Sigma-1 Receptor Imaging in the Brain

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Summary

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The sigma-1 receptor is a unique orphan receptor, strongly expressed in neurons and glia. Proposed endogenous ligands include neurosteroids, sphingosine and dimethyltryptamine. Sigma-1 receptors are now considered as ligand-regulated molecular chaperones modulating the activity of voltage-regulated and ligand-gated ion channels, intracellular calcium signaling, and the release of various neurotransmitters including acetylcholine and glutamate. Sigma receptors are involved in several central nervous system (CNS) disorders like depression, anxiety, psychosis, schizophrenia, Parkinson's disease, Alzheimer's disease, addiction and neuropathic pain. Several CNS drugs like haloperidol, donepezil, rimcazole, fluvoxamine, sertraline and clorgyline have moderate to high sigma receptor affinity. Pharmaceutical companies currently involved in R&D and clinical trials of sigma-1 drugs include Anavex Life Sciences Corp., M's Science Corp. and Laboratorios del Dr. Esteve S.A.

In this thesis we investigated the use of sigma-1 selective agonist tracer ^{11}C-SA4503 and microPET to study changes to sigma-1 receptors in the rat brain in normal aging and in models of cognitive deficit. We investigated the effect of pharmacological treatments on sigma-1receptor occupancy and also estimated the receptor occupancy required for efficacy in an animal model of cognitive deficit. Population pharmacokinetic modeling was performed on data obtained from the tracer ^{11}C-SA4503 to learn more about the pharmacokinetics of the drug cutamesine (SA4503).

Chapter 1 provides a general introduction to sigma-1 receptors, the imaging of sigma-1 receptors using the agonist tracer ^{11}C-SA4503 and drug discovery for sigma-1 ligands.

Chapter 2 provides an overview of present knowledge regarding the relationship between the cholinergic system and sigma-1 receptors, and discusses potential applications of sigma-1 receptor agonists in the treatment of memory deficits and cognitive disorders.

In **Chapter 3** we investigated sigma-1 receptors in the living rat brain to test the hypothesis that sigma-1 binding sites are upregulated in the ageing brain to compensate for age-related losses of activity in other neurotransmitter systems. The necessity of kinetic modelling and metabolite analysis for this tracer became apparent during this evaluation. When the whole brain of the rat is considered, an age dependent reduction in the total volume of distribution and binding potential of the tracer was observed over the ages of 1.5 to 32 months, suggesting a reduction in the receptor numbers and/or affinity with aging. However, when individual smaller regions of interest within the brain were analyzed, some regions like cortex appeared to have preserved binding potential and therefore preserved receptor numbers and/or affinity with aging. Therefore, our study did not provide support for an upregulation of sigma-1 receptors in healthy aging.

During the aging study, about 40% of the aged rats were found to have spontaneous pituitary tumors. In **Chapter 4**, we assessed the kinetics of ^{11}C-SA4503 in tumor

and brain, and evaluated the utility of this tracer in the detection of pituitary tumors. The tumors were well visualized with this tracer and were found to over express sigma-1 receptors. The over expression of sigma-1 receptors in the spontaneous pituitary tumors was detected as an increase in the uptake and binding potential of ^{11}C-SA4503. Therefore, this tracer may have promise for the detection of pituitary adenomas, using PET.

We then investigated the changes in sigma-1 receptor expression in two models of cognitive deficit, cholinergic lesion of the nucleus basalis and REM sleep deprivation (**Chapter 5**). The feasibility of using these models in conjunction with behavioural studies and PET imaging was also examined.

In the cholinergic lesion model, there was no significant difference in the uptake of the tracer ^{11}C-SA4503 in the cortex of lesioned side compared to the sham-lesioned side. Imunocytochemical analysis of brain slices established that the cholinergic lesion spared cortical sigma-1 receptors and even upregulated them in certain parts of layer V of the parietal cortex. The area affected by the lesion was relatively restricted and therefore could not be detected with PET. Since the cholinergic deficit in AD develops rather slowly, and sigma-1 receptor expression appears to be fairly resistant to cholinergic deafferentation in the neocortex, there may be opportunity for pharmacological treatment with sigma-1 receptor agonists in early AD.

Forty-eight hours of REM sleep deprivation produced cognitive deficits which could be assessed using behavioural tests (**Chapter 5**). This duration of sleep deprivation also led to a reduced tissue to metabolite-corrected plasma ratio of the tracer ^{11}C-SA4503 in the brain and periphery. Western blot analysis confirmed that sigma-1 receptors are in fact down-regulated in the two brain regions analysed. PET studies in this model appear to be feasible however, metabolite analysis would be essential. Sleep deprivation is known to increase aggregation of misfolded proteins and also to trigger the ER stress response while sigma-1 receptors are known to be molecular chaperones at the ER. Treatment with sigma-1 agonists could potentially be beneficial in this model.

In **Chapter 6**, the sigma-1 receptor occupancy by donepezil was investigated. Donepezil, the most widely prescribed drug for symptomatic treatment of Alzheimer's disease, is also a sigma-1 agonist. It is the gold standard against which new drugs are compared. Using ^{11}C-SA4503 and microPET, we evaluated the sigma-1 receptor occupancy by donepezil in rats. Plasma levels of the drug were determined using UPLC-MS analysis and ED_{50} and EC_{50} for receptor occupancy were calculated. Donepezil, in the usually used dose range, was found to occupy a significant fraction of the sigma-1 receptor population in the rat brain, in a dose- and concentration-dependent manner. In future tests of sigma-1 ligands as cognitive enhancers, antidepressants and antipsychotics, the tracer ^{11}C-SA4503 and microPET can be used to examine the relationship between target occupancy and therapeutic effects.

Sigma-1 receptor occupancy required for efficacy in a model of cognitive impairment was estimated in **Chapter 7**. Treatment with cutamesine was found to be effective in overcoming REM sleep deprivation-induced memory deficit. The sigma-1 receptor occupancies at the tested doses were then evaluated using ^{11}C-SA4503 and microPET. Plasma and brain levels of the drug were determined using UPLC-MS analysis. Virtually complete sigma-1 receptor occupancy (over 90%) was found to be necessary for effectiveness in this model. Combining behavioral studies with pre-clinical PET studies and pharmacokinetic analysis can give valuable insights in to the relationship between exposure of tissue to drug, drug-target interaction and efficacy.

While information about the kinetics of the tracer ^{11}C-SA4503 is available from several studies, less was known about the pharmacokinetics of the drug cutamesine. Therefore, microPET data for ^{11}C-SA4503, obtained from rats of varying ages, were analyzed using a population-based approach (nonlinear mixed-effects modeling, NONMEM) to obtain pharmacokinetic profile of cutamesine in plasma and brain (**Chapter 9**). Actual data obtained after i.p injection of cutamesine (**Chapter 8**) was used for comparison. The plasma and brain time profile for cutamesine obtained from the NONMEM analysis was found to be a reasonable match after correction for route of administration. Cutamesine was also found to have favorable plasma and brain pharmacokinetics allowing once daily treatment in the case of rats. This model structure could potentially be extrapolated to human studies.

In conclusion, the tracer ^{11}C-SA4503 could be used to study changes to sigma-1 receptors, not only in the brain, but also in tumors. The tracer can be used to assess the dose-dependent sigma-1 receptor occupancy of CNS drugs. Cutamesine was found to be effective in the treatment of memory deficit. The occupancy to aim for, when a novel sigma-1 agonist drug is evaluated in cognitive tests was also established. The data obtained from the tracer was found to be useful in evaluating the pharmacokinetics of the drug cutamesine. Therefore, sigma-1 receptor imaging has potential uses in the study of human diseases and in drug discovery.