Scope and Objective
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AD is epidemic with an estimated 33.9 million people worldwide having the disease (Corrada et al., 2010). The incidence rate increases exponentially with aging so that at age 90 about 12% of people have AD, but about 40% of those over age 100 have it (Barnes et al., 2011). Several factors that put persons at increased risk of AD are a history of head injury, obesity, diabetes mellitus, hypertension, renal disease, and histories of smoking, traumatic brain injury, or depression. As the occurrence of many of these factors is becoming more common, the incidence of AD may increase even more. Clearly, interventions that prevent, stabilize, remediate, or cure AD are desperately needed (Banks, 2012).

In the context of AD, the muscarinic acetylcholine receptor of the subtype 1 (M1 mAChR1 or simply M1 receptor) was proposed to be a suitable target and subsequent research fostered this assumption since in AD the degeneration of basal nucleus of Meynert cholinergic neurons leads to deficits of ACh neurotransmission in regions expressing M1 (Langmead et al., 2008; Wess et al., 2007). The muscarinic acetylcholine receptors are involved in an amazing number of very diverse physiological processes made possible on the one hand by specific tissue distribution and on the other hand by the existence of five receptor subtypes (M1, M2, M3, M4, M5): regulating heart rate and exocrine glands, mediating broncho and vasoconstriction, regulating dopaminergic neurotransmission and many more (Langmead et al., 2008). Not very surprisingly, early on the various M receptor subtypes became therapeutic targets. And also not very surprisingly, the medicinal chemists involved immediately encountered the problem of pronounced unwanted pharmacological effects caused by non-sufficient subtype selectivity (Wess et al., 2007). This lack of selectivity for many compounds synthesized is more prominent than for other GPCRs due to the fact that the orthosteric recognition (or endogenous ligand binding) site of all subtypes of the muscarinic receptors is structurally very similar. Concerning the M1 receptors, basal forebrain cholinergic neurons in both the cortex and hippocampus are responsible for memory function, and in AD the number of these neurons – the M1 containing neurons are the most abundant one – is strongly decreased (Langmead et al., 2008). This observation prompted the clinical application of AChEIs which are up to now the treatment of choice for AD: they increase the amount of the neurotransmitter ACh in the synaptic cleft (Johnstone et al., 2011). However, even though new AChE inhibitors with better pharmacological and pharmacokinetic properties were developed in the last few years they are only able to improve cognitive deficits of AD for a couple of months; maybe
the only short-lasting beneficial clinical effect is due to a decrease of AChE in the relevant brain regions (Giacobini, 2003).

For medicinal chemists the M1 receptor represents a highly interesting target since a direct and selective activation of this receptor subtype modulates cognition and memory in the brain proven by M1<sup>−/−</sup> knockout mice (Anagnostaras et al., 2003). Several findings about the M1 subtype also make it an attractive target for antipsychotic agents: on the one hand cognitive impairment is also associated with psychotic patients and on the other hand M1 receptor expression is decreased in schizophrenia (Sellin et al., 2008). Therefore, there has been a considerable effort to develop high affinity and selective M1 agonists.

Recently, several substances targeting the M1 receptor have been under development. While the development of orthosteric agonists was stopped at the clinical trial level because of lack of efficacy and/or side effects (McArthur et al., 2010), research focused on allosteric agonists believed to have better profile. In comparison to orthosteric agonists, they do not show receptor internalisation or down-regulation (Thomas et al., 2009). Recently, a third category of M1 receptor ligands, positive allosteric modulators, were introduced. They enhance the action of orthosteric agonists such as acetylcholine thereby strengthening synaptic transmission without changing its dynamics (Caruana et al., 2011; Christopoulos et al., 1998; Jakubík et al., 1997). Among them, BQCA was tested at M2–M5 receptors without showing any effect up to 100 mM (Ma et al., 2009). Moreover, BQCA has been described to improve learning in animal models (Ma et al., 2009; Shirey et al., 2009). We confirmed this finding showing that BQCA attenuated the natural forgetting in a novel object recognition task (Chambon et al., 2011). Such a positive effect of BQCA in an episodic memory task is a further argument supporting the M1 receptor positive allosteric modulator approach for the treatment of Alzheimer’s disease.

Clearly, allosteric modulators offer a number of advantages over their orthosteric counterparts, although both PAMs and NAMs rely upon the presence of the endogenous ligand. It is also worth noting that drug discovery programs centered on small molecules, be it orthosteric or allosteric, share common problems concerning solubility and formulation, generation of active metabolites, clearance and lack of brain penetrance (Gregory et al., 2010).

Central nervous system drug efficacy depends upon the ability of a drug to cross the blood–brain barrier and reach therapeutic concentrations in brain following systemic administration. The clinical failures of most of the potentially effective therapeutics to treat
the central nervous system disorders are often not due to a lack of drug potency but rather shortcomings in the method by which the drug is delivered (Wilson et al., 2008a).

Hence, considering the importance of treating Alzheimer's disease, we made an attempt to target a Selective Allosteric Potentiator of the M1 Muscarinic Acetylcholine Receptor, BQCA in the brain by using PLGA NPs followed by slow release and/or reduced metabolism of BQCA in the Brain over a period of days or weeks after injection to Restore Reversal Learning Management of AD.

**The further objectives of the work were as below**

- To develop a simple, sensitive and selective high performance liquid chromatography method for quantitative estimation of BQCA in plasma and tissue samples
- To perform pharmacokinetics and tissue distribution of BQCA in Sprague-Dawley (SD) rats following intraperitoneal (i.p) administration
- To develop and optimize of BQCA loaded PLGA NPs
- To perform physicochemical characterisation of the developed nanoparticulate formulation and its *in vitro* release studies
- To study comparative pharmacokinetics and tissue distribution of developed NPs formulation with BQCA solution
- To induce AD in rat model using intracerebroventricular injection of streptozotocin (icv-STZ).
- To evaluate the effect of developed NPs formulation on learning and memory