Ex vivo Evaluation of [^{18}F]MH.MZ

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Introduction: Serotonergic 5-HT$_{2A}$ receptors are of central interest in the pathophysiology of schizophrenia and other diseases, including Alzheimer's disease and personality disorders.[1] The serotonergic system is also implicated in sleep, aging and pain. *In vivo* studies of 5-HT$_{2A}$ receptor occupancy would provide a significant advance in the understanding of the mentioned disorders and conditions. Positron emission tomography (PET) is an appropriate tool to measure *in vivo* directly, non-invasively and repetitively the binding potential of radio tracers for neuroreceptors.

Aim: The aim of this study was to evaluate the *ex vivo* behaviour of [^{18}F]MH.MZ in autoradiographic experiments and in blood metabolism studies.

Experimental: Autoradiography experiments were carried out at room temperature in reaction buffer (50 mM Tris/HCl buffer, pH 7.4 containing 120 mM $NaCl_2$ and 5 mM KCl) with [^{3}H]MDL 100907 and [^{18}F]MH.MZ. Sections with [^{18}F]MH.MZ were washed 2x2 min in reaction buffer containing 0,01 % Triton X-100 and 1x2 min in reaction buffer, shortly dipped into deionized water and quickly dried in a stream of cold air. Sections with [^{18}F]altanserin were washed in pure ice cold reaction buffer 2 x 10 min, sections with [^{3}H]MDL 100907 were washed in pure buffer 2 x 2 min. Sections were exposed to Fuji phosphor screen for 3 h when ^{18}F was used and for 5 days when ^{3}H was used. Blood samples were taken at 5, 10, 30 and 60 min and analysed via radio-TLC.

Results: Autoradiographic images of the 5-HT$_{2A}$ receptor obtained with [^{18}F]MH.MZ showed excellent visualization results in rat brain sections (fig. 1). Competition autoradiography assays (data not shown) with 5 nM [^{18}F]MH.MZ and 10 µM of fallypride, WAY 100635 and prazosin showed that [^{18}F]MH.MZ is highly specific for 5-HT$_{2A}$ receptors.

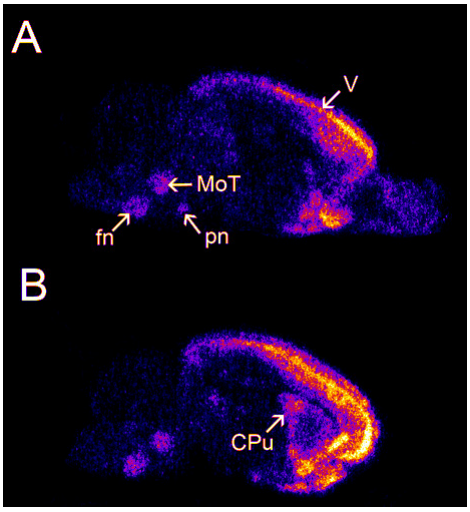

Fig. 1: Images of an autoradiography of [^{18}F]MH.MZ binding rat brain sections; **A** & **B** total binding.

A comparison of the binding of [^{18}F]altanserin and [^{18}F]MHMZ (fig. 2) displays that [^{18}F]MHMZ is in no way inferior to [^{18}F]altanserin in terms of specificity for 5-HT$_{2A}$ receptors. Figure 2 also shows the complete agreement of the binding of [^{3}H]MDL 100907 and [^{18}F]altanserin.

The metabolite analyses of rat plasma showed that [^{18}F]FE1-MDL 100907 underwent fast metabolism. One polar metabolite was found in rat plasma which is not likely to cross the blood-brain-barrier because of its hydrophilicity. The percentage of unmetabolized fractions were 43%, 32%, 16%, 7% and at 5, 10, 30 and 60 min, respectively.

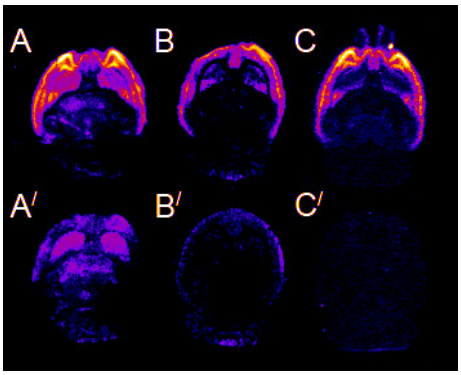

Fig. 2: Autoradiographic images of the total binding and non-specific binding respectively of **A**/**A**/ [^{18}F]altanserin, **B**/**B**/ [^{3}H]MDL 100907 and **C**/**C**/ 5 nM [^{18}F]MH.MZ 100907 at 14 µm rat brain sections. Non-specific binding was determined in the presence of 10 µM ketanserin.

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Conclusion: New auspicious results concerning the *in vitro* studies of [^{18}F]MH.MZ justify further experiments like *in vivo* small animal PET studies to verify the potential of this new 5-HT$_{2A}$ imaging ligand.

References

[1] Kristiansen et al. (2005); Synapse 58: 249