Infection and Drug Resistance

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ORIGINAL RESEARCH

Amorphous Roxithromycin Loaded in-situ Gel for the Treatment of *Staphylococcus aureus* Induced Upper Respiratory Tract Infection

Li Ji[1], He Bai[2], Ning Tao[2], Yanpeng Lei[2], Anyin Li[2], Cheng Wang[2], Pengfei Cui[2], Xiaofeng Gu[1]

[1]Department of Otolaryngology, The Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University, Changzhou, 213003, People's Republic of China; [2]School of Pharmacy, Changzhou University, Changzhou, 213164, People's Republic of China

Correspondence: Pengfei Cui; Xiaofeng Gu, Email cuizy1990@cczu.edu.cn; xiaofenggu2006@163.com

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Objective: Upper respiratory tract infections are among the most prevalent respiratory diseases, imposing both financial and physical burdens on affected individuals. Roxithromycin (ROX), a primary drug for treating bacterial-induced respiratory tract infections, is typically administered orally due to its hydrophobic nature. However, the non-specific distribution resulting from oral administration reduces bioavailability and can cause side effects such as diarrhea.

Methods: In this study, we prepared a thermo-sensitive in-situ gel using a facile and highly reproducible method by simply mixing two types of poloxamers with ROX.

Results: The ROX can be well dissolved in the poloxamer matrix in amorphous state to give solution. Upon intranasal administration, the ROX solution undergoes a phase transition to form in-situ gel under body temperature. This gel remains in the nasal cavity for an extended period, releasing the drug directly to the site of infection and minimizing non-specific distribution. Pharmacokinetic experiments revealed that, compared to oral administration, the bioavailability of local nasal administration increased by 1.5 times, and the drug concentration in the local nasal cavity increased by 8 times. In contrast, concentrations in the liver and small intestine did not significantly differ from those following oral administration. In vivo antibacterial experiments also showed that the ROX in-situ gel has superior antibacterial efficacy and excellent biocompatibility.

Conclusion: These results suggest that the thermo-sensitive ROX in-situ gel is a promising formulation for treating bacterial upper respiratory tract infections.

Keywords: roxithromycin, in-situ gel, bacterial infection, upper respiratory tract

Introduction

Upper respiratory tract infections, such as pharyngitis, laryngitis, nasopharyngitis, and rhinitis, are among the most common diseases.[1,2] The most common symptoms include cough, sore throat, nasal congestion, and headache.[3,4] Although these symptoms are usually self-limiting and not fatal, they may lead to complications such as pneumonia, otitis media, glomerulonephritis, and myocarditis, posing additional risks to human health. Bacterial infections are a leading cause of upper respiratory tract infections, with *Staphylococcus aureus* and *Streptococcus* species being the most common sources of infection.[5,6]

Roxithromycin (ROX) is a semi-synthetic 14-membered carbocyclic macrolide antibiotic with characteristics of general macrolide.[7] As a representative macrolide drug, ROX is one of the most widely used antibiotic in clinical practice for the treatment of bacterial upper respiratory tract infections. Due to its poor water solubility, ROX is mostly formulated as tablets, capsules, and granules, with oral administration being the primary administration route.[8] The drug is mainly absorbed in the intestine, which has the potential to cause gastrointestinal reactions such as nausea, abdominal pain, and diarrhea, and also disrupting the gastrointestinal flora, upon high dosing,[9] especially for patients with liver dysfunction.[10] However, upper respiratory tract infections typically occur in the nasal cavity to the throat, whereas most

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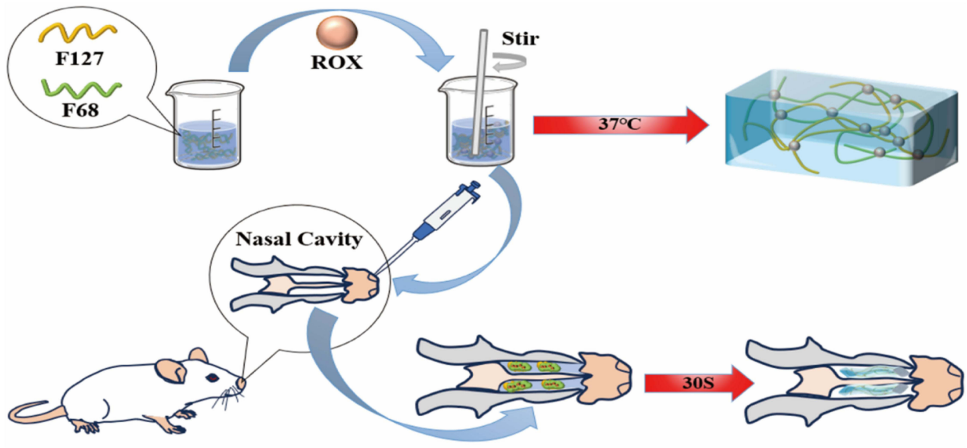
orally administered drugs are absorbed in the small intestine, requiring them to pass through the bloodstream to reach the affected organs, resulting in delayed effects and increased liver burden. The drug concentration at the lesion site can only be maintained at a high level for a short period, and treating variant drug-resistant strains requires larger doses and more frequent administration. This can prevent patients with abnormal liver function from taking medication normally. Therefore, developing appropriate drug administration methods to enhance the efficacy of ROX and reduce its toxicity and side effects is worthwhile. This study aims to develop an in-situ gel for the local nasal administration to reduce the toxicity and side effects of ROX, while increase its local drug concentration, and extend its retention time.

The in-situ gel is a preparation that, after administration in the sol state, undergoes an immediate phase change at the administration site, transforming the liquid into a cross-linked semi-solid gel.[11,12] The application of in-situ gels in biomedical fields has been widely practiced.[13,14] Ha et al combined resveratrol nanosuspension (Res NS) into an ion-sensitive gel for intranasal administration to the brain, increasing its bioavailability by 2.88 times. No significant volume expansion was observed during the sol–gel transition, with an expansion coefficient of less than 4%. Therefore, the small increase in volume when the gel is formed in the nasal cavity will not compromise patient compliance.[15] Zeng et al developed a thermo-sensitive in-situ gel delivery system for oral administration based on poloxamer 407, poloxamer 188, and xanthan gum. The sol–gel transition temperature is 28–34°C, and the methylcyano-5 derivative (Me-Cy5) was used to label hydrogels. This was the first in vivo research on mice using optical imaging, with the fluorescence intensity time–response curve in the oral region reflecting the formulation's residence time. The area under the curve (AUC) value of the in-situ gel delivery system was twice that of the drug solution.[16]

Local nasal administration effectively avoids gastrointestinal tract stimulation during drug absorption, avoids the first-pass effect, and increases bioavailability. It also offers the advantages of simple preparation, convenient use, and accurate dosing.[17,18] In-situ gels can be divided into thermo-sensitive, pH-sensitive, and ion-sensitive types.[19] In this study, we aim to develop a thermo-sensitive ROX in-situ gel suitable for local nasal administration. It was expected that the thermo-sensitive in-situ gel, when injected or dripped into the nasal cavity in the sol state, can undergo phase-transformation to gel state at body temperature with appropriate biological adhesion and slowly release the drug.[20] After a deep literature survey, we employed poloxamer 407 (F127) and poloxamer 188 (F68) as the matrix of the thermo-sensitive gel while, at the same time, serving as surfactant to solubilize hydrophobic ROX.[21] We found that the preparation of in-situ gel is facile and highly reproducible by simply mixing ROX with both poloxamers in PBS to produce a uniform solution that is ready for use. Using in vitro drug release and in vivo pharmacodynamic assays, it was revealed that compared to oral ROX administration, the drug in the sol state is in a free molecular state, which facilitates faster dissolution and absorption. F127/F68 not only improves drug solubility but also induces gel transformation in response to body temperature, increasing retention in the nasal cavity (Scheme 1). Finally, the in vivo antibacterial assay was employed to further confirm our hypothesis.



Scheme 1 Scheme of the preparation and nasal delivery of thermo-sensitive ROX in-situ gel.

Materials and Methods

Materials

Roxithromycin (ROX, 98%) was purchased from Dalian Meilun Biological Co. (Dalian, China). Poloxamer 407 (F127) and Poloxamer 188 (F68) were purchased from BASF (Schweiz AG, Germany). *Staphylococcus aureus* (ATCC-6538) was obtained from ATCC (USA). Tryptone soy broth (TSB) and tryptone soy agar (TSA) medium were procured from Haibo Biological Co. (Qingdao, China). All other unspecified chemicals and reagents were provided by Adamas (Shanghai, China).

BALB/c mice (6–8 weeks old, 18–22 g) were sourced from the Shanghai Laboratory Animal Center (Shanghai, China). All animal experiments were conducted in accordance with the NIH guidelines and the experiments were approved by the Animal Protection and Utilization Committee of Changzhou University (No. 2023030128). The mice were maintained in a rigorously controlled environment with a stable temperature of 23°C ± 0.5°C and a relative humidity range of 45% to 55%. They were consistently supplied with sterile water and standardized feed to ensure optimal living conditions.

Determination of ROX by HPLC

Detection Parameters of ROX

The ROX molecule, lacking delocalized electronic structure, does not emit fluorescence. The ROX has a maximum absorption peak at 210 nm, hence HPLC coupled with UV detector was selected as the detection method for ROX. The mobile phase was prepared by mixing 400 mL of acetonitrile, 200 mL of methanol with 400 mL of a 0.01 mol/L ammonium acetate solution in water. ROX determination in human plasma was performed using an HPLC chromatograph (Agilent 1260). The flow rate was set at 1.0 mL/min, with a column temperature at 32°C and a UV detector at an absorption wavelength (Ex) of 210 nm. Samples (20 μL) were injected using an autosampler. Separation was conducted on a Grace Smart RP 18C (4.6 × 250 mm, 5 μm) liquid phase column.

Sample Preparation

Stock solutions of ROX at 1 mg/mL were prepared using acetonitrile as the solvent. Standard solutions of ROX at concentrations of 0.5, 1, 2, 4, 8, 12, 24, 50, 75, 100, 150, 200, and 400 μg/mL were also prepared in acetonitrile. Aliquots (100 μL) of these samples were transferred to glass vials containing 10 μL of blank mouse serum. The resulting serum samples contained 0, 0.5, 1, 2, 4, 8, 12, 24, 50, 75, 100, 150, 200, and 400 μg/mL of ROX and were processed as described below.

Healthy mouse blood was centrifuged at 4500 rpm for 10 minutes at room temperature, and the supernatant was taken. To 100 μL of ROX solution with varying concentrations, 10 μL of serum and 10 μL of Na_2CO_3 solution (0.2 mol/L) were added. The solution was mixed, extracted 1–2 times with 500 μL of dichloromethane, and centrifuged at 3000 r/min for 5 minutes after each extraction. The organic phase was separated for nitrogen flow drying in a water bath at 40°C. The product was redissolved in 100 μL of mobile phase, followed by HPLC detection.

Preparation and Characterization of ROX in-situ Gel

Poloxamer 407 (F127) and Poloxamer 188 (F68) were dispersed into a PBS solution and stirred at 4°C for 24 hours to achieve a homogeneous solution, with final polymer concentrations of 20.5% (w/w) for F127 and 5.5% (w/w) for F68, respectively. Various amounts of ROX were mixed with a certain amount of hydrogel to prepare ROX in-situ gel (sol state) at concentrations of 2, 4, 6, 8, 10, 12, and 20 mg/mL. The mixture was stirred on a magnetic stirrer for 12 hours in the dark, then placed in a refrigerator to observe for sedimentation. Finally, a concentration of 10 mg/mL ROX was selected for subsequent experiments.[22]

ROX, blank gel, and ROX in-situ gel were characterized using an X-ray diffractometer and a scanning electron microscope (SEM, Regulus-8100, Tokyo, Japan). In brief, samples were prepared by placing a small amount of the gelling system on a conductive adhesive tape and allowing it to dry under ambient conditions. The dried samples were then sputter-coated with a thin layer of gold to enhance conductivity and minimize charging effects during imaging. SEM analysis was operated at an accelerating voltage of 20 kV. High-resolution images were captured to provide detailed

insights into the microstructure of the gels. The blank gel and ROX in-situ gel were also characterized using a high-speed rheometer (AR 1500 ex, TA Instruments, New Castle, DE, USA).

In vitro Antibacterial Effect

Bacteria Culture and Antibacterial Research

The bacteria were cultured in LB medium and harvested by centrifugation during the exponential growth phase. The bacterial concentration was monitored photometrically by measuring the optical density (OD) at a wavelength of 600 nm. Before the experiment, the OD600 value of the bacterial stock solution was adjusted to 0.1, equivalent to 10^8 CFU per mL of *Staphylococcus aureus*. Since ROX is poorly soluble in water, a 5% Tween 80 solution was first used to prepare an aqueous solution of ROX, resulting in a 1 mg/mL stock solution. Afterward, 500 μL of the diluted bacterial solution was mixed with 500 μL of ROX solution for 1 hour, with ROX concentrations ranging from 0 to 16 μg/mL. After incubating the ROX solution and bacterial solution for 1 hour, 100 μL of the sample from each concentration was taken and incubated at 37°C in a bacterial culture incubator. After 12 hours, the culture plate was removed, and the inhibition rate was calculated based on the number of colonies using the following formula.

$$\text{Antibacterial rate} = \frac{\text{number of colonies in the experimental group}}{\text{number of colonies in the control group}}$$

The bacterial solution, diluted up to 40,000 times with tryptic soytone liquid medium based on the measured OD value, was spread evenly on pre-prepared tryptic soytone agar medium with 100 μL of the diluted solution. Pre-prepared absorbent paper was dipped into the prepared ROX aqueous solution and ROX in-situ gel, left for 1 minute to ensure complete saturation, and then placed on the smeared tryptic soytone agar culture plate in an equilateral triangle pattern, with three pieces per plate. The plates were pressed lightly to ensure the paper fitted completely. The culture dishes were inverted and placed in a 37°C constant temperature bacterial incubator overnight. The next day, the area of the inhibition zone was measured and recorded.[23]

Sustained Drug Release of ROX in-situ Gel in Artificial Nasal Fluid

Artificial nasal fluid was prepared according to previous literature. In brief, prepare 7.91 g of sodium chloride, 2.56 g of sodium bicarbonate, 3.68 g of potassium chloride, and 0.51 g of calcium chloride, dissolved in 1 L of deionized water and stored at 4°C for use.

ROX was dissolved in acetonitrile to prepare a 1 mg/mL stock solution, and the prepared artificial nasal fluid was used as a diluent to achieve final ROX concentrations of 0, 0.25, 0.5, 1, 10, 50, 100, 200, 300, and 500 μg/mL as standard solutions for the establishment of standard curve.

The ROX in-situ gel (1 mL containing 10 mg of ROX) was placed in a dialysis bag (WM 3500), with both ends sealed. The bag was immersed in 20 mL of artificial nasal fluid in a constant temperature water bath of 37°C with shaking speed set at 100 rpm. At intervals of 5, 15, 30, and 60 minutes, and 3, 9, and 24 hours, 1 mL of artificial nasal fluid was extracted and replaced with preheated artificial. The extracted release medium was used for HPLC detection as mentioned above.

The ROX + F68 and ROX powder at the same drug content and subjected to the same treatments were also employed as control groups.

In vivo Antibacterial Experiments

In vivo Pharmacodynamic Assay

Experimental mice were randomly divided into four groups, as detailed in Table 1 (n = 3 in each group). Each mouse received a daily drug administration based on body weight (average 20 g) at a standard of 10 mg/kg, which equals to about 200 μg of ROX.

Blood was collected from the eye socket and centrifuged at 4500 rpm for 10 minutes to obtain plasma samples at 15, 45, 90, 60, 150, 240, and 480 minutes after drug administration. Subsequently, the animals were sacrificed, and the liver, heart, brain, nose, throat, and small intestine were extracted. After washing away surface blood with PBS, the organs were placed in

Table 1 Grouping and Dosage for Pharmacokinetics Experiment in vivo

Group	Preparation	Drug Concentration (mg/mL)	Administration Routes	Dosage
A	ROX gel	10	Nasal	20 μL
B	ROX solution	1	Oral	200 μL
C	ROX+F68	10	Nasal	20 μL
D	Blank gel	0	Nasal	20 μL

24-well plates and stored at −80°C. To treat the plasma samples, 20 μL of a Na_2CO_3 solution (0.2 mol/L) was added to 20 μL of plasma. The mixture was extracted with 500 μL of dichloromethane to obtain ROX. The samples were then centrifuged at 3000 rpm for 10 minutes, and the organic phase was collected. The collected organic phase was dried using nitrogen flow in a water bath at 40°C, and the dried product was redissolved in the mobile phase (200 μL). After reconstitution and centrifugation at 3500 rpm for 10 minutes, the supernatant was collected for HPLC detection. For tissue samples, the samples were first weighed and then placed in a tissue homogenizer. Extraction medium (acetonitrile/water = 40/60, v/v) (0.4 mL) was added to each 100 mg of tissue for homogenization. The process was conducted in an ice water bath. After homogenization, the tissue fluid was sonicated for 5 minutes and then centrifuged at 3500 rpm for 10 minutes at 4°C. The supernatant was transferred to a new test tube. The supernatant was dried as mentioned above and then re-suspended with the mobile phase, followed centrifugation and finally subjected HPLC analysis.

In vivo Antibacterial Assay

The experimental mice were randomly divided into five groups, with n = 3 in each group, as detailed in Table 2.

To establish the *Staphylococcus aureus* infected animal model, the *Staphylococcus aureus* solution at the density of 10^8 CFU/mL was injected (50 μL per mouse) into the nasal cavities of mice (groups B, C, D, and E) once a day for three consecutive days to ensure infection. After a different treatment for three days, 100 μL of PBS was used to lavage the nasal cavities, and the collected solution was subjected to centrifugation (1500 rpm for 10 min) to isolate the bacteria. The isolated bacteria were further dispersed with PBS and cultured for 12 hours to observe colony growth (Scheme 2).

Finally, blood samples were taken from the eye sockets for routine testing of white blood cells, lymphocytes, and neutrophils. The mice were then euthanized, and nasal, liver, and kidney tissues were stained with H&E and sliced for a comprehensive evaluation of the treatment's effect. In brief, tissue sections were obtained from paraffin embedded samples. The sections were deparaffinized and rehydrated through a series of xylene and graded alcohol solutions. Subsequently, the sections were stained with hematoxylin for 5–10 minutes to visualize cell nuclei, followed by a brief rinse in running tap water to remove excess stain. The sections were then counterstained with eosin for 2–3 minutes to highlight the cytoplasm and extracellular matrix. After staining, the sections were dehydrated through graded alcohol solutions and cleared in xylene. Finally, the sections were mounted with a coverslip using a permanent mounting medium and then subjected to examination under a light microscope.

Table 2 Grouping and Dosage for Antibacterial Experiment in vivo

Group	Preparation	Drug Concentration (mg/mL)	Administration Routes	Dosage (μL)
A	Control	/	/	/
B	Blank gel	0	Nasal	20 μL
C	ROX solution	1	Oral	200 μL
D	ROX+F68	10	Nasal	20 μL
E	ROX gel	10	Nasal	20 μL

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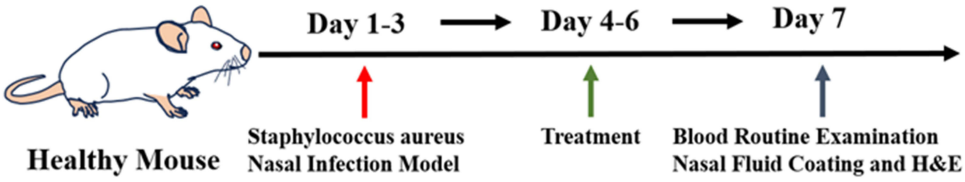

Scheme 2 Schematic diagram of in vivo antibacterial assay.

Statistical Analysis

Data are presented as mean ± standard deviation (SD) with n = 3 if not specifically stated. The *t*-test or one-way analysis of variance (ANOVA) was employed to compare the significance of different groups with $*P < 0.05$ and $**P < 0.01$.

Results

Preparation and Characterization of ROX in-situ Gel

The gelation temperature as well as gelation time were first optimized. To ensure adequate hydrogel formation and extend the in vivo drug retention time during local treatment, a hydrogel matrix consisting of 20.5% (w/w) F127 and 5.5% (w/w) F68 was selected (Table 3).

As depicted in Figure 1A, the blank in-situ gel is liquid at room temperature and can transform to a gel state near body temperature, with a gelation time of approximately 0.5 minute. The storage modulus (elastic modulus, G′) and loss modulus (viscous modulus, G″) of the blank in-situ gel were analyzed in Figure 1B. It was revealed that at above 25°C, the G′ values for blank in-situ gel were higher than the G″ values, suggesting the formation of gel state. Similarly, the ROX loaded in-situ gel's G′ and G″ were also analyzed, and the results are shown in Figure 1C. It was demonstrated that at above 29°C, the G′ values were higher than the G″ values, suggesting the feasibility of forming in-situ gel upon in vivo applications.

The XRD scanning was performed on ROX, blank gel, and ROX in-situ gel, as shown in Figure 1D. The disappearance of ROX's diffraction peak suggests that most of the drug in the gel is amorphous, which indicates that ROX is fully dissolved in the gel matrix. This is beneficial as high specific surface area and porosity of amorphous drug molecules facilitate rapid drug dissolution to realize antibacterial drug concentration threshold.[24]

The SEM observations also reached similar conclusions. As shown in Figure 1E, the morphology of blank in-situ gel was shown as porous structure, while that of ROX was shown as aggregate of multiple irregular small crystals (Figure 1E). The SEM image of ROX in-situ gel showed similar appearance to that of blank in-situ gel without obvious observation of the ROX crystals, which aligns with the XRD results in Figure 1D that most of the ROX was dissolved in the gel matrix in an amorphous state.

Simulated Release of ROX in-situ Gel

To verify the release of ROX in-situ gel in the nasal cavity, in vitro release experiments were conducted using artificial simulated nasal fluid as the release medium, with ROX powder and solution (ROX + F68) serving as the control. The results, as shown in Figure 2, indicated that the release rate of the ROX powder was relatively slow, while that of ROX + F68 was too quick. In contrast, the ROX in-situ gel showed moderate drug release with sustained manner for a long period of time. ROX is a highly

Table 3 Poloxamer Ratio Screening and Corresponding Gel Performance

Groups	F127 (g)	F68 (g)	Deionized Water (g)	Gel Temperature (°C)	Gelation Time (min)
1	2.0	0.2	7.8	>38	>5
2	2.0	0.4	7.6	>38	>5
3	2.0	0.6	7.4	27	<2
4	2.2	0.2	7.6	>38	>5
5	2.2	0.4	7.4	<30	<2
6	2.05	0.55	7.4	34	0.5

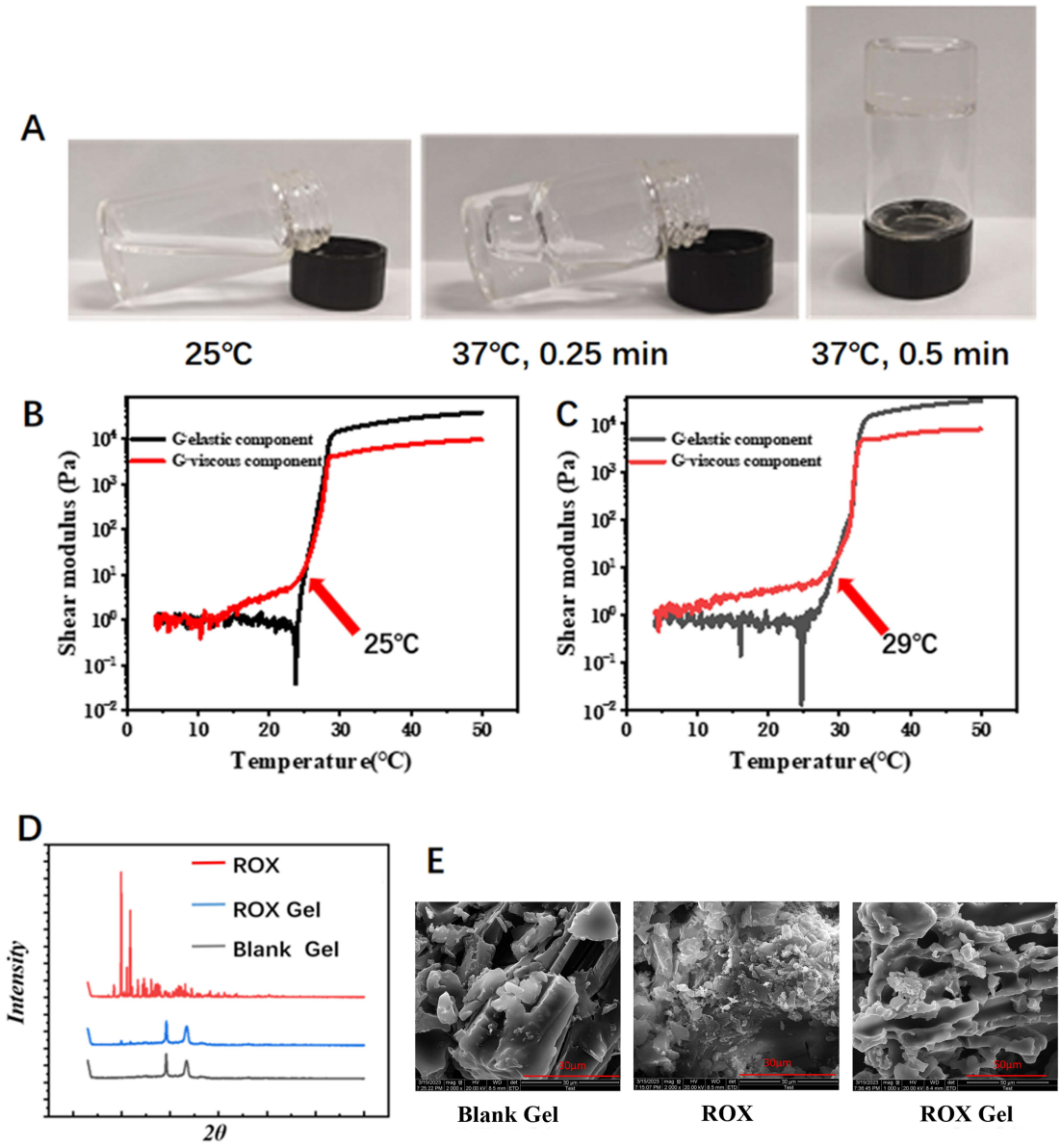

Figure 1 (**A**) Phase transition diagram of blank in-situ gel at room temperature and 37 °C. The changes of elastic modulus and viscous modulus with the variation of temperature for blank in-situ gel (**B**) and ROX in-situ gel (**C**). The XRD spectrum (**D**) and SEM images (**E**) for different samples. The scale bar is 30 μm.

hydrophobic drug with poor solubility in water, which corresponded to its slow release in the artificial nasal fluid whose main component is water. The faster release of the ROX in-situ gel group, compared to the ROX group, is suggested to be attributed to the amorphous state of ROX in the gel, which increases the release rate, as confirmed by the results in Figure 1D and E. After gel formation, the gel with porous structure effectively slowed down drug release, demonstrating a good sustained release effect.

In vitro Antibacterial Effect of ROX in-situ Gel

An in vitro inhibition zone experiment was conducted to determine if the gel formulation affected the antibacterial efficacy of ROX. Figure 3 shows that the control group and blank gel exhibited no antibacterial activity, while the ROX solution with F68 produced an inhibition zone of about 9.3 mm in diameter. In contrast, the ROX in-situ gel yielded a significantly larger inhibition zone of around 13.5 mm, indicating that the gel not only preserved but also enhanced the antibacterial activity of ROX, likely due to its sustained release properties.

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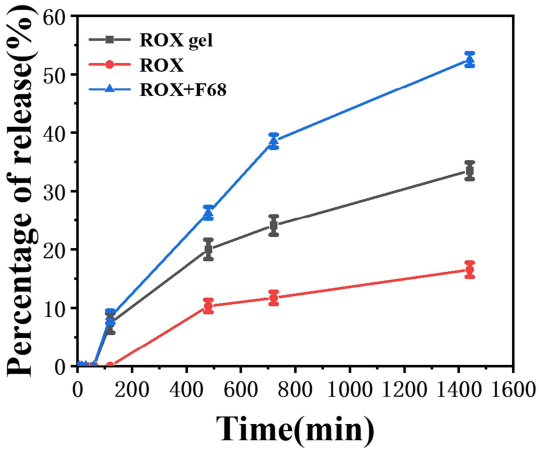

Figure 2 The release profile of different formulations.

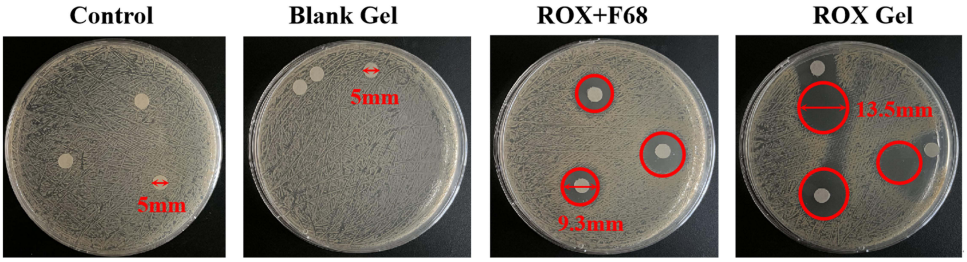

Figure 3 In vitro inhibition zone experiment of different formulations.

In vivo Pharmacokinetics and Drug Distribution

We further assessed the in vivo pharmacokinetics of ROX and its organ distribution after 8 hours post administration of different formulations. Following a 12-hour fast, the ROX in-situ gel was administered to the experimental group, with the free drug groups subjected to different administration routes (ROX + F68 group for nasal and ROX solution for gavage administration) serving as controls. Blood samples were collected at various time points, and after the final collection, the mice were sacrificed for organ sampling.

The blood drug concentration test (Figure 4A) revealed the highest concentrations in the group that ROX in-situ gel is administered nasally, with sustained high drug levels over time. The AUC value for the experimental group was 1.5 times that of the ROX + F68 group and 5 times that of the gavage administration group, indicating that our preparation and administration method effectively improved drug bioavailability.

The drug concentration in the internal organs (Figure 4B) showed that the local drug concentration in the nasal cavity of the ROX in-situ gel group was 4 and 8 times higher than that of the ROX + F68 and gavage administration group, respectively, which significantly enhance local drug concentration without notable increase to the drug concentrations in the liver and intestine, thus avoiding heightened drug toxicity and side effects.

In vivo Antibacterial Assay

To evaluate the in vivo antibacterial efficacy of the ROX in-situ gel, the nasal fluid from the mouse of each group before and after treatment was taken and subjected to plate culture to evaluate the infection profile. Figure 5A shows no significant improvement in the blank gel group, while the other groups, particularly the ROX in-situ gel group, demonstrated improved inhibition of *Staphylococcus aureus* in the nasal cavity. H&E staining of nasal tissue revealed that treatment with the ROX in-situ gel ameliorated bacterial infection-induced nasal environment disruptions, as indicated by a restoration of normal nasal structure that is similar to that observed in the control group.

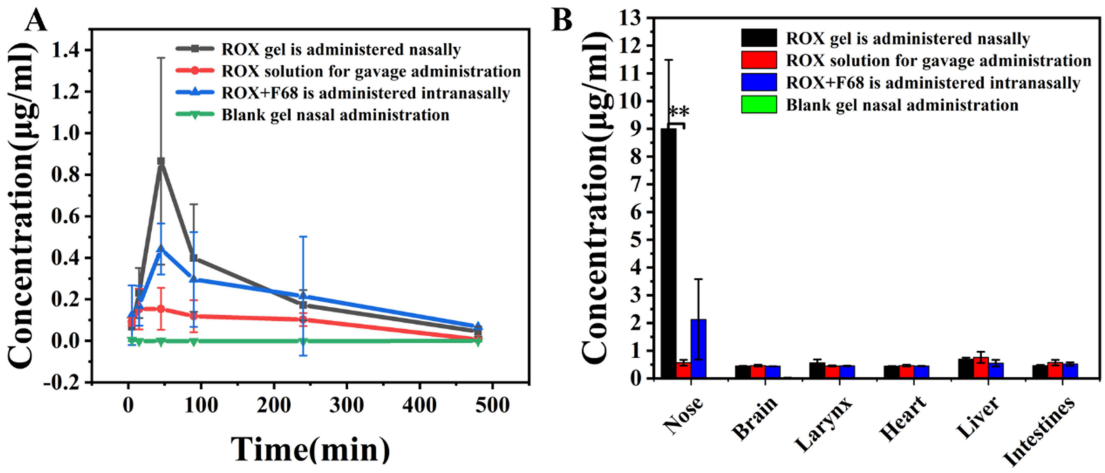

Figure 4 In vivo pharmacodynamic assay of ROX in-situ gel. (**A**) Changes of drug concentration in blood samples from different groups; (**B**) Distribution of drugs in organs samples from different group. Data are presented as mean ± standard deviation (SD) with n=3, $^{**}P < 0.01$.

H&E staining of liver and kidney sections (Figure 5B) from different groups showed no significant differences between treatment and control groups, suggesting no notable adverse effects on these organs. The heatmap of blood parameters (Figure 5C) indicated a more pronounced decrease in inflammatory markers such as white blood cells, lymphocytes, and neutrophils in the ROX in-situ gel group compared to the other treatment groups, especially the blank gel group, suggesting a positive therapeutic effect.

Discussion

The need for developing in-situ nasal drug delivery formulations of ROX for upper respiratory tract infections is significant. The nasal delivery can provide quick therapeutic action compared to oral administration, which is beneficial for time-sensitive treatments of infections like acute bacterial rhinosinusitis. Nasal delivery also bypasses hepatic first-pass metabolism and can directly target the upper respiratory tract infections, which is advantageous to increase the bioavailability while at the same time avoiding the potential side effects upon oral administration.[25,26] Therefore, in our study, a thermo-sensitive in-situ gel employing poloxamer as the matrix to dissolve ROX was designed and prepared, which is capable of transforming from solution to a gel state at body temperature, thereby allowing for in-situ delivery of ROX for effective antibacterial treatment.[27] The results in Figure 1 proved the successful preparation of the desired gel, which can respond to the body temperature for in-situ application. Moreover, the ROX can be facilely loaded within the gel in an amorphous state, which can balance the need for drug dissolution, release as well as stability.[28]

Sustained release is an advanced technology that is widely adopted in pharmaceutical science for the delivery of various drugs.[29–31] Recent advancements in in-situ gel drug delivery systems highlight the successful development and application of these technologies across various therapeutic areas for sustained release.[32,33] For instance, thermosensitive hydrogels have been effectively utilized for ocular drug delivery, demonstrating their ability to form a gel at body temperature and thereby prolonging the residence time of drugs on the eye surface.[34] Studies have also shown that nanoparticle-laden in-situ gels can enhance the efficacy of ocular treatments by providing sustained release and improving drug bioavailability.[35] In another area, intranasal administration has benefited from the formulation of in-situ gels, which improve drug absorption and pharmacokinetic profiles compared to conventional formulations. This approach has been particularly effective for enhancing brain targeting and treating central nervous system disorders.[36] Moreover, the use of in-situ gelling systems in local drug delivery for post-surgical tumor treatment has seen progress, with hydrogels offering high drug loading capacity and controlled release properties. These systems enable direct administration of therapeutics during surgery, ensuring localized action and minimizing systemic side effects.[37] The field has witnessed an increase in publications and patents related to in-situ gels, reflecting growing interest and

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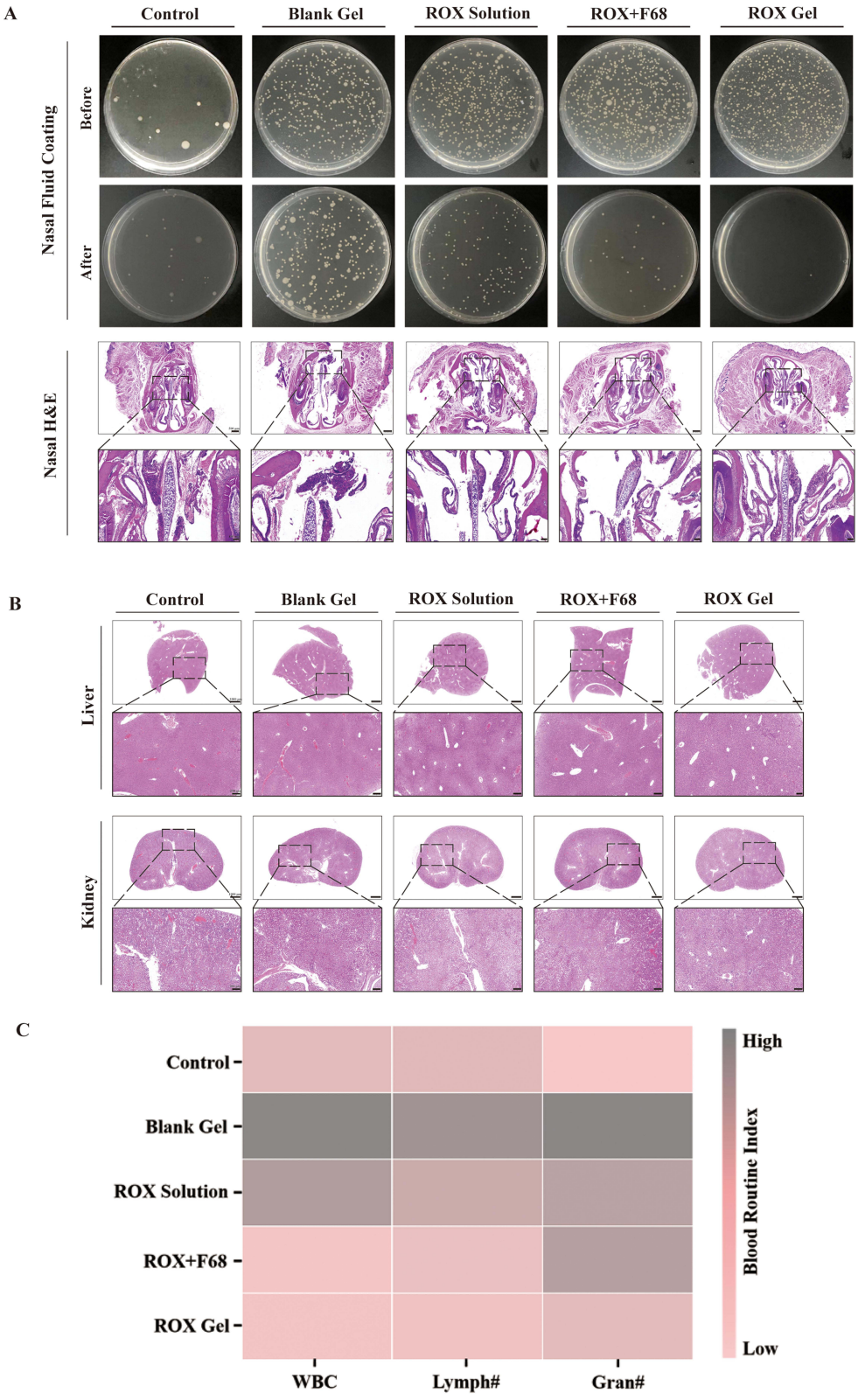

Figure 5 In vivo antibacterial and safety experiments of ROX in-situ gel. (**A**) Therapeuutic effets eveluated by colony forming (upper) and H&E staining (lower) assays. The scale bar is 500 μm for upper panel and 100 μm for lower panel. (**B**) Safety assay eveluated H&E staining of mouse liver and kidney. The scale bar is 1000 μm for upper panel and 200 μm for lower panel. (**C**) Heat map related to blood routine test after different treatments.

investment from both academia and industry. The expanding body of research indicates that in-situ gel technology is not only versatile but also capable of delivering improved therapeutic outcomes across diverse medical conditions.

Therefore, it was expected that nasal formulations with sustained release profiles can provide a controlled and gradual release of ROX, ensuring therapeutic drug levels are maintained over an extended period. This can lead to improved efficacy and reduced risk of resistance development, especially for infections that require prolonged treatment. Furthermore, the convenience and potential for self-administration associated with nasal delivery can improve patient compliance, a critical factor in the successful treatment of bacterial infections.[38,39] In the release experiment, the ROX in the formulation was isolated to that of the release medium using dialysis bag. The released ratio was calculated by determining the ROX concentration in the outer release medium. The results in Figures 2 and 3 indicate that the ROX in-situ gel provides a moderate release rate, effectively inhibiting *Staphylococcus aureus* and offering long-acting treatment for bacterial upper respiratory tract infections, which shares similar conclusions with previous reports on sustained release formulations.[40,41]

The nasal cavity is lined with a highly vascularized mucosa, which provides a rich blood supply and a large surface area for drug absorption, offering a rapid and extensive absorption pathway for drugs.[42] This method avoids degradation within the gastrointestinal tract and first-pass metabolism in the liver, which is particularly beneficial for drugs like ROX that require high tissue concentrations.[43] The pharmacokinetic characteristics of nasal delivery include rapid onset of action due to direct absorption into the bloodstream, which is advantageous for treating conditions that require swift intervention, such as respiratory infections.[44,45] These merits further ensure the suitability of ROX in-situ gel to be a candidate for the treatment of bacterial upper respiratory tract infections. Figure 4 shows that the thermo-sensitive ROX in-situ gel delivered nasally has higher bioavailability, with an 8-fold increase in local nasal concentration compared to oral administration, without increasing drug levels in non-target organs like the liver and small intestine, indicating strong targeting and retention effects and no exacerbated side effects.

From the results of Figures 2 and 4, it was suggested that temperature-sensitive gelation occurs upon nasal delivery, followed by erosion under the influence of nasal fluids. This process maintains a high local drug concentration within the nasal cavity for an extended period, with the gel disappearing after several hours. Therefore, the obtained nasal fluid from mice contains only low concentration of ROX. Moreover, we further employ centrifugation to collect the bacteria within the nasal fluid and separate them from the remaining ROX, if possible. The in vivo antibacterial experiment in Figure 5 also shares a similar conclusion to that of Figure 4. In the colony forming experiments, the ROX in-situ gel treatment significantly reduced *Staphylococcus aureus* in the nasal cavity, with nasal tissue structure closest to normal and no adverse effects on liver or kidneys, confirming its potent antibacterial efficacy and targeted delivery without burdening non-target organs, as supported by H&E staining and blood routine tests. All the above results were in line with previous reports to further confirm the advantages of nasal delivery of drugs for bacterial upper respiratory tract infections over conventional routes.[46,47]

The research encompassed the preparation and characterization, drug release determination, in vitro antibacterial effect detection, and in vivo pharmacokinetic and antibacterial assays of ROX in-situ gel. Detailed experimental analysis of the micromorphology, crystal morphology, and rheological properties of ROX in-situ gel confirmed the effectiveness and stability of the new formulation. Both in vivo and in vitro experimental results confirmed the superiority of this new formulation in treating bacterial upper respiratory tract infections, which holds the potential for enhancing therapeutic effects while reducing systemic side effects and non-specific distribution of ROX.

Conclusion

In summary, our research results demonstrate that the ROX in-situ gel offers significant advantages in treating bacterial upper respiratory tract infections. By improving the bioavailability and local drug concentration of ROX through in-situ gel preparation and local administration, our formulation effectively sustains the release of ROX, enhances its concentration in the nasal cavity, and reduces drug concentrations in the non-target liver and intestines. This approach significantly minimizes drug side effects on subjects while effectively inhibiting bacterial infections in the upper respiratory tract, showcasing the great potential and practical value of ROX in-situ gel as a treatment for bacterial upper respiratory tract infections.

Declaration of Generative AI in Scientific Writing

During the preparation of this work, the authors used Kimi AI in order to improve the readability of the article. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Disclosure

The authors report no conflicts of interest in this work.

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