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An Integrated Ciliotoxicity and Mucosal Safety Assessment of Amisulpride-Loaded Nanostructured Lipid Carriers for Nose-To-Brain Delivery

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ABSTRACT
Intranasal nanostructured lipid carriers (NLC) are an attractive platform for amisulpride (AMS), a benzamide antipsychotic whose oral absorption is incomplete and whose entry into the brain is further restricted by P-glycoprotein-mediated efflux. Because nose-to-brain therapy requires repeated dosing, the tolerance of the ciliated nasal epithelium, not permeation efficiency alone, determines whether such a platform is viable for development. Blank NLC were prepared by hot-melt emulsification followed by probe ultrasonication and converted into amisulpride-loaded NLC (AMS-NLC) and then into a mucoadhesive AMS-NLC in situ gel. Wistar albino rats were allocated and dosed once daily by the intranasal route for 28 consecutive days with nasal saline pH 6.4 (G1, vehicle control), blank NLC (G2), AMS-NLC (G3), or AMS-NLC in situ gel (G4). All three dispersions remained homogeneous with mean particle sizes of 118.4 ± 4.2 nm (blank NLC), 133.7 ± 5.6 nm (AMS-NLC) and 178.2 ± 6.1 nm (gel on dilution), zeta potentials of −24.6 to −30.1 mV, pH 6.1–6.3, and a sol–gel transition at 34.4 ± 0.6 °C. After 28 days, repeated intranasal administration of an anionic, poloxamer-stabilised AMS-NLC and of its in-situ gelling variant is therefore well tolerated by the rat nasal mucosa, and prolonging mucosal residence by thermoresponsive gelation imposed no detectable additional epithelial damage. The study demonstrates the tolerability of the formulations, not a protective effect of encapsulation, as it omitted both a free-drug arm and a positive irritant control.

1. INTRODUCTION:

Treatment of central nervous system (CNS) disorders is constrained less by the discovery of active molecules than by the difficulty of delivering them. The blood–brain barrier excludes essentially all large molecules and most small ones, and compounds that do cross are frequently returned to the circulation by ATP-binding cassette efflux transporters [1].

The intranasal route is a pragmatic alternative, being the only non-invasive arrangement in which neural tissue is separated from the environment by a single epithelial layer. Illum's demonstration that molecules deposited in the nasal cavity appear in cerebrospinal fluid at concentrations disproportionate to plasma levels established the principle of a direct, blood-independent route [2].

The adult nasal cavity offers roughly 150 cm² of absorptive surface, of which the olfactory region — the gateway to the direct pathway — represents only 3–10%, situated in the roof of the cavity and along the superior turbinate and adjacent septum [3,4].

Success depends on the deposition pattern of the device, the residence time of the formulation, partitioning across the epithelium, and degradation by nasal cytochrome P450 isoforms, carboxylesterases and peptidases [5]. Residence time is the variable most amenable to formulation

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control — and precisely the one that brings the delivery system into conflict with the clearance machinery of the nose. Two constraints apply throughout: the administration volume is limited to about 25–200 µL per nostril, so the drug must be presented at high thermodynamic activity, and preparations should be isotonic and buffered near pH 5.5–6.5, since deviations provoke stinging, reflex secretion and epithelial injury [5,6].

Mucociliary clearance is the principal innate defence of the upper airway. Each ciliated cell carries 100–250 cilia, 5–7 µm long, beating at 8–15 Hz in a coordinated metachronal wave, with a rapid effective stroke in the low-viscosity periciliary layer and a slower recovery stroke beneath the mucus gel [7].

This defense is a double-edged constraint. It bounds the time available for absorption, which is why mucoadhesive polymers, viscosity modifiers and in situ gelling systems have become standard components of nasal products [8]. It is also the function most sensitive to formulation-induced injury, since beating depends on an intact axonemal dynein–ATP system, regulated intracellular calcium and the physical properties of the periciliary fluid. The relationship is bidirectional: a formulation that prolongs residence by slowing clearance may increase absorption in a single-dose experiment while progressively compromising the epithelium that performs the absorption, and only a study measuring ciliary structure and function alongside permeation can distinguish deliberate, reversible retardation from incipient toxicity [7,9].

The excipients that make nasal absorption efficient are largely those that make it hazardous. Bile salts, surfactants, fatty acids and cationic polymers enhance permeation by fluidising membrane lipids or opening tight junctions, and their potency generally tracks their capacity to damage the epithelium [7]. Products intended for chronic use are therefore assessed for their effect on the mucociliary apparatus, and current guidance identifies ciliary beat frequency, mucociliary transport time and histopathology as the relevant endpoints, preferably measured in combination [10].

An amisulpride-loaded NLC gelling system is a rational candidate for this approach because its biodegradable matrix, small size and amenability to surface modification suit the nasal route, and enhanced brain targeting has been demonstrated for several CNS-active drugs delivered as NLC [11,12]. Encapsulation should reduce the free-drug concentration contacting the epithelium, yet the carrier introduces surfactants that are known ciliotoxicants at higher concentrations, and the gelling variant deliberately prolongs their contact with the mucosa. Safety therefore cannot be assumed from the safety of the individual excipients; it has to be measured on the assembled product under the dosing regimen that will actually be used [13–15].

This study therefore aimed to (i) prepare and characterise AMS-NLC and a thermoresponsive AMS-NLC in situ gel; (ii) determine, by once-daily intranasal administration to rats over 28 consecutive days, whether these formulations injure the ciliated nasal epithelium, using haematoxylin–eosin histopathology as the endpoint; (iii) establish whether the in situ gelling step, which is intended to prolong mucosal residence, carries an additional epithelial cost relative to the ungelled dispersion; and (iv) relate the histological findings to measurable formulation attributes- particle size, surface charge, surfactant load- so that the conclusions generalise beyond this drug.

2. MATERIALS AND METHODS:

2.1 Materials

Amisulpride (purity > 99.5%) was received as a gift sample from Talent India Private Ltd., Ahmedabad, India. Glyceryl monostearate (GMS), polysorbate 20, poloxamer 407 and hydroxypropyl methylcellulose (HPMC) were of pharmaceutical grade. Sodium chloride, potassium chloride, and calcium chloride dihydrate were of analytical grade and obtained from commercial sources. Dissection kits were procured locally.

2.2 Preparation and characterisation of the nasal formulations

2.2.1 Nasal saline solution, pH 6.4

Simulated nasal fluid was prepared by dissolving sodium chloride (0.745 g), potassium chloride (0.129 g) and calcium chloride dihydrate (0.036 g) in distilled water and making the volume up to exactly 100 mL, giving nominal concentrations of 7.45, 1.29 and 0.36 mg/mL respectively. The pH was adjusted to 6.4 and the solution was filtered before use [16].

2.2.2 Blank and drug-loaded nanostructured lipid carriers

Blank NLC were prepared without drug by hot-melt emulsification followed by probe ultrasonication. The solid lipid to give a total lipid content of 5% w/v, and an aqueous phase containing 1% w/v polysorbate 20 was heated under high-shear stirring to form a coarse pre-emulsion, which was immediately probe-ultrasonicated. Amisulpride-loaded NLC (AMS-NLC) was prepared by the identical procedure, with amisulpride dissolved in the molten lipid phase before emulsification. The gelling variant (AMS-NLC gel) was obtained by

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dispersing poloxamer 407 and HPMC in the cold dispersion [17–19].

2.2.3 Physicochemical characterization
The formulation had previously been developed and optimised following a Quality by Design (QbD) approach, with the quality target product profile for nasal delivery defined as a mean particle size below 200 nm, a polydispersity index of approximately 0.3 or less, and a zeta potential of at least ±30 mV in absolute terms, these being the attributes associated with colloidal stability and effective nasal delivery [11,15].

2.3 Animals and ethical approval
Wistar albino rats of either sex, weighing 150–200 g, were obtained from the institutional animal house and used for the in vivo nasal mucosal toxicity investigation. Animals were acclimatised for one week with free access to a standard pellet diet and water, under a 12 h light–dark cycle at 23 ± 2 °C and 60 ± 5% relative humidity. The protocol was authorised by the Institutional Animal Ethics Committee (approval no. 1088/PO/Re/S/07/CPCSEA, dated 24 February 2024), and every procedure adhered to the recognised guidelines for the care and welfare of laboratory animals [11,20,21]

2.4 Experimental design for the ciliotoxicity assessment
The nasal ciliotoxicity study was performed to generate safety data for the AMS-NLC formulations on the nasal lining following repeated administration. Twelve rats were randomly allocated to four groups of three animals each (Table 1). Group G1 received pH 6.4 nasal saline as vehicle control, G2 received blank NLC, G3 received drug-loaded NLC at 1 mg/kg, and G4 received the AMS-NLC in situ gel at 1 mg/kg. Dosing was by the intranasal route, once daily for 28 consecutive days, with the animals restrained in a supine position and the dose instilled into the nostrils using a micropipette [11,20]. Animals were observed daily for nasal discharge, sneezing, local irritation and general condition.

Table 1. Group allocation for the 28-day repeated-dose nasal ciliotoxicity study (n = 3 animals per group)

Group	Treatment	Dose	No. of animals
G1 (normal control)	Nasal saline, pH 6.4	—	3
G2 (test control 1)	Blank NLC	—	3
G3 (test control 2)	AMS-NLC	1 mg/kg	3
G4 (test control 3)	AMS-NLC in situ gel	1 mg/kg	3

AMS, amisulpride; NLC, nanostructured lipid carrier. All groups were dosed intranasally once daily for 28 consecutive days.

2.5 Histopathological evaluation
At the end of the 28-day treatment period the animals were humanely sacrificed and the nasal mucosa was isolated. The collected tissue was cleaned thoroughly and preserved in 10% neutral buffered formalin. Following routine processing and paraffin embedding, sections of 5 µm were cut, mounted on slides and stained with haematoxylin and eosin [22]. The prepared sections were examined by light microscopy at 10× magnification for changes in cellular structure, with particular attention to the presence and regularity of cilia, continuity of the pseudostratified columnar epithelium, goblet cell appearance, and oedema, congestion or inflammatory infiltration of the lamina propria. Sections from treated groups were compared with those from the vehicle control [20,22,23].

3. RESULTS:

3.1 Physicochemical characteristics of the formulations
Blank NLC had a mean diameter of 118.4 ± 4.2 nm; drug loading increased this to 133.7 ± 5.6 nm, and incorporation into the poloxamer matrix gave 178.2 ± 6.1 nm on dilution. All three values lie below the 200 nm target set during optimisation. Zeta potentials were strongly negative −24.6 ± 1.4, −29.3 ± 1.8, and −30.1 ± 1.6 mV respectively — reflecting ionised fatty-acid residues partially screened by the poloxamer corona and indicating adequate electrostatic stabilization [11,15].

The pH of all formulations lay between 6.1 and 6.3, within the 5.5–6.5 window regarded as physiologically acceptable for nasal instillation. The in situ gel underwent a sharp sol–gel transition at 34.4 ± 0.6 °C, below nasal cavity temperature but above ambient, so that it remains instillable at room temperature and gels on contact with the mucosa [24].

3.2 Nasal tissue histopathology and ciliotoxicity
No animal showed nasal discharge, persistent sneezing, signs of local irritation or any change in general condition, body weight, or grooming behaviour during the 28-day dosing period, and there were no deaths.

Histological examination of the nasal mucosa after 28 days of repeated intranasal administration (Figure 1) revealed no appreciable alteration in the cellular morphology of the treated sections relative to the normal control. In all four groups, the pseudostratified columnar ciliated epithelium was continuous and of normal thickness; the ciliary border was present and regularly arranged, goblet

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cells were unremarkable; and the lamina propria showed neither oedema and vascular congestion nor inflammatory cell infiltration. The only departures from normal architecture were a few minor superficial lacerations, and these were distributed without regard to treatment group — a pattern characteristic of microtome and handling artefact rather than of chemical injury. No group-related loss of cilia, epithelial erosion, necrosis or squamous metaplasia was observed. These findings indicate that all three formulations- blank NLC, AMS-NLC, and the AMS-NLC in situ gel- were well tolerated by the nasal mucosa on repeated daily administration and did not produce detectable ciliotoxicity. In particular, the in situ gelling variant, which is designed to prolong contact with the mucosa, was indistinguishable from the un-gelled dispersion and from the saline control [20,22].

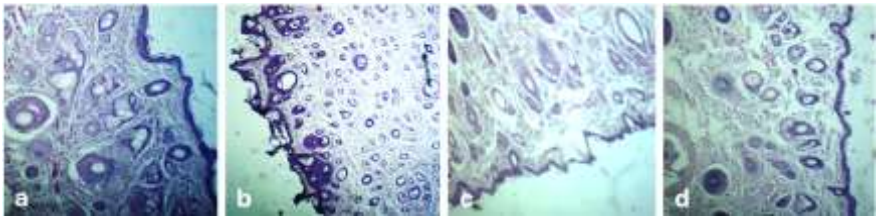

Figure 1. Histopathology of rat nasal mucosa (H&E, 10×) after 28 days of once-daily intranasal administration of (a) nasal saline solution pH 6.4, (b) blank NLC, (c) drug-loaded NLC and (d) AMS-NLC in situ gel.

4. DISCUSSION:

This experiment included four sets as a vehicle control and three test formulations. The blank NLC carries the entire excipient burden without the drug, and it produced no change relative to saline. The lipid matrix and its stabiliser corona are therefore not appreciably ciliotoxic at 5% w/v lipid under this dosing regimen, which localises any drug-related contribution to the difference between G2 and G3. No difference was observable at 1 mg/kg. Encapsulation lowers the free, ionised drug concentration at the epithelial surface [10,25].

The most practically useful comparison is between AMS-NLC and its gelling variant. Thermoresponsive gelation prolongs the residence of the applied dose on the mucosa, which by itself would be expected to increase cumulative exposure of the epithelium to both drug and surfactant. However, the gel matrix simultaneously restricts diffusion of drug and surfactant to the epithelial surface, so retention and instantaneous exposure move in opposite directions and largely cancel [26,27].

The incorporated anionic and cationic NLC into poloxamer in situ gels and administered them intranasally to rats. The present anionic AMS-NLC gel (particles of comparable size below 200 nm and an absolute zeta potential of −30.1 ± 1.6 mV) produced no appreciable change in nasal mucosal lining [26].

The principal outcome of this work is negative in the statistical sense and positive in the developmental sense: after 28 consecutive days of once-daily intranasal dosing, neither the blank NLC, nor the amisulpride-loaded NLC, nor the in-situ gelling variant produced histological changes that distinguished them from the pH 6.4 nasal saline control. A 28-day interval is long relative to the turnover time of the nasal epithelium, so cumulative injury, had it occurred, would have been expected to declare itself by the end of the study as ciliary loss, epithelial erosion, and subepithelial inflammation. None of these was seen [11,20,23].

5. CONCLUSION:

Repeated once-daily intranasal administration for 28 days of blank NLC, amisulpride-loaded NLC and AMS-NLC in situ gel produced no histologically detectable injury to the ciliated nasal epithelium of Wistar rats relative to pH 6.4 nasal saline. Cilia, epithelial continuity and lamina propria were preserved in every treated group; the occasional superficial lacerations that were observed occurred independently of treatment and are best interpreted as processing artefact. Because the study included neither a free-drug arm nor a positive irritant control, it demonstrates tolerability rather than protection; the claim that lipid encapsulation actively shields the epithelium remains an inference drawn from the wider literature rather than a result of this experiment, and the same caution applies to the role of surfactant load, which was not varied here. Subject to that confirmation, AMS-NLC and AMS-NLC in situ gel represent mucosal tolerable candidate platforms for nose-to-brain delivery of amisulpride and merit progression to a combined efficacy-and-safety evaluation in which brain uptake and epithelial integrity are measured in the same animals.

ABBREVIATION:

AMS- amisulpride
AMS-NLC- amisulpride- nanostructured lipid carrier
NLC- nanostructured lipid carrier

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