Sigma-1 Receptor Imaging in the Brain

Kuzhuppilly Ramakrishnan, Nisha

Future perspectives

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The studies presented in this thesis demonstrated the usefulness of ^{11}C-SA4503 and microPET to evaluate sigma-1 receptors in the rat brain. Increased and decreased binding of the tracer to sigma-1 receptors could be estimated in normal aging brain (Chapter 3) and spontaneous pituitary tumor (Chapter 4) respectively. In Chapter 5, REM sleep deprivation was found to cause reduced sigma-1 expression in the brain, while in Chapter 7, treatment with cutamesine (SA4503) was shown to overcome REM sleep deprivation induced cognitive deficits. The receptor occupancy by agonist drugs, donepezil and cutamesine, at doses effective in behavioral studies of cognition were estimated using ^{11}C-SA4503 in Chapters 6 and 7. In Chapters 8 and 9, the kinetics of the drug cutamesine in plasma and brain was modeled using classical and non-linear mixed effects modeling (NONMEM) for the cold drug and its radioactive form (^{11}C-SA4503) respectively. Based on the findings in this thesis, several questions were raised. In this chapter, future avenues for the use of this tracer will be explored.

True status of sigma-1 receptors in Alzheimer's disease

In our aging study (Chapter 3), we found that the binding potential of sigma-1 agonist tracer ^{11}C-SA4503 was reduced in several brain regions in rats. However, cholinergic lesion of the nucleus basalis did not reduce the sigma-1 receptor density, but rather increased it in one of the regions studied (Chapter 5). A study in early Alzheimer's disease patients with the same tracer showed a reduction in tracer binding potential *(1)*. However, three out of the five patients included in that study were being treated with donepezil which we know is a sigma-1 receptor agonist (Chapter 6). It is possible that the reduction observed was partially due to the blocking of sigma-1 receptors by donepezil. If the sigma-1 receptors are preserved in Alzheimer's disease, treatment with sigma-1 agonists would be a possibility. Therefore, it would be beneficial to study drug naive early Alzheimer's disease patients with this tracer in order to determine the true status of sigma-1 receptors.

Distinguishing types of pituitary tumors

In chapter 4 we demonstrated that spontaneous pituitary tumors in aged rats can be detected and distinguished from normal pituitary and brain by microPET imaging with the sigma-1 ligand, ^{11}C-SA4503. The very high uptake of ^{11}C-SA4503 in pituitary adenomas indicates that it may be worthwhile to test ^{11}C-SA4503-PET in a clinical setting. Even very small tumors were clearly detected. This suggests that ^{11}C-SA4503 may be applied for the detection of microadenomas. Future studies are needed to answer the question whether ^{11}C-SA4503-PET can discriminate between symptomatic, hormone-secreting and non-functioning (non-symptomatic, non-secretive) tumors.

Role of sigma-1 receptors in sleep deprivation, depression and diabetes

Sleep deprivation is known to cause accumulation of protein aggregates and endoplasmic reticulum (ER) stress leading to unfolded protein response *(2, 3, 4)*.

While acute ER stress could upregulate sigma-1 receptors leading to a protective effect, chronic stressors could alter the stability and subcellular distribution of sigma-1 receptors *(5)*. Treatment with sigma-1 agonists have been shown to be protective in ER stress *(6, 7, 8)*.

Forty-eight hours of REM sleep deprivation in rats produced cognitive deficits (Chapter 5) which were associated with a reduced tissue to metabolite-corrected plasma ratio of the tracer ^{11}C-SA4503 in the brain and periphery. It would be informative to compare the effects of different durations of sleep deprivation on sigma-1 receptors. In terms of ER stress, is forty-eight hours of sleep deprivation a chronic stress? Would a shorter duration of sleep deprivation, of say, 6 or 24 hours, have a different, perhaps beneficial, effect on sigma-1 receptors?

It would also be interesting to investigate if the rapid beneficial effect of short term sleep deprivation in depression *(9)* is related to its effects on sigma-1 receptors. Under certain forms of stress, sigma-1 receptor knockout mice display a depressive-like phenotype. *(10)* The sigma-1 receptor gene was found to be involved in the pathophysiology of depression in the Japanese population *(11)*. Sigma-1 agonists have antidepressant effects in pre-clinical models and several marketed antidepressants are sigma-1 agonists *(12)*. Recently, sigma-1 receptor concentration in plasma of depressed patients was found to increase after antidepressant treatment *(13)*. Therefore, a comparative study which looks at normal individuals and depressed patients before and after sleep deprivation using ^{11}C-SA4503 and PET would be informative on the role of sigma receptors in depression.

In the case of peripheral tissues, the reduction in tissue to metabolite-corrected plasma ratio of the tracer in pancreas of sleep deprived rats was especially striking. Sleep restriction, especially when it is chronic, has metabolic consequences which could lead to the development of type-2 diabetes *(14, 15, 16)*. In an animal model of diabetes mellitus, neuronal sigma-1 receptors were found to be reduced, suggesting that changes in sigma receptors may play a role in diabetes related abnormalities *(17)*. The role of sigma-1 receptors in the development and treatment of diabetes would be an interesting and novel avenue of research that could be undertaken using the tracer ^{11}C-SA4503.

Sigma-1 receptors in drug effects

We have shown that a single i.p. dose of donepezil and cutamesine, in the dose range commonly used in pre-clinical behavioral studies, occupies a significant fraction of sigma-1 receptors in the brain (Chapters 6 and 7). Virtually complete receptor occupancy was found to be required for efficacy of cutamesine in the sleep deprivation induced cognitive deficit model. However, the donepezil dose that produces a similar occupancy is associated with significant cholinergic side effects. Therefore, co-treatment with a sigma-1 agonist like cutamesine might improve the treatment effects of donepezil without increasing side effects. Designing of new molecules which incorporate sigma-1 receptor affinity in addition to established

targets for treatment of (cognitive deficits in) CNS disorders might be worth considering. In the case of antidepressants, selective serotonin reuptake inhibitors which also have sigma-1 affinity are better at improving cognition *(18, 19, 20)*.

Another point to consider is that drug administration need not be in a single dose. Repeated administration of smaller doses, that does not produce side effects, but leads to sufficient receptor occupancy due to accumulation of drug in target tissues, is possible. Further studies, behavioral, pharmacokinetic and PET, would be required to evaluate the effects of co-treatment and to identify ideal dosing regimens. However, pharmacokinetic-pharmacodynamic modeling and simulation, using PET and pharmacokinetic data available from this thesis and literature could be used to predict the ideal dose ranges before expensive and time consuming experiments are performed.

Use of NONMEM in the analysis of PET data

There is no getting away from the fact that PET studies are expensive, time consuming and labor intensive. However, it also produces vast amounts of data that should be completely exploited. Non-linear mixed effects modeling uses pooled data from all sampled individuals. Therefore, it allows the quantification of population-typical values as well as two separate kinds of variability. The inter-individual variability, that is explainable, arising from biological processes, and intra-individual variability, that is not explainable, arising from different sources (e.g., measurement error, dosing errors and model misspecification) *(21)*. This methodology may additionally identify covariates influencing the variability. Use of NONMEM should be encouraged in the analysis of PET data, both prospectively and retrospectively.

Neurodegenerative disorders

Sigma-1 receptors are molecular chaperones at the endoplasmic reticulum (ER)-mitochondrion interface (Hayashi and Su, 2007). Molecular chaperones promote the proper folding of newly synthesized proteins and prevent the accumulation of toxic protein aggregates by transferring misfolded proteins to ER-associated degradation machinery (Schroder, 2005). Misfolded proteins that escape degradation could form aggregates that ultimately cause cellular degeneration.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease wherein degeneration of motor neurons in brain and spinal cord leads to progressive weakness and paralysis. Recently, a mutation in the sigma-1 receptor gene was identified as a cause of familial juvenile ALS and cells expressing the mutated receptor protein were found to be less resistant to apoptosis induced by endoplasmic reticulum stress *(22)*. Levels of the sigma-1 receptor protein were found to be reduced in lumbar ALS patient spinal cord and they were abnormally accumulated in enlarged C-terminals and endoplasmic reticulum structures of alpha motor neurons *(23)*. Further, sigma-1 receptor gene mutations were found to be causative for the co-ocurrence of frototemporal lobar degeneration with motor neuron disease or ALS *(24)*. In pre-

clinical studies, knockout of sigma-1 receptors in a mouse model of ALS was found to decrease the lifespan of the animals *(25)* and treatment with sigma-1 agonists PRE-084 *(23, 26, 27)* and cutamesine *(28)* was found to have protective effects in both *in vitro* and *in vivo* models of ALS. PET studies with ^{11}C-SA4503 in pre-clinical models and patients could shed light on the status of sigma-1 receptors during the course of the disorder rather than at the end point.

Therapeutic potential of sigma-1 antagonists

While the focus so far has been on sigma-1 agonists, interesting research is ongoing into the therapeutic potential of sigma-1 antagonists especially in the field of pain and addiction. Formalin induced pain was reduced in sigma-1 knock-out mice *(29)*, while treatment of animals with the sigma-1 antagonist haloperidol had an antinociceptive effect that is mediated through sigma-1 receptors *(30)*. Capsaicin was unable to induce mechanical hypersensitivity in sigma-1 knockout mice, while sigma-1 antagonists inhibited the mechanical allodynia induced by capsaicin *(31, 32)* in wild type mice. Several selective sigma-1 antagonists are being developed for the treatment of neuropathic pain *(33)*. Sigma-1 antagonists are also being investigated for the treatment of substance abuse and addiction. Behavioral effects of many drugs of abuse including cocaine, methamphetamine, and alcohol are ameliorated by sigma-1 antagonists *(34)*. Antagonism of sigma-1 receptors also blocked addiction-like behavior of binge eating *(35)*. The extent of receptor occupancy required by antagonists to achieve these effects is not known. PET studies using ^{11}C-SA4503 would be useful in this scenario.

In conclusion, the sigma-1 agonist tracer ^{11}C-SA4503 has potential uses in the study of various disorders, both of the CNS and periphery, as well as in the evaluation of potential treatments and drug effects.

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