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Review article

Cell cytotoxicity evaluation of silver microemulsion for intranasal antibacterial use in acute bacterial rhinosinusitis: A pharmacological study

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ABSTRACT

Acute bacterial rhinosinusitis is a commonly observed condition marked by inflammation and infection of the sinuses, prompting the exploration of alternative therapeutic approaches, such as the utilization of topical antibacterial agents incorporating silver nanoparticles. However, prior to clinical translation, a comprehensive evaluation of the safety profile is indispensable. This review article places emphasis on diverse safety pharmacological investigations, specifically cell cytotoxicity assays, conducted to assess the potential adverse effects of silver microemulsion on human cells. Additionally, this review highlights other pharmacological methodologies aimed at ensuring safety prior to efficacy testing and subsequent formulation development of an appropriate preparation.

Keywords: cell cytotoxicity, silver microemulsion, acute bacterial rhinosinusitis

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Introduction

Acute Bacterial Rhinosinusitis (ABRS) arises from bacterial infection and inflammation in the paranasal sinuses, typically triggered by viral upper respiratory infections, allergies, or sinus obstructions, leading to symptoms like nasal congestion, facial pain, and headaches.[1-3] Timely medical attention is pivotal to prevent complications. Management involves evidence-based practices and antimicrobial stewardship principles, with antibiotic therapy tailored to specific pathogens, favoring amoxicillin or amoxicillin-clavulanate for primary treatment, and considering doxycycline or levofloxacin for beta-lactam allergies, mindful of regional resistance patterns and patient factors.[4-6] In cases of persistent disease or complications, otolaryngology-guided endoscopic sinus surgery addresses anatomical issues, promoting recovery.[7-8]

Responsible antibiotic use is crucial to combat resistance. In the context of ABRS, where the infection primarily affects the nasal and sinus regions, the use of topical antibiotics offers a targeted advantage by directly addressing localized bacteria, minimizing systemic spread, reducing side effects, and curbing antibiotic resistance. This approach not only maintains the body's microbial balance but also contributes to combating the growing issue of antibiotic resistance.[9-11] While the use of topical antibiotics, such as mupirocin,[12] in ABRS treatment has shown promise, there is a need for further research to determine their efficacy, optimal application methods, and long-term consequences, including the risk of antibiotic resistance. Clinical decisions should consider individual patient factors, symptom severity, and regional antibiotic resistance patterns, with systemic antibiotics remaining the standard treatment for ABRS.

Overall, ongoing research is necessary to establish the precise role and guidelines for topical antibiotics in ABRS management. Silver's potent antimicrobial properties make it an appealing option for combatting infections, especially those involving antibiotic-resistant bacteria.[13-16] Researchers have explored the delivery of drugs to the nasal cavity and sinuses to effectively treat local infections. Silver combats microorganisms through multiple mechanisms, including damaging their outer structures, hindering DNA replication, and disrupting enzymes, showcasing its broad-spectrum activity against bacteria, fungi, and viruses while preserving human cell viability.[13-16]

The nasal route offers advantages like rapid absorption, bypassing hepatic metabolism, and non-invasiveness, and silver nanoparticles can be formulated into stable microemulsions or nanosuspensions for targeted and sustained release. However, questions about appropriate dosage, duration, long-term safety, accumulation, and toxicity require comprehensive laboratory and clinical investigations. While silver's versatility makes it an intriguing option for nasal drug delivery, further research is needed to validate its efficacy and safety in this context. Notably, topical antibiotics, despite their potential, lack robust support from medical research, and there are currently no universally recommended treatments. Nevertheless, silver nanoparticles as a nasal spray hold promise for bacterial eradication, warranting further safety evaluations.[16-17]

This review aims to accomplish two key objectives. Firstly, it evaluates the safety of a silver microemulsion concerning its potential cytotoxicity to the delicate nasal and sinus tissues, ensuring patient well-being. Secondly, given the efficacy of antibiotics, it investigates the silver microemulsion's capacity to combat bacterial infections in ARBS, with a focus on its antibacterial mechanisms, molecular interactions, and performance within the nasal and sinus environments.

Cellular interactions with silver nanoparticles

To comprehend how silver nanoparticles affect cells, a critical initial step is assessing their safety. This helps determine the right range of concentrations for further testing and establishing safe dosage levels for potential therapies. Researchers use lab-based models, like human nasal, bronchial, alveolar cells, immune cells, and skin cells, to gain insights into how these nanoparticles interact with cells and whether they cause inflammation.[17] Understanding how silver nanoparticles get into cells is essential and involves various mechanisms influenced by nanoparticle properties and cell types. Evaluating cell toxicity is crucial as it helps identify potential harm, set safety limits, and ensure compatibility. Different tests can be used to investigate the impact of silver nanoparticles as shown in table 1.[17-18]

Investigating cell cytotoxicity of silver microemulsion

According to literature review as shown in table 1, studying the effects of a silver microemulsion on cells involves several important considerations. Firstly, researchers need to choose the right concentrations of nanoparticles to test, covering a wide range to see how cell health changes with different amounts of silver. They should also study cells over various time periods to understand both immediate and long-term effects. Specific tests, such as those for cell energy, cell membrane health, and damage to cells, should be used to assess different aspects of cell health. Having robust control groups is vital for making valid comparisons and confirming that observed effects are caused by silver nanoparticles.[19-20] Researchers should also investigate how stable the liquid nanoparticles are and how their size and charge affect their interactions with cells. To make the research relevant, they should translate the experimental concentrations to real- world situations to determine potential harm levels and safety guidelines. Using cell types like those in the body part being treated makes the findings more meaningful.[19-21] By looking at data from various tests and examining factors like oxidative stress and inflammation, researchers can get a better understanding of how silver nanoparticles impact cells.[21-23] Following established research standards ensures rigor and reliability.

All these considerations together help researchers thoroughly evaluate the potential harm that a silver microemulsion may cause to cells, providing insights into how cells respond to nanoparticles and their implications for human health.

Unraveling nanoparticle-based therapies for successful clinical applications

The transition of nanoparticle-based therapeutics from laboratory experimentation to practical clinical deployment necessitates a meticulous exploration of their physical and chemical attributes, firmly grounded in the principles of pharmacology. Central to this investigation is an in-depth examination of nanoparticle size and dispersion, which profoundly influences their interactions with biological entities, systemic distribution, and eventual clearance mechanisms. Ensuring precise and consistent control over nanoparticle size assumes critical significance as it underpins the predictability of their interactions with cells and tissues, constituting a fundamental prerequisite for achieving the intended therapeutic outcomes.[21-23]

Additionally, examining the chemical makeup and surface charge of nanoparticles, while following established pharmacological principles, dictates how they interact with molecules, cells, and tissues. By carefully adjusting these surface properties, we can make treatments more specific, improve their ability to target cells precisely, and ensure they remain stable within the body. This helps deliver therapies where they are needed while minimizing unintended effects.[21-23]

A comprehensive investigation into the behavior of nanoparticles within the biological milieu and their inherent stability is pivotal. This entails probing into tendencies for aggregation or degradation, as these aspects profoundly impact the duration of therapeutic action, spatial distribution within the body, and overall therapeutic efficacy. Proficiency in controlling these stability-related parameters empowers precise modulation of treatment profiles, thereby exerting a significant influence on clinical success.[21-23]

Moreover, understanding how nanoparticles interact with living organisms and their potential effects on the immune system is crucial, following the principles governing pharmacological responses. We need to systematically explore immune reactions, potential harm to cells, and inflammatory responses to ensure the ongoing safety of these therapies and gain confidence in their clinical use.[24, 25] Equally important is mastering how therapeutic agents are loaded into nanoparticles and how they are released over time, which are fundamental aspects of pharmacology. Especially for treatments designed for drug delivery, it's essential to ensure efficient encapsulation in nanoparticles and controlled release. Understanding how the physical and chemical properties of nanoparticles impact drug loading, release rates, and bioavailability is crucial for optimizing therapies.

In the field of pharmacology, the intricate relationship between nanoparticle properties and the body's transport pathways significantly influences the effectiveness and safety of therapies. Customizing nanoparticle surfaces by attaching specific ligands, antibodies, or targeting components aligns with pharmacological principles. This precision in surface engineering enhances therapeutic effectiveness while minimizing unwanted side effects, ensuring the success of clinical interventions.

Furthermore, maintaining the stability of nanoparticle-based therapies over time, especially during storage and transportation, is a critical but often overlooked aspect of pharmacology. Ensuring that both nanoparticle properties and their enclosed substances remain constant until clinical use is essential for consistent and dependable performance. Adhering to quality standards and regulatory requirements, including techniques like transmission electron microscopy (TEM), dynamic light scattering (DLS), zeta potential measurements, and spectral analyses, provides insights into the physical and chemical characteristics of therapeutic agents.[23-25] These analytical insights confirm the uniformity, precision, and compliance of therapeutic agents with regulatory standards. Strict adherence to established standards and regulatory criteria is a cornerstone in the field of pharmacology, ensuring that the physical and chemical attributes of therapeutic agents align with rigorous clinical benchmarks. The convergence of pharmacology and chemistry holds the potential for significant advancements in clinical practice, guided by the foundational principles shared by these closely related disciplines.

Table 1 Exploring Silver Nanoparticle-Induced Cytotoxicity Through Cell Viability Assays[26]

Assay	Principle	Results and Pharmacological Implications	Limitations	Significant Considerations
MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay)	To assess cell viability by measuring the conversion of MTT dye into formazan crystals by metabolically active cells.	Provide insight into cell metabolic activity, guiding drug screening and evaluating cytotoxic effects in pharmaceutical research.	Interference from compounds affecting mitochondrial activity and inability to distinguish between cytotoxicity and growth inhibition.	Several crucial considerations must be rigorously addressed, including the selection of appropriate cell lines and culture conditions, preparation of diverse nanoparticle concentrations, inclusion of essential control groups, and precise determination of exposure durations. A standardized MTT assay protocol with

Assay	Principle	Results and Pharmacological Implications	Limitations	Significant Considerations
				strict adherence to incubation times and accurate spectrophotometric measurements is essential, along with the use of replicates and robust data analysis for statistical validity. Researchers should be vigilant for potential nanoparticle interference, assess assay component biocompatibility, adhere to ethical guidelines for cell culture, and prioritize comprehensive reporting to ensure transparency and reproducibility. Addressing these factors systematically enables scientifically sound evaluations of Silver Nanoparticle-Induced Cytotoxicity, advancing our understanding of nanoparticle toxicity in biomedical applications.
Trypan blue exclusion or propidium iodide staining	To assess cell viability by detecting dye uptake in nonviable cells with compromised cell membranes.	Reveal cell viability, aiding drug testing and understanding treatment effects on cell health.	Inability to distinguish between early apoptotic and necrotic cells, and potential impact on live cell populations during staining.	It's crucial to select relevant cell lines, prepare nanoparticle concentrations, include control groups, and determine exposure durations accurately. Follow standardized protocols, employ replicates and robust data analysis, be cautious about potential nanoparticle interference, consider biocompatibility, adhere to ethical guidelines, and ensure comprehensive reporting for reliable results and improved understanding of nanoparticle toxicity

Assay	Principle	Results and Pharmacological Implications	Limitations	Significant Considerations
				in biomedical applications. Propidium Iodide Staining: Standardized staining protocols and instrument settings help maintain result consistency across experiments.
ATP assay	The key principle of an ATP assay involves using the luciferase enzyme system to measure the amount of ATP in a sample. Luciferase catalyzes the reaction between ATP and luciferin, producing light whose intensity corresponds to ATP concentration. This assay assesses cellular energy and is valuable for various applications, such as studying cell health and drug effects.	The results of an ATP assay provide insights into cellular energy levels and metabolic activity, guiding pharmacological decisions and drug impact assessments.	ATP assay's limitations include sensitivity to experimental conditions, potential interferences, and inability to differentiate between ATP sources.	Key considerations include appropriate cell line selection, consistent culture conditions, diverse nanoparticle concentrations, essential control groups, and precise exposure durations. Adhering to a standardized ATP assay protocol, accurate incubation time monitoring, and rigorous spectrophotometric measurement are crucial.
LDH Assay	The principle of LDH (Lactate Dehydrogenase) assay is to measure the enzymatic activity of LDH, which catalyzes the interconversion of lactate and pyruvate, indicating cell damage or tissue injury.	Elevated LDH levels suggest cell damage or disease, guiding diagnosis and monitoring treatment response in various medical conditions.	LDH assay's limitations include lack of tissue specificity and inability to identify the precise cause of elevated levels.	Important considerations include selecting suitable cell lines, maintaining consistent culture conditions, preparing various nanoparticle concentrations, using control groups, and accurately determining exposure durations. Adhering to a standardized LDH assay protocol with precise incubation times and thorough spectrophotometric measurements is crucial.

Assay	Principle	Results and Pharmacological Implications	Limitations	Significant Considerations
Flow cytometry	Rapid, single-cell analysis based on light scattering and fluorescence, enabling detailed characterization of cell populations.	Provide information about cell types, markers, and functions, guiding drug development and personalized treatments in various diseases.	Need for skilled operators, potential for spectral overlap, and limited ability to analyze rare cell populations.	Main factors to address involve the selection of suitable cell lines, culture conditions, nanoparticle concentrations, control groups, and exposure durations. It's vital to follow a standardized protocol with precise incubation times and accurate measurements using fluorescent markers. Ensuring replicates and robust data analysis is essential for maintaining statistical validity.

Conclusion

The development of intranasal nanoparticle treatments for ARBS faces significant challenges. These treatments hold promise for replacing oral medications and combating antibiotic resistance. But before moving forward, we need to conduct thorough safety tests in preclinical trials. The first step in safety testing involves studying cells in a test tube. We commonly use the MTT assay to check if cells are alive, but it doesn't tell us how well they're doing. So, we also need other tests like ATPase and cell morphology studies to get a deeper understanding of cell health. The LDH assay helps us figure out why cells may have died, and flow cytometry gives us a comprehensive view of cell safety data. Using these different tests together gives us a complete picture of cell safety. This helps us feel confident about the safety of the treatment and helps us find the right concentration for further effectiveness testing. In conclusion, using a variety of testing methods is crucial to thoroughly check cell safety. It gives us confidence in the treatment's safety and helps us determine the best concentration for further testing.

Conflicts of Interest

The authors declare no conflict of interest.

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