, in the brain and the whole body.

Modeling of the PET signal - Making a PET image

When measuring transmitter systems, the PET signal has to be divided into different components. The signal is not only composed of the tracer that is bound to a receptor site but also tracer in blood, metabolized or degraded tracer that is labelled, free tracer in tissue, and tracer in tissue that is non-specifically bound. All these compartments compose the PET signal.

![Schematic diagram of a two-compartment model](image)

**Figure 1.** Schematic diagram of a two-compartment model

*Abbreviations:* $C_{wb}$, tracer concentration in whole blood; $k_1$, transport of ligand from plasma to tissue; $k_2$, transport of ligand from tissue to plasma; $k_3$ and $k_4$ transport of ligand between free and specifically bound

To describe or separate the activity of the specifically bound tracer from all other activities, a model is required that describes all the other compartments. In the simplest case, it is assumed that the tracer is unchanging and that there are no downgrading products with radioactive metabolites. This is often true in brain measurements, because most metabolites are converted
into more polar products and do not cross the blood–brain barrier. This may be tested in detailed experiments where the metabolites are measured during the scan. It is also assumed that a rapid equilibrium is attained between the non-specific binding and free fraction in the tissue. This gives a model with 2 compartments, the specifically bound tracer and free tracer together with the non-specifically bound tracer in tissue.

First, to produce an input function for a dynamic measurement whole blood samples are taken. These samples have to be taken rapidly and regularly to produce a time activity curve (TACT) and from these samples, a TACT curve of the activity in plasma is made. Secondly, the fraction of metabolites is measured and subtracted from the plasma curve. This gives the metabolite corrected plasma curve that is used as one input function. Thirdly, measurement in the PET image is done with a volume of interest (VOI) to produce a TACT curve of the tissue activity. This activity consists of tissue activity (free and bound) and intravascular activity from whole blood. With these 3 inputs, i.e. the metabolite corrected plasma curve, time course of the vascular component (uncorrected whole blood), and TACT curve of the tissue activity, it is possible to make a quantitative PET image.

The units in which a PET receptor study is measured could be the binding potential (BP) or volume of distribution (VD). The BP is the product of receptor density (B_max) and affinity (K_d) for the ligand to the receptor. In general, it is thought that the affinity for the receptor is unchanged, and therefore, a change in BP reflects a change in the number of available receptors.

Sometimes, it is not possible to obtain a proper BP. This means that when examining a number of normal healthy subjects, the BP has to be robust (small standard errors of the fitted BP values) and stable (small standard deviations in average BP). If they are not, then the model is not valid, and it may be better to fit for VD.

VD is the relation between tissue and plasma concentration at equilibrium and is dimensionless. For example, a VD of 15 means that the concentration of ligand in tissue is 15 times more than that in plasma.

The reference tissue model (RTM) – PET imaging without blood sampling
If some part of the examined tissue is devoid of receptors for the tracer that is used, the information from a specific VOI measurement of that region can be used as reference or input functional in the calculation of BP. It is also possible to calculate the delivery or perfusion (R1) of tracer into specific areas relative to the reference region. The delivery of small tracer molecules is often proportional to the tissue perfusion. This reference tissue model (RTM) avoids the need for plasma sampling and measuring of tracer concentrations and metabolites. There are advantages and disadvantages with all
models. RTM often gives a robust estimate of BP, but R1 often has high standard errors.

The simplified reference tissue model
To produce a stable R1, the simplified RTM (SRTM) was developed. In this model, there is an assumption that the concentration of free and specifically bound tracer cannot be distinguished, and therefore, reduces the number of parameters needed. Tracers that typically can be used within this model are raclopride (dopamine D2) and flumazenil (FMZ, GABA A).

Several different implementations on SRTM have been developed for calculation of BP and R1 on a voxel-by-voxel basis. Different receptor parametric mapping models (RPM), SRTM2, and multilinear RTMs (MRTM, MRTM2) can be used.

The differences between the models are various estimations and simplifications compared to SRTM. In RPM, a limited set of predefined solutions of SRTM is used to linerize and speed up calculations, in MRTM2, the clearance rate of the ligand from the reference tissue is fixed, thereby reducing the variability in BP.

PET with $^{11}$C-methionine in Low Grade Glioma
PET with $[^{11}\text{C}]$-methionine (MET) is highly valuable in the planning of treatment for patients with glioma. The uptake of the tracer in the tumour is associated with proliferation activity. PET with MET has shown to be useful for patients with low-grade glioma in various clinical situations such as guiding stereotactic biopsies, differentiating between low-grade tumour and non-tumour lesion, and as a prognostic marker for disease progress and response to treatment.

PET studies in epilepsy
Localisation of interictal cerebral dysfunction with $[^{18}\text{F}]$-fluoro-deoxy-glucose (FDG) PET is the most used method for delineation of epileptogenic regions in MRI-negative patients. FDG is a glucose analog that is extracted by the cells in proportion to metabolic needs. FDG is phosphorylized intracellularly but not metabolized further. Imaging with PET provides an overview of neuronal activity in all brain regions. FDG PET detects decreased interictal glucose metabolism in 60-90% of patients with TLE and can determine prognosis following anterior temporal lobe resection. The method has lower sensitivity for extratemporal epilepsy. Sixty percent of patients with frontal lobe epilepsy have areas with hypometabolism. However, in MRI-negative cases, the finding of a hypometabolic area may help placing intracranial electrodes for ictal recording.
Investigations with PET ligands that bind to specific neuro-receptors are used to give information on abnormalities of neurotransmission involved in the pathophysiology of the epilepsies. Data always need to be interpreted in the light of structural imaging with MRI. Central benzodiazepine binding with $^{11}$C-Flumazenil (FMZ) has been studied most and is used in the clinical routine, although the need for a cyclotron has hampered its use. Traditionally the parametric images (volume of distribution ($V_D$) or binding potential (BP) shows less widespread receptor availability that the hypometabolic area of FDG $^{78-80}$. FMZ PET may be particularly useful in the case of bitemporal pathology in unilateral cryptogenic frontal lobe epilepsy $^{78}$. Furthermore, FMZ PET can show hippocampal damage in patients with no remarkable hippocampal abnormalities on quantitative MRI $^{81}$. As with FDG there is also a seizure related plasticity of the uptake of the tracer depending on time in relation to last seizure $^{82}$. It is therefore important to note time from last seizure to avoid false lateralizing $^{83}$.

In recent years PET imaging of serotonin $^{84-104}$, opioid $^{105-110}$, dopamine $^{111-118}$, glutaminergic $^{119}$ receptors and astrogliosis $^{120}$ have been performed to investigate the neurochemical basis, the role for the epileptogenesis as well as the spread of seizure activity $^{121}$.

**Substance P and epilepsy**

Substance P (SP) is a neurotransmitter and neuromodulator that mediates its effects in the brain predominantly via the neurokinin 1 (NK1) receptors $^{122}$. Activation of NK1 receptors have been implicated in epileptogenesis and modulation of epileptiform activity within the temporal lobe $^{123-125}$. Intrahippocampal administration of SP cause damage in the hippocampus resembling the pathology seen in human epilepsy $^{126}$. Agonists of the NK1 receptor system can promote release of the excitatory transmitter glutamate $^{127}$. Moreover, seizure activity can be blocked by antagonists of the NK1 receptor $^{128}$. Evidence for the involvement of NK1-SP complex in humans include increased SP immunoreactivity in hippocampus in post-mortem $^{125}$ and resected tissue $^{123, 124, 129}$. Altogether, it is likely that the NK1-SP receptor system is involved in excitatory cortical network in epilepsy. Imaging NK1 receptors in the brain using PET with the high-affinity, selective NK1 receptor antagonist $[^{11}C]$-GR205171 has previously been shown to be effective for investigation of NK1 receptor distribution $^{130-132}$.

**$^{11}$C Autoradiography – phosphorimaging**

Autoradiography may be done with an x-ray film, where the pattern is produced by emitting beta particles or gamma rays. Alternatively, the autora-
diograph is produced as a digital image. In these systems, the signal detection is made with scintillation gas detectors or as in our study, within phosphor imaging systems.

\(^{11}\)C radiolabelled ligands can be used to determine biological systems such as distribution of receptors, activity of enzymes, cell metabolism, or protein synthesis from tissue samples. This can be done in vitro, where the ligand, diluted to appropriate concentration, is applied onto tissue sections. It gives the opportunity to do functional imaging in viable brain slices \(^{133-135}\). It may also be done ex vivo, where the ligand is administered into the circulation with subsequent tissue removal and sectioning \(^{136}\).

Phosphorimaging is a faster, more sensitive technique with a greater dynamic range but with less resolution than traditional x-ray film. Although the imaging plates are reusable, this technique is more costly than x-ray filming.

Phosphor is a substance that exhibits luminescence, i.e. emission of light caused by, for example, electric energy. Photostimulated luminescence is release of stored energy from a phosphor. The release may not start until the phosphor is exposed to visible light. A phosphor imaging plate contains phosphorous material and can record and store weak radioactive signals over a prolonged period. The energy stored in the plate is not released until it is exposed to visible light. Therefore, plates may be stored (in darkness) for a period and then be read. The reading of the pattern of radioactivity is performed in phosphorimagers, wherein the plate is exposed to light, causing the stored energy to be released.
Aims of the thesis

The general aims of the thesis were to improve PET imaging and find new PET tracers that can increase the diagnostic yield and understanding of pathophysiological mechanisms in structural epilepsy with focal seizures. The specific aims were to investigate,

- the correlation between seizure activity and tumour metabolic rate measured by MET PET in patients with glioma and focal epilepsy and to assess the prognostic impact of early seizure manifestation on patient survival.

- whether neurobiological changes linked to SP-NK1 receptor system are associated with hyperexcitability in patients with TLE, and to investigate the relationship between seizure frequency and NK1-receptor availability in the temporal lobes.

- whether the same pathological brain areas in patients with focal epilepsy could be identified using FDG and parametric blood flow images estimated from dynamic $^{11}$C-FMZ PET images.

- dynamic changes in microglia, astrocytes, and central benzodiazepine during epileptogenesis.
Materials and Methods

Paper I

Subjects

In total, 128 patients aged ≥ 16 years with supratentorial grade II glioma, who had been examined by PET with MET (PET MET) scan at the department of Neurosurgery, Uppsala University Hospital, between 1983 and 2005, were included.

Study design and collection of clinical data

The following information was retrospectively collected from patient files:
- Age at disease onset at the time of PET investigation
- First symptom of the disease
- The level of patients preoperative activity and medical care requirements (Karnofsky performance status scale, KPS)\(^{137}\)
- Tumour localisation (cortical/subcortical or central)
- Tumour size (as measured with MRI)
- Extent of diagnostic surgery (biopsy, subtotal, or total resection based on postoperative CT scan or the surgeon’s postoperative notes)
- Histological diagnosis (astrocytoma, oligoastroglioma, or oligodendro-glioma)
The study design is illustrated below:

![Study Design Diagram](image)

*Figure 2.* Illustration of the study design showing the time-axis for which clinical data were collected retrospectively and the definitions of patient survival.

**Abbreviations:** KPS: Karnofsky performance status; PET MET: $^{11}$C-metionine positron emission tomography

Survival was calculated at 2 different time-points:
1. **Survival at disease onset** was defined as the time-period between the presenting symptom and date of death or end of the study.
2. **Survival at early stage of disease** was defined as the time-period between the PET scan and date of death or end of the study.
Positron emission tomography

Tree different cameras were used during the time period of 1983-2005 as shown in Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Name</th>
<th>No of slices/slice thickness</th>
<th>Period of use</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>(II) Scanditronix/GEMS PC2048-15B</td>
<td>15/6.5/6.5/6</td>
<td>1991–1999</td>
<td>51</td>
</tr>
<tr>
<td>(III) Siemens ECAT Exact HR+ scanner (CTI, Knoxville, Tennessee)</td>
<td>63/2.46/2.46/4-5</td>
<td>1999–2005</td>
<td>35</td>
</tr>
</tbody>
</table>

Patients were positioned in the camera and 10-min transmission scan was performed. A bolus of $^{11}$C-methionine (Camera I: 50–200 MBq; camera II and III: 300–400 MBq) was injected intravenously, and a dynamic emission scan was started. Summation images covering 20–40 min after the injection were used for the analysis. All patients fasted for at least 4 hours prior to investigation.

PET image analysis

The mean uptake of MET in the tumour was measured and related to a reference area in the healthy hemisphere (hot spot/cortex ratio) \(^{49}\). The reference area consisted of six cortical areas covering the frontal, temporal and occipital cortices in the healthy hemisphere at the level of the thalami, each approximately 30 ×10 mm. The hot spot was defined as ten connected pixels with the highest MET uptake within the tumour, representing an area of 55–60 mm\(^2\).

Statistical analysis

Survival analysis was performed through life tables in the Cox regression model. Each variable at the time was tested first by a univariate analysis and then, a multivariate analysis. The latter was performed, using backward logistic regression for variable selection. For both analyses, the probability for entry/removal was 0.05/0.1. Variables were dichotomised and included in the following order: KPS, \(\leq 80/>80\); age, \(\leq 40/>40\) year; histology, astrocy-
toma-oligoastrocytoma/oligodendroglioma; seizure at disease onset, yes/no; localisation, cortical-subcortical/central; and largest diameter, \( \leq 6 \text{ cm} \).

In the analysis at the early stage of the disease, seizure free at PET yes/no and methionine uptake ratio hotspot/normal cortex \( \leq 2.1 \text{ cm} \), were added. Analysis was performed in SPSS 16.0.1.

**Paper II**

**Subjects**

Nine patients (7 females and 2 males) with medically refractory TLE participating in an epilepsy surgery program at the Uppsala University Hospital were included. The diagnosis and focus lateralisation was based on seizure monitoring with video-EEG and MRI. A thorough validation of seizure frequency in the months prior to the PET examination was performed by scrutinizing patients’ seizure diaries and by interviews with the next of kin. In addition, 18 healthy control subjects (9 females and 9 males) were included for comparison.

**Positron Emission Tomography**

A Siemens ECAT Exact HR+ scanner was used for the PET scans. The radioligand \([^{11}C]\)-GR205171 was synthesised according to standard procedures \[^{130}\]. A 10-min transmission scan was acquired for attenuation correction. Thereafter, the radioligand was given as a rapid bolus injection simultaneously with the initiation of data acquisition. The amount of tracer injected was approximately 5 MBq/kg body weight (average dose, \( 350 \pm 100 \text{ MBq} \)). Dynamic data were collected in 17 progressively increasing time frames over 60 min (4 \( \times \) 60 s, 3 \( \times \) 120 s, and 10 \( \times \) 300 s).

Dynamical frames were realigned within scan to adjust for movements during scanning. After realignment, a summation image was generated for the time interval of 5–60 min. In this image, the cerebellar cortex was manually delineated as a region of interest (ROI), for use as a reference region. The cerebellum was chosen as a reference region, because previous studies have demonstrated negligible in vivo specific receptor binding in the human cerebellar cortex \[^{130,138}\]. Parametric PET images were generated using a Patlak RTM \[^{139,140}\], with the cerebellum as reference region and a time window of 20–60 min. NK1 Patlak slope values were interpreted as a measure of NK1 receptor availability, reflecting NK1 receptor density minus endogenous SP occupancy.
Data analysis

Registration to Montreal Neurological Institute (MNI) space and statistical analysis of parametric slope images for patients and control-subjects were performed using the statistical parametric mapping software, SPM2. The realigned summation images (5–60 min) were used for registration to improve the spatial resolution. Images were then smoothed using a 12-mm full-width-at-half-maximum (FWHM) Gaussian kernel to improve the signal-to-noise ratio. The parametric images for 2 patients were reversed before normalization so that for each subject, the seizure onset zone was on the left side. Reported co-ordinates are MNI and anatomical locations were determined using the Talairach Daemon system (http://www.talairach.org/ daemon.html) after transformation of the MNI co-ordinates to Talairach space using the function mni2tal. Objective voxel-by-voxel statistics in the whole brain was performed for comparison. The level of significance was set to an uncorrected p value of p < 0.001 with a minimum cluster size of 50 voxels. Statistical analyses of group differences were conducted in SPM2 with a between-group ANCOVA, using age as a nuisance variable to dissociate the differences between patients and healthy control subjects. The magnitude of receptor binding in relation to seizure frequency was estimated using linear regression analyses.

Paper III

Subjects

In this study, 15 out of 40 patients with medically refractory focal epilepsy that participated in the preoperative epilepsy surgical program in Uppsala between 2007 and 2010 were analysed. Due to non-converging and localising results from video-EEG monitoring and neuroimaging with MRI, they also underwent PET with FDG and FMZ. Exclusion criteria were seizures during the day of scanning, different scanning days, and anaesthesia or non-localising image information on the FDG scan.

Positron emission tomography

All patients underwent a 40-min dynamic scan after intravenous (i.v.) administration of 4 MBq/kg FMZ and a 20-min static scan at 30 min after i.v. administration of 3 MBq/kg FDG on the same day. The images were acquired using an ECAT Exact HR+ scanner.

For rCBF image calculations from the FMZ data, a VOI was drawn over the pons on a co-registered MRI scan, and that area was transferred to the
dynamic PET dataset to obtain a pons time-activity curve. Next, rCBF images were constructed on a voxel-by-voxel basis using a linearised version of SRTM (MRTM2). rCBF and FDG images were subsequently co-registered using a mutual information algorithm (Vinci v. 3.30, Max-Planck Institute for Neurological Research, Cologne, Germany).

Comparison between rCBF and FDG images

We performed 3 different tests:

- A computer-based pixel-by-pixel comparison with linear regression between grey matter tissue from 5 patients in rCBF and FDG images.
- An asymmetry measurement (left and right) within the scan with paired VOIs. All FDG and rCBF images were globally and spatially normalised into a Montreal Neurological Institute template (MNI space) and a VOI template with 11-paired (left and right) regions were applied. The normalized mean rCBF and FDG standard uptake value (SUV) was calculated for each VOI, and the contrast between the right and left hemispheres was calculated from the equation \[ \frac{(R - L)}{R} \times 100\% \]. The percentage difference of rCBF was plotted against FDG SUV.
- A blinded visual examination where significant changes in different lobes were assessed by experienced nuclear medicine physicians.
- A blinded visual examination where significant changes in different lobes were assessed by experienced nuclear medical physicians.

Paper IV

Animals - Iron-induced posttraumatic epilepsy model

Twenty-four male Sprague–Dawley rats were used. They were anaesthetised during surgery and decapitation with isoflurane mix of 30/70 O2 and N2O. Neurosurgery was performed as described by Willmore with the injection of 5 µL of 100 nM iron chloride solution at 5 mm to the right of the midline and 2 mm behind the bregma to produce a focal epileptogenic lesion. Ten animals were sham operated receiving 5 of artificial CSF at the same location.

The animals were sacrificed on days 1, 5, 100, and 180 after intracortical injection, and their brains were removed and frozen onto cryostat chucks.

Tissue collection and autoradiography

Coronal sections (2-µm thick) were cut with a cryostat microtome, mounted onto gelatine-coated glass slides, dried, and stored until autoradiography
experiments. The $^{11}$C-labelled traces were produced according to standard procedures at the Uppsala PET centre. A semi-quantitative experiment was performed in the brains sacrificed after 1, 5, 100, and 180 days. In addition, a quantitative measurement was performed with samples from 100 days to determine maximal binding ($B_{max}$) and dissociation constant ($K_d$).

For autoradiography, slides were removed from the freezer and air-dried at room temperature for 1 h. Sections used for $[^{11}$C]-PK11195, $[^{11}$C]-(L)-deprenyl, and $[^{11}$C]-FMZ labelling were pre-incubated at 24°C for 6 or 7 min in an incubation buffer (50 mM Tris-HCL, pH 7.4). To define the specific binding, sections were incubated at 24°C for 30 min in 1 nM tracer concentration for $[^{11}$C]-PK11195, 100 nM for $[^{11}$C]-(L)-deprenyl, and 3 nM for $[^{11}$C]-FMZ as indication of total binding. Non-specific binding in all experiments was determined in adjacent sections incubated in the presence of 1 μM PK11195, (L)-deprenyl or Ro 15-1788. In the quantitative experiments, tracer concentrations of 0.3, 1, 3, and 10 were used.

After incubation, the slides were washed, dried, and exposed for 40 min at room temperature to recyclable phosphorimaging plates together with 20-μL drops of known tracer concentration for calibration. The imaging plates were scanned with a laser beam in a Phosphor imager.

Regions of interest were delineated in the autoradiographic dataset at the site of injection, in the contralateral region and in areas with known concentrations. The raw data sets were used to calculate tracer binding in molecular concentrations.

Histology and immunohistochemistry

Frozen sections corresponding (± <200 μm) to the section used in the autoradiographic studies were stained with Luxol fast blue, CD11b, and GFAP. Sections from each time point (1, 5, 100, and 180 days) were visually examined in conjunction with the autoradiographic data.

Statistical analysis was performed by using the independent samples $t$ test for comparison of binding to the lesioned and sham operated rats. A significance level of $p < 0.05$ was used.
Results

Paper I

Patient and tumour characteristics
The mean ages of the patients at disease onset was 41.9 years (range: 16.4–74.4 years); at PET examination, 42.3 years (range: 16.9–74.7 years), and at the time of surgery, 42.8 years (range: 16.9–74.8 years). The histological diagnosis was astrocytoma in 34 cases (34%), oligodendroglioma in 49 (49%), and oligoastrocytoma in 18 (18%).

Seizures at disease onset
Epileptic seizures were the most common presenting symptom and occurred in 88 of the 101 patients (88%). For those 13 patients who did not have seizure at disease onset, headache was the most common symptom. The majority of these patients had tumours with central localization.

Seizure manifestations at early stage of disease
All 13 patients that presented with symptoms other than seizures at disease onset were also seizure free at the early stage of the disease. Of the 88 patients that presented with seizures, 34 were seizure free at the early stage of the disease (34%), resulting in 47 seizure-free patients (13 + 34) at the early stage of the disease.

PET with methionine and seizure manifestations
The overall mean hot spot/cortex ratio of the uptake of MET measured by MET PET was 2.3 (range: 0.9–5.4). In the statistical analyses, patients with astrocytoma and oligoastrocytoma were grouped together, as previously described. The MET PET was 2.2 for the astrocytoma/oligoastrocytoma group and 2.5 for the oligodendroglioma group. We found a slightly lower MET PET for patients with no seizures compared with those patients that
had seizures at disease onset, which was the case for the whole patient population, as well as for the 2 histological subgroups, but the differences were not significant. Also, at the early stage of the disease, no significant differences were found between MET PET values for patients that were seizure free compared to patients with recurrent seizures.

Survival analysis at disease onset

In the multivariate analysis, 2 variables, the histological subtype of the tumour (oligodendroglioma) and seizures as presenting symptom (Figure 3), were found to contribute significantly to the prognosis for survival. Good clinical performance, as measured by a high Karnofsky score 137 (KPS > 80) did almost reach a significance level. The parameters young age (≤40 years) at disease onset, non-central tumour localisation, and small tumour diameter (≤6 cm) did not show any trends towards favourable prognosis.

![Survival analysis at disease onset](image)

*Figure 3. Kaplan-Meier estimates of cumulative survival at disease onset by presenting symptom. Seizure at onset versus no seizures at onset*

Survival analysis at the early stage of the disease

A multivariate analysis was made at the early stage of the disease, in which the time-point for PET was used as a reference point. In this model, all pre-
vious parameters from the disease onset model together with MET PET and seizure manifestations at early stage were used. All patients, including the ones that were seizure free at disease onset, were included in this analysis.

The following 3 variables contributed significantly to a favourable prognosis: histological diagnosis of oligodendroglioma, seizure free at the early stage of disease (*Figure 4*), and low MET PET uptake quota. The variable, high Karnofsky score (KPS > 80) (measured at disease onset), was significant in the univariate analysis but did not fully reach significance (P = 0.052) in the multivariate analysis. The parameters young age at early stage of disease (<40 year), non-central tumour localisation, and small tumour diameter (<6 cm) were not identified as favourable prognostic parameters in the Cox analysis.

*Figure 4*. Kaplan–Meier estimates of cumulative survival at the early stage of the disease by seizure free or not
Paper II

NK1 receptor availability in TLE
The highest receptor availability in both patients and control subjects was seen in the putamen and the caudate nucleus, and the lowest was observed in the cerebellum. Patients had significantly higher receptor availability than the control subjects in both the ipsi- and contralateral hemispheres. These areas were

- Ipsilateral cingulate gyrus
- Contralateral precuneus
- Contralateral insula
- Contralateral occipito-temporal area
- Ipsilateral postcentral gyrus
- Ipsilateral cuneus
- Ipsilateral anterior cingulate
- Contralateral caudate head
- Contralateral cuneus
NK1 receptor availability in relation to seizure frequency

The mean seizure frequency in the patient group was 17 ± 20 (Mean, SD). A significant and positive correlation between NK1 receptor availability and seizure frequency was observed in a cluster area in the mesial ipsilateral temporal lobe (Figure 5).

*Figure 5. Regression analysis of patients with temporal lobe epilepsy, showing a positive correlation between tracer uptake and seizure frequency. The hair cross indicates the parahippocampal gyrus, Brodmann area 28 (threshold, \( p = 0.001 \); minimum cluster size, 50 voxels), ipsilateral to seizure onset. Colour bar represents \( t \)-statistics*
Paper III

Pixel-by pixel assessment
The correlation curve for a typical patient between cortical rCBF and FDG are shown in Figure 6. For all subjects R was 0.88 ± 0.01 (mean, SD), slope was 1.12 ± 0.09, and intercept was −0.12 ± 0.09.

![Graph showing correlation between rCBF and FDG](image)

*Figure 6*. Pixel-wise correlation between the normalised grey matter values of FDG and rCBF in one patient with focal epilepsy.

*Abbreviations*: rCBF, Relative cerebral blood flow; FDG SUV: Fluorodeoxyglucose, Standard Uptake Values.

VOI-based assessment
The correlation curve and coefficient between the VOI-defined FDG uptake values and rCBF are shown in Figure 7. R was 0.80, the slope was 0.78, and the intercept was 0.6%.
Figure 7. The difference between the right and left FDG uptake compared to rCBF in 15 patients with focal epilepsy. All brains were divided into 11 paired cortical regions. Each dot represents one paired region. In areas with pathology, there is an asymmetric signal with reduced FDG uptake and rCBF in the pathological (epileptic cortex) as compared to the normal (healthy cortex).

Abbreviations: R=right; L=left. The paired cortical regions were anterior- and posterior cingulate gyri, prefrontal area, pre- and post-central gyri, parietal lobe, precuneus, mesial- and lateral temporal lobe, occipital lobe, and cerebellum.

Visual assessment
Inter-rater agreement was calculated for the first rater and the consensus result of the second and third raters. The first rater only assessed the FDG scan, and the second and third raters only viewed the rCBF scans. The agreement values for the frontal, parietal, temporal, and occipital lobes in the right hemisphere were 93%, 87%, 100%, and 93%, respectively, and the corresponding values for the left hemisphere were 93%, 100%, 100%, and 100%. The second and third raters did not have any false-positive results.
Paper IV

PK11195, (L)-deprenyl, and flumazenil $^{11}$C-autoradiography

In the semi-quantitative part of the study, the binding pattern of PK11195 showed a significant increase after 5 days and a decrease to the same levels as the control animals after 180 days. The binding of (L)-deprenyl was lower than the sham operated animals at day 1 but after 180 days, a significant increase was found. For FMZ there was an immediate significant decrease of binding at day 1. This decrease was persistent throughout the study.

The results from the quantitative measurements at day 100 were similar to that shown in the semi-quantitative measurements with the most prominent findings in the FMZ part of the study.

Histology and immunohistochemistry

The site of injection was histologically determined in all rats. In 3 rats, the injection had been given extra-cerebral. Data from these animals were excluded from further analyses.

In the iron-injected areas, there was an instant formation of oedema and occasional minor haemorrhages. After 5 days, an infiltration of peripheral lymphoid cells was seen in the Luxol-stained slides and corresponding binding to PBR using the immunohistochemical staining of CD11b-positive cells. There was also a scar formation with vacuoles and surrounding astrocytes seen in the GFAP-stained slides. In the sham-operated animals, there was a small amount of oedema and invasion of peripheral lymphoid cells, but no increase of PBR or activation of astrocytes nor formation of a scar was seen.
Discussion and Conclusions

Paper I

In this study, we studied the presence of seizures at disease onset and in the early stage of the disease in patients with low-grade glioma. We chose these time points because our hospital has a long tradition in using PET MET in the evaluation of such patients, and reliable clinical data were available in the hospital files at the time for PET. This gave us the opportunity to (i) study the uptake of MET in relation to seizure manifestations and (ii) to study if seizure manifestations had any impact on survival in this patient group.

We did not find any correlation between MET PET and epileptic manifestations either at disease onset or early in the disease at the time of PET. According to our knowledge, no assessment between seizure status and MET PET has been made before. It has been studied in conjunction with epilepsy and specific types of brain tumours such as dysembryoplastic neuroepithelial tumours, gangliogliomas, and low-grade gliomas \(^{142, 143}\). However, these assessments only addressed the tumour type and outcome after surgery and not the preoperative seizure status or localisation of the seizure onset zone.

We found a positive relation between the presence of seizures at disease onset and more favourable prognosis in our patient group. This may be due to a higher proportion of tumours with cortical or subcortical involvement. Consistently, we also found a higher proportion of tumours with central localisation in the group of patients that did not have seizures (8/13 = 62%) than in the group that had seizures at disease onset (9/88 = 88%). Central localisation of the tumour is associated with a poorer prognosis \(^{144}\).

This study also showed that patients with seizures in the early stage of disease had a shorter survival time than those that were seizure free. It is likely that seizure freedom in patients that experienced seizures earlier is related to factors other than the location of the tumour. It is, of cause likely that a more advanced clinical stage would favour poor prognosis, and the proportion of patients with large tumours was higher in the group with recurrent seizures (24/54 = 44%) than in the seizure-free group (13/47 = 28%).

In conclusion, in this retrospective study, we found that seizures as the first symptom of disease and seizure freedom in the early stage of the disease
is correlated to a favourable prognosis. Seizure as first symptom may reflect a more peripheral tumour, and thereby, a better prognosis as compared to central tumours. Seizure freedom at early stage for those that presented with a seizure may indicate a slower growth rate or earlier phase in tumour development. The study did not reveal any positive or negative correlation between seizure activity and MET uptake. This may be because epilepsy depends more on localisation, i.e. spatial relation to cortical structures rather than the rate of tumour growth.

Paper II

This PET study showed that NK1 receptor availability was significantly increased in structures located in both the ipsilateral and contralateral hemispheres in patients with TLE. This could imply a decreased level of endogenous synaptic SP, an increased number of NK1 receptors, or their combination. TLE is characterised by seizures that often begin in limbic structures, and the present study suggests both remote and local changes. This is in agreement with previous animal studies using EEG, FDG autoradiography and fMRI\textsuperscript{145-148}. In humans, similar remote changes in blood flow have been found using subtracted ictal and interictal SPECT\textsuperscript{149}.

In this study we also showed novel results where an increased NK1 receptor availability was found to correlate to the number of seizures in ipsilateral medial temporal structures. In experimental models of epilepsy an increased activity of NK1-SP signalling pathway has been demonstrated\textsuperscript{126, 150, 151}. It is possible that the NK1-SP system has a role in cortical network and may function to promote excitatory communication between neurons in TLE. Furthermore, antagonists of NK1 receptors are known to inhibit seizure activity\textsuperscript{128}. It is possible that antagonists of the NK1 receptor have a potential to moderate the spread of seizure activity. This possibility has been explored in an animal model and treatment with the antagonist GR205171 (vofopitant) potentiates the anticonvulsant effect of sodium channel blockers\textsuperscript{152}. In humans the effect of NK1 receptor antagonists has yet to be explored.

There were some limitations to the present study. None of our patients had any history of psychiatric problems but no formal evaluation of anxiety or depression was performed. Therefore we cannot exclude that psychiatric problems with endogenous release of SP may affect the availability of NK1 receptors. Also, there is a possibility that antiepileptic drugs may influence the endogenous SP levels or protein binding of the tracer in the blood.

The results from this study suggests that there is an epileptiform network that uses the NK1-receptor system for synaptic transmission in TLE, and it is likely that availability of NK1 receptors varies with time from last seizure.
Paper III

The results show a close relationship between glucose metabolism and cerebral blood flow. This has been found before in interictal studies with PET and single photon emission computed tomography (SPECT) \cite{71,153}. This relationship may, however, be disrupted in epileptogenic regions \cite{154}. In this study we also noticed that the areas with perfusion abnormalities were less pronounced than the area with hypometabolism. This is shown in the VOI assessment with a slope value $< 1.0$ and in the visual examination where there was a tendency towards false negative results with an underestimation of the hypoperfused areas as compared to hypometabolism but no false positive results. This is in agreement with a PET study of patients with TLE comparing $^{15}$O water and $^{18}$FDG \cite{155}. Depending on the method the epileptogenic zone was localized in 15/25 patients with $^{15}$O water and 21/25 or 23/25 with $^{18}$FDG \cite{156}.

Blood flow changes during and after a seizure will complicate image interpretation. Acute effects of seizures or ictal examinations will increase cerebral blood flow. Postictal hypoperfusion may last for 10-30 min \cite{157} but depending on the type and duration of the seizure clinical experience shows us that it as in Todd’s paralysis may stay longer than that. For this reason all scans were performed at least 24 hours after a seizure.

In the preoperative evaluation of epilepsy patients it is important to get as detailed localizing information as possible. In this respect also sublobar information would be valuable. To this end a group of control subjects would be needed.

This explorative PET study used novel methods to further improve the presurgical investigation in patients with intractable epilepsy. The results indicate that it is possible to simplify the investigational protocol and reduce costs and patient radiation dose.

Paper IV

In this study, we analyzed the dynamic binding characteristic of $[^{11}]$PK11195, $[^{11}]$-(L)-deprenyl, and $[^{11}]$FMZ to visualize changes in microglia, astrocytes, and inhibitory neuronal cells between acute TBI and the development of posttraumatic epilepsy. For this purpose, we used Willmore’s model of posttraumatic epilepsy. This model is well characterized \cite{158} and more than 90 % of the rats develop chronic focal epileptic activity during the first 12 months \cite{159}. This makes it suitable for longitudinal studies of epileptogenesis despite the fact that EEG was not monitored in this study.

Earlier studies have shown that microglia are activated in human and experimental epilepsy \cite{160-162}. In this study, a peak of $[^{11}]$PK11195 binding was seen at day 1 and 5 at the iron chloride-injected sites. According to re-
sults from the histological examination, this increase represents an invasion of peripheral lymphocytes reflecting an early inflammatory phase. The increase in \([^{11}C]PK11195\) binding was most prominent on the ipsilateral side, but there was also a contralateral increase. We know from prior studies that epileptic activity occurs not only on the side of the lesion, but also in the contralateral hemisphere. Accordingly, an acute lesion may trigger chronic epileptiform activity in other parts of the brain. An alternative explanation might be that specific neuroinflammatory cells with a putative role in the epileptogenesis are activated in response to head trauma.

It has previously been shown that L-Deprenyl can be used to quantify gliosis in TLE. In this study the binding of \([^{11}C\)-(L)-deprenyl decreased 1 and 5 days after TBI, and then increased at 100 and 180 days. This increase is due to activation of astrocytes. Increased binding of \(^{11}C\)-labeled deprenyl after TBI has also been demonstrated in PET studies in humans as well as in patients with TLE. Astrocytes have an important role in the regulation of extracellular neurotransmitters, particularly for the excitatory neurotransmitter glutamate. To remove glutamate from the synapse, astrocytes use the transporter protein GLT-1. Measurements of GLT-1 levels by using immunoblotting have demonstrated decreased levels at day 1 and 5 after iron chloride lesion but not at 3 months. The same dynamic changes were seen in the deprenyl binding in the present study, and it is possible that the initial acute and later chronic epileptic discharges are related to this transformation.

Abnormalities of flumazenil binding has been reported in autoradiographic and numerous PET studies in patients with focal epilepsy. In this study, we noticed an immediate and persistent decrease of the binding of \([^{11}C]\)flumazenil in the lesioned region. In experimental animal models and in humans this has been shown to correlate to the loss of neurons. The observed decrease, however, extended far beyond the histologically verified loss of neurons.

Calculations of the \(K_d\), \(B_{max}\), and \(BP\) were performed in order to compare quantitative binding data to previous experiments. Because of the limited number of sections available, this was only carried out once on the day 100. The result were in line with the semi-quantitative measurements and with data reported in the literature.

This autoradiographic study shows that \(^{11}C\)-labelled PET-tracers for visualization of glial cells and benzodiazepine binding may be useful in future investigation of molecular pathways involved in epileptogenesis after an initial trauma.
Concluding Remarks

We have used new applications of $^{11}\text{C}$-labelled tracers in PET and autoradiographic investigations with an aim to improve the diagnostic yield and understanding of pathophysiological mechanisms in focal epilepsy. The main findings were

- no correlation exists between seizure activity and metabolic rate as measured with PET with MET in patients with low-grade glioma
- an increased NK1 receptor availability as measured using PET with $[^{11}\text{C}]-\text{GR205171}$ were seen in both the hemispheres, most pronounced in the anterior cingulate gyrus, ipsilateral to seizure onset region in patients with TLE
- there is evidence of an intrinsic network using the NK1-SP receptor system in TLE
- images of relative cerebral blood flow can be produced by pharmacokinetic modelling of dynamic $[^{11}\text{C}]-\text{FMZ}$ PET data, and these images resembles the uptake images of $^{18}\text{FDG}$
- autoradiography with $^{11}\text{C}$-labelled ligands can be used to visualise dynamic changes in the microglia, astrocytes, and GABAergic neurons during epileptogenesis
Future perspectives

The present research is a starting point for further prospective studies. The results from the PET study with $[^{11}\text{C}]$-GR205171 suggest that there is an intrinsic network that uses the NK1-SP receptor system for synaptic transmission in TLE. These data are new and more studies will have to be performed. It is likely that the availability of NK1-SP receptor varies with time to the last seizure. Studies comparing inter- and postictal images of the same patients would, therefore, contribute to the knowledge of seizure propagation in preoperative epilepsy surgery investigations.

A prospective study of consecutive patients undergoing epilepsy surgery with PET with $[^{11}\text{C}]$-FMZ and $^{18}\text{F}$-FDG will be of great importance to evaluate the correlations between relative blood flow measured with dynamic PET with FMZ and metabolism measured with FDG. The clinical utility of the method will have to be assessed in relation to the outcome after epilepsy surgery.

Studies of cerebral blood flow would benefit from multiple comparisons with different modalities. Soon systems with combined PET and MRI will be available with the possibility to get excellent anatomical resolution. Above all it will also be possible to simultaneously measure blood flow with PET and haemodynamic and metabolic properties by MRI.

The results from the autoradiographic study shows that it is possible to measure and monitor important steps in the molecular pathways crucial to the development of epilepsy. A study of the effect of drugs such, as minocycline that may decrease the microglial activation (e.g. translocator protein) is highly interesting.
Sammanfattning på svenska

Epilepsi är en vanlig neurologisk sjukdom med en prevalens på 0.7% vilket motsvarar 70 000 individer i Sverige. Orsaken till epilepsi kan vara många, till exempel är hjärntumörer och skallas. Merparten av epilepsipatienter svarar bra på läkemedelsbehandling men cirka 30 % är medicinskt refraktära, dvs. fortsätter att ha anfall trots behandling. För dessa patienter kan kirurgisk behandling vara ett alternativ under förutsättning att man kan lokalisera det område i hjärnan som genererar anfallen. Avhandlingens syfte är att med nuklearmedicinska tekniker förbättra omhändertagandet och utveckla nya diagnostiska metoder och behandlingar för patienter med svår epilepsi. Tekniker som använts är positronemissions tomografi (PET) samt autoradiografi med olika 11C-märkta spårsubstanser.

Aktivering av neurokinin-1 receptorn (NK1) med substans P kan inducera och förlänga epileptiska anfall i experimentella modeller av epilepsi. I delarbete II studerades bindningen av en antagonist till NK-1 receptorn på patienter med temporallibsepilepsi (TLE) och jämfördes med friska försökspersoner. Vidare undersökte vi om grad av bindning korrelerade till anfallsfrekvens. Nio patienter med TLE och 18 friska försökspersoner undersöktes med PET med den selektiva NK1-receptor antagonisten \([^{11}C]GR205171\). Parametriska PET-bilder beräknades enligt en grafisk analysmetod (Patlak metoden) med cerebellum som referens. Bilderna normaliserades och omformades därefter en standard hjärna för att möjliggöra beräkningar för varje bildpunkt. Jämförelse mot kontrollmaterial och korrelation mot anfallsfrekvens gjordes med ”statistical parametrical mapping” (SPM). Resultaten visade av att det finns en relativ ökning av NK-1 receptorer hos personer med TLE, dels i området i hjärnan där anfallet startar och dels i centrala samt kontralaterala strukturer som kan förväntas vara inblandade i spridningen av anfallsaktivitet. Då antalet tillgängliga receptorer hos patienter med TLE jämfördes med anfallsfrekvens sågs en tydlig positiv korrelation in mediala strukturer i temporalloben och i nukleus lentiformis. Vår tolkning är att det finns epileptiska nätverk som verkar via NK-1 receptorer och att dessa uppregleras i samband med anfall.

PET är en viktig metod i utredningen av patienter med terapiresistent epilepsy och används för att definiera området från vilket de epileptiska anfallen startar. I klinisk rutin används oftast en kombination av PET med flumazenil (FMZ) som en markör för GABA\textsubscript{A}-receptorer och fluorodeoxyglukos (FDG) som mäter cerebral glukosmetabolism. I delarbete III ville vi undersöka om ett dynamiskt insamlat dataset från FMZ-PET kan användas för att beräkna det relativa cerebrala blodflödet (rCBF) och om denna beräkning korrelerar till metabolism mätt med FDG-PET. 15 patienter som genomgått epilepsikirurgisk utredning inklusive FMZ- och FDG-PET ingick i studien. På varje patient beräknades rCBF enligt en multilinjär referensvävnadsmodell (MRTM2) med pons som referensregion och resultaten jämfördes med FDG-PET undersöknings. Tre olika beräkningsätt användes: 1) pixel för pixel jämförelse, 2) jämförelse av områden (VOI, volume of interest) i sjuk och frisk lob samt 3) visuell bedömning av PET-bilder utfört av tre erfarna nuklearmedicinare. För samtliga beräkningsmetoder förelåg en god korrelation mellan rCBF och metabolism. Resultatet ger ett bra underlag för en prospektiv studie för att säkrare svara på om FMZ kan ersätta FDG i epilepsikirurgiska utredningar. Om metoden kan användas kommer det att innebära en avsevärd förenkling av utredningen med PET vid epilepsy samt en minskning av total mängd radioaktiv exponering.
Delarbete IV är en djurexperimentell studie där sambandet mellan skall-trauma och uppkomsten av epileptiska anfall undersöcktes. Förändringar i celler med inhibiterande aktivitet samt stödjeceller (gliaceller) kan påverka den kedja av händelser som leder fram till epileptiska anfall och epilepsi. Vi undersökte det dynamiska uttrycket av GABA\textsubscript{A} receptorer (flumazenil), aktiverade mikroglia celler (PK11195) och astrocyter (L-deuterium deprenyl) med \textsuperscript{11}C-autoradiografi i en modell av posttraumatisk epilepsi. Tjugofyra Sprague Dawley råttor fick en intrakortikal injektion av järnklorid eller placebo. Djuren delades in i 4 grupper och avlivades efter 1, 5, 100 respektive 180 dagar efter injektionen. Hjärnvävnaden samlades in, frystes ner och snittades. Autoradiografiska studierna utfördes med \textsuperscript{11}C-märkt flumazenil, PK11195 och L-deprenyl. Mätningar av upptagen av de olika liganderna utfördes i den järnkloridinjicerade hemisfären och jämfördes mot den kontralaterala friska hemisfären samt mot placebobehandlade djur. Resultaten visade en ipsilateral ökning av mikrogliaktivitet ([\textsuperscript{11}C]PK11195) vid dag 1 och 5 som därefter successivt minskade. Vidare noterades en initial minskning åtföljt av en successiv ökning av receptorupptag för astroglios markören [\textsuperscript{11}C](L)-deprenyl samt en omedelbar och kvarstående minskning av upptaget av [\textsuperscript{11}C]flumazenil. Konklusionen är att förändringar i olika receptorsystemen kan följas kontinuerligt under epileptogenesen med \textit{in vitro} \textsuperscript{11}C-autoradiografi. I en tidig fas efter en skada uppträder en akut neuroinflammation med aktivering av mikroglia och senare under förloppet en årrbildning med ökad mängd aktiverade astrocyter. Modellen kan användas för att följa effekter av behandling med neuroprotektiva eller antiepileptogena substanser.
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