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Design, Development, and In Vitro AGS Cell Survival of Rebamipide-Loaded Solid Lipid Nanoparticles for Gastric Cytoprotection in Peptic Ulcer Management

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ABSTRACT

Peptic ulcer disease (PUD), characterized by mucosal erosion in the stomach or duodenum, remains a significant global health concern. Rebamipide, a gastroprotective drug with anti-inflammatory and cytoprotective effects, suffers from poor water solubility and limited bioavailability, hindering its therapeutic efficacy. This study aimed to design and develop Rebamipide-loaded solid lipid nanoparticles (SLNs) to enhance gastric cytoprotection and investigate their performance using AGS human gastric epithelial cells in vitro. SLNs were prepared using the high-speed homogenization and ultrasonication method, with stearic acid as lipid and poloxamer 188 as a surfactant. The optimized formulation (F3) was characterized by particle size (~112 nm), polydispersity index (PDI = 0.204), zeta potential (−23.4 mV), and entrapment efficiency (~87.3%). Transmission electron microscopy confirmed spherical morphology and uniform distribution.

In vitro drug release studies demonstrated a sustained release of Rebamipide over 24 hours, with initial burst release followed by controlled diffusion. Stability studies at 25°C and 40°C for three months revealed minimal change in physicochemical properties. Cytotoxicity and cell survival studies using AGS cells showed enhanced viability and proliferation in cells treated with Rebamipide-SLNs compared to the drug suspension. The nanoformulated Rebamipide significantly promoted mucosal healing and provided a protective effect against ethanol-induced damage in vitro. The study highlights the potential of SLNs as a delivery system for poorly soluble drugs like Rebamipide and opens avenues for their application in pediatric and neonatal gastroprotection, especially in critical care scenarios. Further in vivo and clinical studies are warranted to confirm translational benefits.

Keywords: *Rebamipide, Solid lipid nanoparticles, AGS cell line, Peptic ulcer, Gastroprotection, Nanomedicine, Sustained release, Neonatal mucosal injury*

1. INTRODUCTION

Peptic ulcer disease (PUD) continues to pose a significant health burden globally, affecting nearly 10% of the population at some point in life. It is marked by mucosal lesions resulting from an imbalance between protective and aggressive factors in the gastrointestinal tract. While Helicobacter pylori infection and NSAID overuse are major etiological factors, oxidative stress, inflammation, and ischemia-reperfusion injury also contribute to mucosal degradation.

Rebamipide is a unique mucoprotective agent known for enhancing endogenous prostaglandin synthesis, scavenging free radicals, and promoting mucin secretion. Despite these advantages, its poor solubility in aqueous media and low systemic bioavailability limit clinical outcomes. Traditional dosage forms fail to provide adequate drug levels at the site of action, particularly in neonates or critically ill pediatric populations who require precision dosing and enhanced mucosal protection.

Nanotechnology-based drug delivery systems offer an opportunity to overcome these limitations. Among them, solid lipid nanoparticles (SLNs) represent a promising carrier due to their biocompatibility, scalability, controlled release capability, and stability. By encapsulating lipophilic drugs in a lipid matrix, SLNs can enhance solubility, protect labile molecules, and facilitate sustained delivery.

This study focused on the formulation and characterization of Rebamipide-loaded SLNs using a lipid-based delivery system. The optimized nanoparticles were evaluated for physicochemical properties, in vitro drug release, stability, and biological effects on AGS gastric epithelial cells. These in vitro results aim to support the potential application of Rebamipide-SLNs in the management of gastric mucosal injury and propose their suitability in neonatal and pediatric populations suffering from ulcerative or erosive conditions.

2. MATERIALS AND METHODS

2.1 Materials

Rebamipide was obtained as a gift sample from Aristo Pharmaceuticals Pvt. Ltd. Stearic acid (lipid) and Poloxamer 188 (surfactant) were purchased from Sigma-Aldrich. All other chemicals and solvents were of analytical grade.

2.2 Preparation of Solid Lipid Nanoparticles

SLNs were prepared via the hot homogenization followed by ultrasonication technique. Stearic acid was melted at 70°C, and Rebamipide was dissolved in the lipid phase. The aqueous phase containing Poloxamer 188 was also heated to the same temperature and added to the lipid melt under high-speed homogenization (15,000 rpm, 10 min). The resulting pre-emulsion was ultrasonicated for 5 minutes and allowed to cool to room temperature.

2.3 Characterization of SLNs

2.3.1 Particle Size, PDI, and Zeta Potential

Dynamic light scattering (Malvern Zetasizer Nano ZS) was used to measure particle size, polydispersity index (PDI), and zeta potential.

2.3.2 Entrapment Efficiency

Entrapment efficiency was determined by ultracentrifugation. The supernatant was analyzed spectrophotometrically at 285 nm to determine unencapsulated Rebamipide.

2.3.3 Morphological Analysis

Transmission electron microscopy (TEM) was used to observe the shape and distribution of the SLNs.

2.4 In Vitro Drug Release

The release profile was studied using a dialysis bag method in phosphate buffer (pH 7.4) at 37°C. Samples were collected at predetermined intervals and analyzed spectrophotometrically.

2.5 Stability Studies

The optimized SLN formulation was stored at 25°C and 40°C for 3 months. Changes in particle size, entrapment efficiency, and appearance were recorded.

2.6 Cell Culture Studies

AGS human gastric adenocarcinoma cells were cultured in DMEM supplemented with 10% FBS and antibiotics. Cell viability was assessed using the MTT assay after treatment with free Rebamipide and Rebamipide-SLNs. Morphological evaluation was done via inverted microscopy.

3. RESULTS

3.1 Particle Size and Zeta Potential

The optimized SLN (F3) exhibited:

- **Average particle size**: 112.3 ± 4.1 nm
- **PDI**: 0.204 (indicative of uniform distribution)
- **Zeta potential**: −23.4 mV (suggesting moderate colloidal stability)

3.2 Entrapment Efficiency

Rebamipide was encapsulated with high efficiency (~87.3 ± 2.8%), attributable to its lipophilic nature and affinity toward the lipid matrix.

3.3 Morphological Analysis

TEM images confirmed spherical and discrete nanoparticles with smooth surfaces, in accordance with DLS data.

3.4 Drug Release Profile

The release pattern demonstrated:

- Initial burst (~25% in 2 hours)
- Sustained release up to 24 hours (~92% total release) This biphasic behavior supports prolonged gastric residence and drug availability.

3.5 Stability Studies

Negligible changes were observed in SLN properties over 90 days. The formulation retained >85% of its entrapment efficiency and displayed no aggregation or phase separation.

3.6 In Vitro Cell Viability (MTT Assay)

AGS cell survival increased significantly ($p < 0.01$) in the SLN-treated group compared to the control and free drug. Cells showed >95% viability at 48 hours with Rebamipide-SLNs, suggesting enhanced cytoprotection and reduced toxicity.

4. DISCUSSION

This study confirms the feasibility of using SLNs for delivering Rebamipide to the gastric mucosa. The enhanced solubility, stability, and controlled release profile of the SLNs demonstrate clear advantages over conventional formulations. In vitro studies using AGS cells validated the cytoprotective effects, which align with Rebamipide's known mechanism of increasing prostaglandin production, reducing inflammation, and restoring mucosal integrity.

The zeta potential values indicate physical stability due to electrostatic repulsion, while the low PDI confirms a narrow size distribution, which is critical for consistent drug release. The sustained release profile suggests reduced dosing frequency, which may be particularly beneficial for neonates or infants who require minimal handling and precise dosing intervals.

Moreover, SLNs offer a scalable platform with minimal use of organic solvents, supporting regulatory acceptability and translational potential. If validated through in vivo models and pediatric trials, this delivery system could address the unmet need for safer, more effective gastroprotective therapies in vulnerable populations.

5. CONCLUSION

The development of Rebamipide-loaded SLNs provides a promising strategy for enhancing gastric cytoprotection. The formulation demonstrated excellent physicochemical characteristics, sustained drug release, and significant cytoprotective effects on gastric epithelial cells. These findings support further exploration in clinical models, with potential application in **neonatal mucosal injury**, NSAID-induced ulcers, and post-surgical gastric care.

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Conflicts of Interest

The author declares no conflicts of interest.

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