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Research paper

# Haloperidol-loaded intranasally administered lectin functionalized poly(ethylene glycol)–block-poly(D,L)-lactic-co-glycolic acid (PEG–PLGA) nanoparticles for the treatment of schizophrenia

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ABSTRACT

Lectin-functionalized, polyethylene glycol–block-poly-(D,L)-lactic-co-glycolic acid nanoparticles loaded with haloperidol were prepared with narrow size distributions and sizes <135 nm. The nanoparticles exhibited high *Solanum tuberosum* lectin (STL) conjugation efficiencies, encapsulation efficiencies, and drug loading capacities. The *in vitro* release of haloperidol was 6–8% of the loaded amount in endo-lysosomal conditions over 96 h, demonstrating minimal drug leakage and the potential for the efficient drug transport to the targeted brain tissue. The haloperidol released upon erosion was successful in displacing [$^3$H] N-propylnorapomorphine and binding to bovine striatal dopamine D2 receptors. Both haloperidol-loaded nanoparticle formulations were found to be highly effective at inducing catalepsy. Intranasal administration of STL-functionalized nanoparticles increased the brain tissue haloperidol concentrations by 1.5–3-fold compared to non-STL-functionalized particles and other routes of administration. This formulation demonstrates promise in the reduction of the drug dose necessary to produce a therapeutic effect with antipsychotic drugs for the treatment of schizophrenia.



## 1. Introduction

Over the past two decades, there has been marked improvement in our understanding of the underlying etiology and treatment of central nervous system (CNS) disorders. However, many of the drugs used to treat these disorders lack an effective means for crossing the blood–brain barrier (BBB) [1]. The BBB presents a great obstacle to the transport of exogenous substances into the brain and has stimulated work toward the customization of nanoparticle drug carriers specific to the preferred route of administration to overcome this issue [2–5]. Nanoparticles increase the likelihood of drugs reaching their target of interest (e.g., preventing binding of the drug to mucus), which could significantly improve the therapeutic efficacy and allow for dosage reduction due to a reduction in premature drug metabolism [5,6]. Customization of nanoparticles to engineer slow degrading, uncharged, functionalized PEG-rich surface that discourages particle aggregation (avoiding biological clearance mechanisms) and promotes disease or cell-specific localization can help address the challenges of BBB transport to effectively transport drugs to their target of interest [2,4,5]. Drug carrier development specialized for various routes of administration is integral for the treatment of a variety of CNS disorders (e.g., schizophrenia, Parkinson's disease, etc.) [4].

Nanoparticles loaded with antipsychotic drugs (APDs) have been tested in rodent models using the intranasal, injectable (sub-

*Abbreviations:* PEG–PLGA, polyethylene glycol–block-poly-(D,L)-lactic-co-glycolic acid; STL, *Solanum tuberosum* lectin; NPA, [$^3$H] N-propylnorapomorphine; BBB, blood brain barrier; IP, intraperitoneal; IN, intranasal; APDs, antipsychotic drugs; SLNs, solid lipid nanoparticles; Methoxy, Me; Mal, maleimide; DCM, dichloromethane; PVA, polyvinyl alcohol; EDTA, ethylenediaminetetraacetic acid; TEM, transmission electron microscope; BCA, bicinchoninic acid; NTA, nanoparticle tracking analysis; HEPES, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); HPLC, high performance liquid chromatography; EE, encapsulation efficiency; DLC, drug loading capacity; PDI, polydispersity index; PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol; ANOVA, Analysis of Variance.

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cutaneous, intramuscular, intraperitoneal (IP), and intravenous), and oral routes of administration to treat schizophrenia [5,7,8]. The availability of multiple routes of administration with nanoparticle drug carriers could provide patients and medical practitioners with the flexibility to choose a preferred route of administration for the patient [7,9]. The intranasal route of administration provides both the fastest route to the brain and higher neural bioavailability. This effect is primarily attributable to the greater uptake of the nanoparticle formulations via the nasal relative to injection or oral administration due to direct nanoparticle uptake by the olfactory nerve cells (leading straight to the olfactory bulb) or via transport of nanoparticles across the olfactory epithelium to gain direct access to a number of proximal vessels to the olfactory epithelium (leading to transport through the endothelial tight junctions with access to the neural circulation) [8–10].

The indirect route of nanoparticle delivery to the brain starts with the endocytosis of nanoparticles via the olfactory epithelial cells following binding to N-acetylglucosamine residues on the cell surface, which has been proposed (in other cell types) to travel through the cell as endosome-like structures (i.e., via transcytosis) and exit through the basal side of the cell via vesicle fusion/exocytosis potentially out into the subepithelial space or fluid [44–46]. The nanoparticle would then bypass the subepithelial connective tissues within this space traveling through the lamina propria to passively diffuse through the endothelial tight junctions of the nasal vascular bed (i.e., supplied via the external carotid and opthalamic artery), which are designed for the rapid exchange of fluid or dissolved substances to later cross the blood brain barrier [45–47]. From here, these particles would either travel across to the opposite side of capillary or potentially flow much further to the Circle of Willis and travel more globally around the brain, explaining the observed spread of dye-nanoparticles as well as the greater olfactory bulb concentrations of dye following intranasal administration [48]. When traveling through the neural side, the particles would traverse the endothelial tight junctions to the basement membrane, potentially followed by passage through the glia limitans gap junctions into the cerebral spinal fluid (CSF) [44,49]. It is then possible for the particles to bind to N-acetylglucosamine residues on the neuronal cell surface (potentially involved in neuronal cell interaction and plasticity) to be endocytosed for use, degradation or to continue travel around the multitude of interconnected neuronal pathways within the brain [52]. During this entire process, it is also possible for the drug loaded within these nanoparticles to leak out, where it may continue to travel to the brain (provided it is non-immunogenic and/or lipophilic) or, if already in the brain near its therapeutic site of action, exert its therapeutic effect [1].

The direct route of nanoparticle transport to the brain involves the receptor mediated endocytosis of nanoparticles via the olfactory receptor cells that extend into the nasal mucosa [45,46,49]. These uptaken nanoparticles could then travel directly up one of the cells (via transcytosis) of the olfactory receptor cell fascicles (receptor cell bundles) extending through the basal membrane, lamina propria, and pores in the cribiform plate to the olfactory bulb [45]. The axons of these fascicles branch out to form glomerular tufts (or glomeruli), where the nanoparticles could exit the cell via exocytosis and cross the synaptic cleft to uptaken at the dendrites of the mitrial cells [45]. The nanoparticles would then travel down the bundles of the mitrial cells present in the olfactory bulb that form the olfactory tract, which could transport these nanoparticles along the olfactory tract projections to the prefrontal cortex, amygdala, thalamus and entohinal cortex (then projecting to the hippocampus) [45,50]. From many of these regions, there are a number of ways that the nanoparticles could reach the striatum (the site of action for many APDs); one potential example is the transport of the nanoparticles via glutaminergic neurons projecting from the prefrontal cortex to synapse on D2 receptor expressing neurons within the striatum [51]. This pathway would therefore allow the nanoparticles to skip the exclusive blood brain barrier and make it to tissue containing the drugs target of interest in producing a clinical effect.

A number of typical and atypical APDs have demonstrated effectiveness when incorporated into nanoparticles, including haloperidol, chlorpromazine, olanzapine, and risperidone [7,9,11,12]. To date there are only two studies that specifically involve the intranasal administration of APDs. Olazapine-loaded poly (D,L)-lactic-co-glycolic acid (PLGA) nanoparticles of size 100–200 nm were demonstrated to achieve low to moderate drug encapsulation and high neural bioavailability *in vivo* compared to other routs of administration [12]. Since these particles lack groups such as polyethylene glycol (PEG) to stabilize the particles through steric interactions, the PLGA particles would likely be subject to aggregation and removal via nasal clearance mechanisms. In another study, risperidone-loaded solid lipid nanoparticles (SLNs) of particle sizes near the 150 nm limit were capable of passive transport across the BBB and were reported to facilitate moderate drug encapsulation [13]. Improved APD loaded nanoparticle formulations need to exhibit smaller particle sizes and lower surface charges (to allow greater transport across the BBB) while facilitating high drug encapsulation efficiencies to account for potential premature drug loss due to nasal clearance mechanisms [14]. Based on these requirements, polyethylene glycol–block-poly-(D,L)-lactic-co-glycolic acid (PEG–PLGA) copolymer nanoparticles that feature a PEG-rich surface around the PLGA nanoparticle core are ideal for intranasal APD administration. The PEG-rich surface has been demonstrated to prevent the nanoparticle aggregation typically seen with uncoated PLGA nanoparticles upon contact with the nasal mucosa [15]. In addition, these particles can be prepared quite reliably at a size <150 nm, prolonging circulation time and inhibiting nanoparticle uptake by the mononuclear phagocytic system [16].

Further improvements in APD-loaded nanoparticle efficacy may be achieved by functionalizing the particle surface for the purpose of cell-specific targeting of either the olfactory nerve cells or the olfactory epithelial cells, increasing the efficacy of nanoparticle uptake at the olfactory epithelium following intranasal administration.

Such targeting has not yet been demonstrated using APD-loaded nanoparticle formulations. By functionalizing maleimide-PEG–PLGA (Mal-PEG–PLGA) polymers through nucleophilic addition with *Solanum tuberosum* lectin (STL), the APD loaded nanoparticles should be more effective at selectively binding to the N-acetylglucosamine residues highly expressed on the nasal epithelial membrane [1,17,18]. Such selective binding has been previously shown to lead to increased nasal epithelial cell uptake and improved nanoparticle bioavailability within the brain [1,19]. Prior research has also shown that STL activity is unchanged upon heating below 50 °C and remains relatively stable over a large pH range (i.e., pH 4–10) [1]. The STL-functionalized PEG-rich nanoparticle surface could prevent aggregation and drug clearance via the beating nasal cilia, which have an average clearance rate of 8.8 min in humans (15–20 min in rats). Similar nanoparticles have been previously shown to be uptaken as early as 15 min via Calu-3 cells and to be uptaken into the rodent olfactory bulb in about 5 min following intranasal administration [1,42,43]. The above properties of the lectin target, coupled with the relatively small nanoparticle surface charge, should inhibit aggregation, reduce interaction with the mucin molecules and thus avoid the nasal clearance mechanisms [14].

The objectives of the current study are to: (1) develop haloperidol-loaded, STL-functionalized PEG–PLGA nanoparticles capable of effective transport across the BBB, (2) demonstrate that haloperidol is still functional following release from the nanoparticles, (3)