Establishment of Radiolabelling Method for the Development of Neurodegenerative Disease Imaging Agent Using 5-HT_{1A} Subtype of Receptor Anatagonist

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1. Introduction

The 5-HT1A subtype of receptors for the neurotransmitter serotonin is predominantly located in the limbic forebrain. And it is involved in the modulation of emotion and the function of the hypothalamus[1,2]. Since 5-HT1A receptors are implicated in the pathogenesis of anxiety, depression, hallucinogenic behaviour, motion sickness and eating disorders, they are an important target for drug therapy and diagnosis of diseases[3,4]. Serotonin is synthesized from the amino acid L-tryptophan by sequential hydroxylation and decarboxylation. It is stored in presynaptic vesicles and released from nerve terminals during neuronal firing. One of the best-characterised binding sites for serotonin is the 5-HT1A receptor. This is mainly due to the relatively early discovery of a selective ligand, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) for this subpopulation. Thus, many researchers have tried to develop a radioligand capable of assessing in vivo changes in 5-HT1A receptors in depressed subjects, people with anxiety disorders, patients with Alzheimer's disease and schizophrenics. In present study, we studied the radioligands which would play a role in visualization and quantification of this important neuroreceptor for single-photon emission tomography (SPET).

2. Methods and Results

2.1. Synthesis of Ligand

New quipazine derivative for imaging 5-HT_{1A} receptor was synthesized to develop neurodegenerative imaging agent. And N_2S_2 moiety was added for the possible radiolabeling

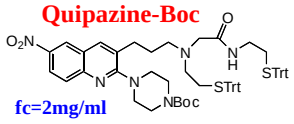


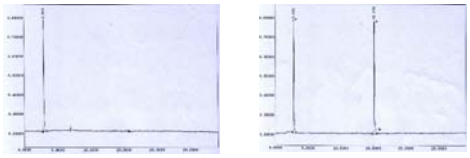
Figure 1. 5-HT1A Receptor Antagonist Analogue

2.1. Analytical Method for Radiolabeled Ligand

For the analysis of radiolabeling efficiency, HPLC (Waters, USA) was used. The X-terra column(Waters, WSA) was used with reverse phase of H_2O and Acetonitle as mobile phase gradient system. The flowrate was 1 ml/min and running time was 40 mins. Data was reported as labeled rate (%) compared to pertechnate level.

2.1. Radiolabelling of Ligand with Tc-99m under the mild condition

Radiolabeling with Quipazine derivative was carried out under the several labeling condition to earn the high labeling efficiency. Under the mild labeling condition, it was not able to deprotect the trityl group from N_2S_2 moiety which lead to low radiolabeling labeling yield which analyzed by HPLC analysis. The retention time at 3 mins was noticed in higher percentage which proves Tc-99m exists in free form($^{99m}TcO_4^{-}$) instead of labeled one.

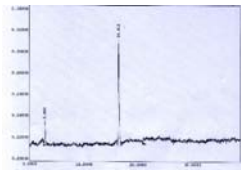


Free Tc-99m Mild condition

Figure 2. Labeling efficiency with mild labeling condition.

2.2. Radiolabelling of Ligand with Tc-99m under the vigorous condition

The more vigorous reaction condition was applied for obtaining the better labeling efficiency. The condition was following; 0.07 uM of ligand with $SnCl_2$ (0.056 uM) and Tartaric acid (1mg) were dissolved in HCl(0.05 N). Final pH was 1-2. Heating block was applied for 1 hr to give 110^{o}C of reaction temperature. This resulted in the possible deprotection of trityl group which made Tc-99m labeling possible.



High labeling condition

Figure 3. Labeling efficiency in acidic labeling condition.

3. Conclusion

The arylpiperazine, Quipazine-boc, has been recognized as the highly selective ligand for binding to the 5-HT_{1A} receptor as competitive antagonist. The profile with highly binding affinity was target of present study to develop radioligands which visualize the malfunction of brain region. Present study established the radiolabeling condition which resulted in high radiolabeling efficiency. But these studies with Quipazine ligand for serotonin receptor 5-HT_{1A} imaging agents would not be enough to prove it is qualified as good imaging agent [5,6,7]. Thereby it is suggested that further study has to be followed related to the improved reaction speed and radiochemical purity without any other extra purification process in spite of activity loss. Since the metabolism of radioligands is crucial fact in developing imaging agents, the further study like in-vivo stability and in-vivo biodistribution ought to be followed by. Also the ability to pass blood brain barrier has to be examined considering structure-activity relationship among other related derviavtives and other antagonist such WAY-100635[8].

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