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Original Article

RL-QN15 Facilitates Vaginal Mucosal Repair by Coordinating Autophagy-Apoptosis Crosstalk

Yue Jia[1,$], Yuying Pang[2,$], Ying Liu[1], Zhiling Yan[1], Youqin Ruan[1], Jinwei Liu[1], Yuye Li[1], Die Li[1], Linlin Yang[1,*], Yan Hu[1,*]

[1]Department of Gynecology, The Third Affiliated Hospital of Kunming Medical University, Yunnan Cancer Hospital, Peking University Cancer Hospital Yunnan, Kunming 650118, China

[2]Department of Infection Prevention and Control, The Third Affiliated Hospital of Kunming Medical University, Yunnan Cancer Hospital, Peking University Cancer Hospital Yunnan

$ These Authors Contributed Equally to this Work

***Corresponding Author: Prof. Linlin Yang**

Abstract:
Vaginal mucosa is a crucial protective barrier in the female reproductive tract, yet it remains susceptible to injury from infection, trauma, or estrogen deficiency, often leading to dysbiosis, chronic inflammation, and associated complications. Current therapies such as antibiotics and topical estrogen are limited by resistance and side effects, underscoring the need for new agents that restore mucosal integrity. Here, we assessed the regenerative potential of RL-QN15, an amphibian-derived pro-healing peptide, in vaginal mucosal repair. Using *in vitro* LPS-injured rat vaginal epithelial cells and an *in vivo* mucosal damage model, we found that RL-QN15 significantly promoted mucosal healing. Mechanistically, it suppressed inflammation via IL-6 downregulation, inhibited apoptosis by rebalancing Bax/Bcl-2 and reducing caspase-3 activation, and enhanced autophagy, as shown by an elevated LC3-II/LC3-I ratio and accelerated p62 degradation. Notably, RL-QN15 outperformed erythromycin in epithelial regeneration and tissue restructuring. Our study identifies RL-QN15 as a novel multi-target peptide that facilitates vaginal mucosal repair by coordinating autophagy-apoptosis crosstalk, highlighting its therapeutic potential for treating mucosal injury.
Key words: RL-QN15, vaginal mucosa damage, autophagy, apoptosis, inflammation

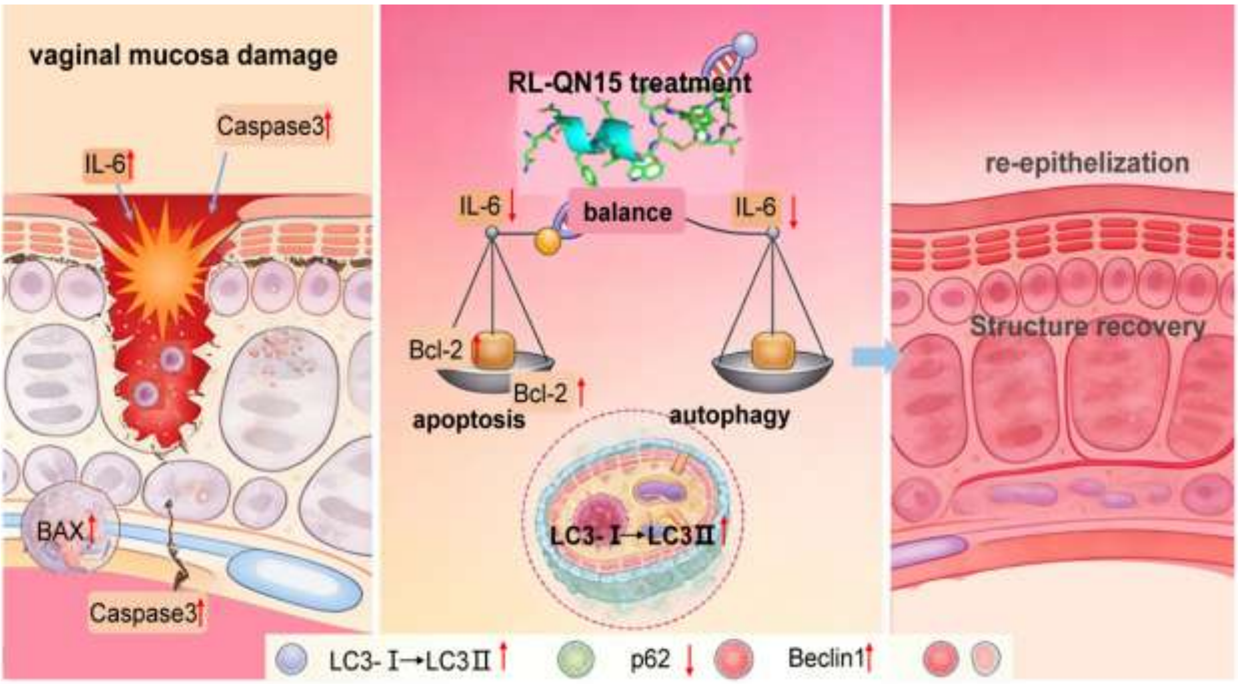
Introduction

As a critical barrier in the female reproductive system, the vaginal mucosa provides physical protection through its stratified squamous epithelium and collaborates with mucosal immune cells (including macrophages and T lymphocytes) to establish a local defense network [1, [2]. However, this barrier remains vulnerable to pathological insults including infection, mechanical trauma, iatrogenic procedures (radiotherapy/surgery), and estrogen deficiency [3, [4, [5]. This impairment induces microbiota dysbiosis, chronic inflammation, and heightened susceptibility to sexually transmitted infections (including HIV), potentially progressing to infertility or pelvic inflammatory disease [3, [5]. Consequently, vaginal mucosal repair is essential for maintaining reproductive health, infection resistance, and life quality [6]. The clinical burden remains substantial, with vaginal infections affecting approximately 40% of reproductive-age women worldwide and estrogen-deficient mucosal atrophy developing in >50% of postmenopausal women [7, [8]. Therefore, developing novel therapeutics and elucidating repair mechanisms for vaginal mucosal injury represent critical research priorities.

Current vaginal mucosal injury management relies on broad-spectrum antibiotics to prevent secondary infection and topical estrogen therapy to promote epithelial regeneration [9, [10, [11]. However, these approaches are limited by antibiotic resistance, estrogen-related risks such as endometrial hyperplasia, and failure to fully restore mucosal barrier structure and function [12, [13]. Molecular analyses reveal that repair mechanisms primarily involve three biological processes: activation of the basal stem cell niche driving epithelial regeneration [14], dynamic remodeling of the stromal extracellular matrix (ECM) to maintain structural integrity [15], and coordinated crosstalk between mucosal immunity and commensal microbiota [16]. Consequently, emerging therapeutic strategies, such as biodegradable hydrogel scaffolds providing physical support through slow-release bioactive factors, probiotics restoring microecological balance, and small-molecule compounds targeting key signaling pathways-offer promising alternatives [17]. Nevertheless, patient heterogeneity and incomplete understanding of multi-target coordination remain major clinical challenges. Elucidating mucosal repair mechanisms and developing novel therapeutics are therefore essential for advancing reproductive medicine and women's health.

Autophagy, an evolutionarily conserved lysosomal degradation pathway, maintains cellular homeostasis (including metabolic balance, inflammation regulation, and oxidative stress responses), by clearing damaged organelles, pathogens, and misfolded proteins [21, [22, [23]. Recent studies demonstrate the promising therapeutic potential of autophagy in mucosal injury repair [24, [25]. For instance, autophagy promotes gastrointestinal mucosal repair via MTOR-ULK1-mediated goblet cell differentiation and facilitates respiratory mucosal repair by inhibiting NLRP3 inflammasome activation to reduce inflammation [26]. Notably, autophagy mediates vaginal mucosal repair by facilitating tissue restoration through pathogen clearance, inflammatory microenvironment modulation via TLR4/MyD88/NF-κB signaling blockade, and epithelial regeneration involving LC3-II-mediated cytoskeletal remodeling that drives basal stem cell differentiation into functional epithelial layers [27]. In addition, reduced expression of key autophagy genes (such as ATG5 and Beclin-1) correlates with recurrent candidal vaginitis [28]. However, tissue-specific regulatory networks, systemic toxicity from non-targeted activator delivery, and uncharacterized autophagy interactions with estrogen signaling and local microbiomes present significant translational challenges [24, [29].

Amphibian bioactive peptides represent a valuable natural peptide library for tissue repair therapeutics, owing to their high bioactivity, stability, biocompatibility, and targeting potential [30, [31]. Notably, the peptide RL-QN15, isolated from *Rana limnocharis* skin secretion by our group, inhibits inflammation and promotes keratinocyte proliferation and migration through modulation of the MAPK/ERK and TGF-β/SMAD2/3 pathways, demonstrating efficacy in diabetic chronic wounds and oral ulcers [32]. This study firstly elucidated the molecular mechanism underlying RL-QN15-mediated promotion of vaginal mucosal regeneration, involving a regulatory network of autophagy, apoptosis, and inflammation. In LPS-induced rat vaginal epithelial cell injury, RL-QN15 treatment significantly suppressed IL-6 expression and enhanced proliferation. Similarly, in a rat vaginal mucosa mechanical injury model, local RL-QN15 administration markedly reduced inflammation and accelerated mucosal injury recovery. Mechanistically, RL-QN15 downregulated the anti-apoptotic protein Bcl-2, upregulated the pro-apoptotic protein Bax, and reduced Caspase-3 activity, thereby facilitating the apoptosis of necrotic tissue to promote mucosal repair. Furthermore, RL-QN15 enhanced the LC3-II/I ratio, increased Beclin-1 expression, and promoted p62 degradation, thus enhancing autophagy and subsequent mucosal tissue regeneration (Scheme 1). Collectively, RL-QN15 promotes functional vaginal mucosal regeneration by activating protective autophagy, restoring apoptosis/pro-survival balance, and remodeling the immune microenvironment, supporting its potential as a novel candidate for peptide-based, mucosa-targeted repair therapies.



Scheme 1. The novel peptide RL-QN15 as a potent promoter of vaginal mucosal repair.

2 Materials and Methods

2.1 Cell Culture

Rat Vaginal Epithelial Cells derived from Wuhan Procell System Life Sciences Co., Ltd (CP-R202). Immortalized rat vaginal epithelial cells were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS; HyClone, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin, at 37 °C in a humidified incubator with 5% CO_2.

2.2 CCK-8 Assay

CCK-8 assay was performed to test the proliferation of rat vaginal epithelial cells after the treatment of RL-QN15 in the excessive inflammatory condition induced by LPS. Cells were seeded into 96-well plates at a density of 2×10^4 cells/well. Following adherence, cells were pre-treated for 2 hours with either PBS (normal model) or LPS (injury model). Subsequently, LPS-treated cells were dealed with PBS, RL-QN15, or DEX for 24 hours. After treatment, 10 μL of CCK-8 reagent was added to each well according to the manufacturer's instructions and incubated at 37°C in the dark for 2 hours. Absorbance was then measured at 450 nm using a microplate reader, and data were analyzed using GraphPad Prism 8 software [33].

2.3 TUNEL Stain

TUNEL fluorescence staining was performed to test the apoptosis in rat vaginal epithelial cells, following previous protocols [34]. Briefly, cells were seeded at a density of 2 × 10^4 cells/well into 4-well plates containing coverslips. Following adherence, cells were treated for 2 hours with either PBS (vehicle) or LPS (injury model). Subsequently, LPS-injured cells were treated with PBS, RL-QN15, or DEX for 24 hours. After treatment, the medium was aspirated and cells were washed three times with PBS, fixed with 4% PFA for 20 min at room temperature (RT), and permeabilized with 0.1% Triton X-100 for 10 min at RT. Following three additional PBS washes, TUNEL staining was performed according to the manufacturer's instructions by incubating cells with the reagent at 37 °C for 60 min. Coverslips were then washed, counterstained with DAPI, and mounted for observation. Cells were visualized using laser scanning confocal microscopy, and fluorescence intensity was quantified using ImageJ software. Statistical analysis was performed using GraphPad Prism 8 software.

2.4 Flow Cytometry

Annexin V-FITC/PI double staining was used to assessed the apoptosis in rat vaginal epithelial cells according to previous study [35]. Rat vaginal epithelial cells were seeded in 6-well plates at 1 × 10^6 cells/well. After adherence, cells were treated with LPS (1 μg/mL) for 2 h to establish inflammatory injury models. Next, LPS-injured cells were treated with PBS, RL-QN15 (10 μM), or DEX (100 nM) for 24 h. Cells were then harvested, stained with Annexin V-FITC/PI kit, filtered through a 40-μm cell strainer. Finally, Flow cytometry analysis was performed to detect the apoptosis of rat vaginal epithelial cells following standard protocols. Apoptosis rates were calculated using FlowJo software (v10.8.1), with statistical analysis conducted in GraphPad Prism 8 software.

2.5 Rat Vaginal Mucosal Injury Model

To establish a rat model of vaginal mucosal injury, female Sprague-Dawley (SD) rats (8 weeks old, 180–220 g) were obtained from Kunming medical university and acclimatized for one week and subsequently randomized into control and model groups. All animal care and handling procedures were approved and performed in accordance with the Kunming Medical University Ethics Committee (kmmu20241183). Rats were anesthetized via intraperitoneal injection of 3% pentobarbital sodium (40 mg/kg), fixed in a supine position, and injected with lipopolysaccharide (LPS; 10 μM) into the vaginal mucosa to induce an excessive inflammatory injury model [36, [37]. On day 2 post-model establishment, treatments were administered: phosphate-buffered saline (PBS; vehicle), RL-QN15, or erythromycin injection (positive control). Following 15 consecutive days of treatment, rat serum and vaginal mucosal tissues were collected for subsequent analyses. Finally, euthanasia was performed following an intraperitoneal injection of an overdose of pentobarbital sodium (150 mg/kg).

2.6 H&E Staining

To assess regeneration in injured rat mucosal tissues, hematoxylin and eosin (H&E) staining was performed following established methods [38]. Paraffin-embedded tissue sections (5 μm thickness) were sequentially dewaxed, rehydrated through an ethanol gradient, and H&E-stained. Regeneration was evaluated by light microscopy at uniform magnification. Mucosal thickness and neo-tissue formation in pathological specimens were quantified using ImageJ software, with statistical analysis conducted in GraphPad Prism 8 software.

2.7 IF Staining

Rat vaginal epithelial cells (2 × 10^4) were seeded in 4-well chamber slides. After adherence, normal and LPS-induced injury models were established by treating cells with PBS or LPS (2 h), respectively. LPS-injured models were then treated with PBS, RL-QN15, or dexamethasone (DEX) for 24 h. Subsequently, cells were washed with PBS, fixed with 4% paraformaldehyde (PFA, 15 min, RT), and permeabilized with 0.1% Triton X-100 in PBS (10 min, RT). Non-specific binding was blocked with 2% bovine serum albumin (BSA, 1 h, RT). After PBST washes, cells were incubated with LC3B primary antibody (1:500 dilution, 4 °C overnight). Following additional PBST washes, species-appropriate fluorescent secondary antibody was applied (1 h, RT) and nuclei were counterstained with DAPI (10 min). Mounted slides were imaged by fluorescence microscopy. LC3B fluorescence intensity was quantified using ImageJ and statistically analyzed in GraphPad Prism 8 software [39].

2.8 Elisa

Rat vaginal epithelial cells (2×10^5) were seeded in 6-well plates. After adherence, normal and injury models were established by treating cells with PBS or LPS, respectively. LPS-injured cells were then treated with PBS, RL-QN15, or DEX for 24 h. Cell culture supernatants were collected, centrifuged (1 000 rpm, 4 °C, 10 min), and stored for inflammatory factor analysis. To evaluate systemic effects, whole blood was collected from rats after 7 days of topical treatment with PBS, RL-QN15, or erythromycin ointment, followed by serum isolation. IL-6 expression levels in both *in vitro* supernatants and in vivo serum samples were quantified using commercial ELISA kits according to manufacturer protocols [40].

2.9 Western Blot

To assess RL-QN15 effects on apoptosis and autophagy in inflamed rat vaginal epithelial cells, we evaluated expression of apoptotic proteins (Bcl-2, Bax, cleaved caspase-3) and autophagy markers (LC3-II/LC3-I, p62, Beclin1). Vaginal epithelial cells (1×10^6/well) were seeded in 6-well plates. Post-adherence, inflammatory injury was induced with LPS, followed by 24 h treatments with PBS, RL-QN15, or DEX. After PBS washing (4 °C), cells were lysed in RIPA buffer containing 1% PMSF and phosphatase inhibitors. Lysates were centrifuged (14 000 ×g, 20 min, 4 °C) and supernatants collected. Moreover, vaginal mucosa tissues from rats treated for 14 days with PBS, RL-QN15, or DEX were homogenized in RIPA buffer and processed identically for protein extraction. Western blot analysis: Protein concentrations were determined by BCA assay. Samples were mixed with 4 × loading buffer (1:4), denatured (95 °C, 15 min), and stored at -80 °C. Proteins were separated on 10% SDS-PAGE gels, transferred to PVDF membranes, and blocked with 5% skim milk (1 h, RT). Membranes were incubated with primary antibodies (4 °C, 12 h) against target proteins, washed with TBST, then probed with HRP-conjugated secondary antibodies (37 °C, 1 h). Signals were developed using enhanced chemiluminescence. Band intensities were quantified with Image Lab software, normalized to β-actin, and statistically analyzed in GraphPad Prism 8 software [41].

2.10 Statistics Analysis

All the data are from three independent experiments and are expressed as mean ±SEM. Statistical analyses were performed using GraphPad Prism 8 software, employing unpaired t-tests or one-way ANOVA as appropriate.

3 Results

3.1 RL-QN15 Suppressed Excessive Inflammation and Apoptosis Induced by LPS and Promoted the Proliferation of Rat Vaginal Epithelial Cells

To explore the function of RL-QN15 in vaginal mucosal injury, we detected its effects against rat Vaginal Epithelial Cell. Firstly, we detected the pro-proliferation effects of RL-QN15 under hyperinflammation condition induced by LPS (Figure 1A). LPS treatment inhibited the proliferation of rat Vaginal Epithelial cell, whereas RL-QN15 alleviated the effects of LPS and promoted the proliferation of rat Vaginal Epithelial cell (Figure 1A). As shown in Figure 1B, compared to the vehicle, LPS significantly promoted the expression of IL-6, indicating that LPS successfully induced the inflammatory injury. However, RL-QN15 suppressed the excessive inflammation induced by LPS, as evidencing by the downregulating expression of IL-6 in RL-QN15 group (Figure 1B). Excessive inflammation cause cell apoptosis, therefore we detected the vaginal epithelial cell apoptosis after treatment of RL-QN15. Compared to vehicle, the treatment of LPS in rat Vaginal Epithelial Cells induced the cell apoptosis, with the apoptosis rate reaching 20.8% (Figure 2A-B). Interestingly, RL-QN15 inhibited the apoptosis induced by LPS, which reduced the apoptosis rate from 20.8% to 12.9% (Figure 2A-B). LPS also inhibited the expression of LC3, indicating that LPS may induce excessive inflammation and inhibit the autophagy of rat Vaginal Epithelial Cell (Figure 2C-D). However, the treatment of RL-QN15 promoted the expression of LC3 and autophagy (Figure 2C-D). Moreover, Tunel staining also indicated that RL-QN15 inhibited the cell apoptosis induced by LPS (Figure 2E-F). Together, RL-QN15 inhibited excessive inflammation and apoptosis, and promoted cell proliferation, thus may exhibit excellent therapeutic effects on vaginal mucosal injuries.

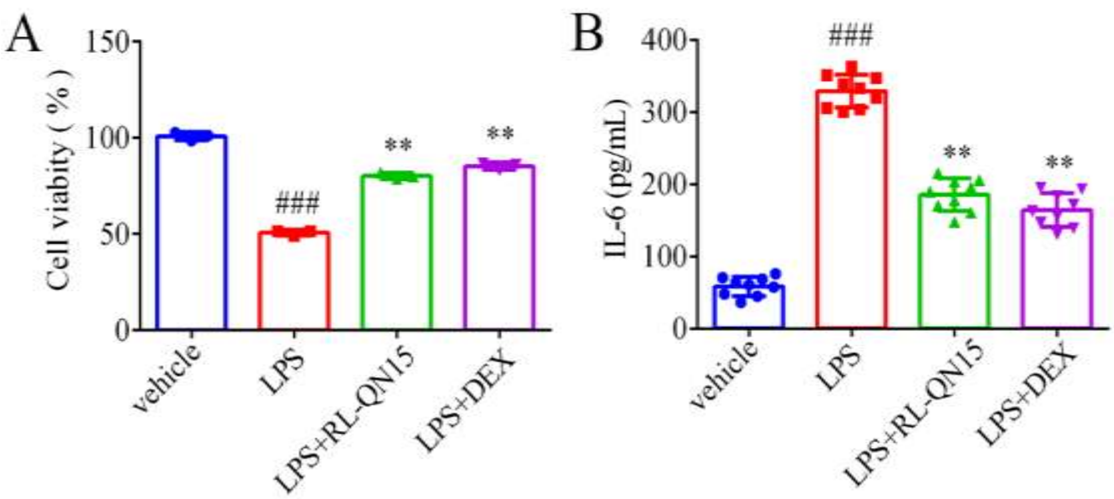


Figure 1. RL-QN15 promoted the proliferation and reduced excessive inflammation of rat vaginal epithelial cells after the treatment of LPS.

A. MTS assay revealed the pro-proliferation ability of RL-QN15 against rat vaginal epithelial cells under the inflammation condition induced by LPS. B. RL-QN15 alleviated the excessive inflammation after the treatment of LPS. $^{###}p < 0.001$ indicated the statistics significance between vehicle and LPS group. **$^{**}p$** < 0.001 displayed the statistics significance compared to LPS group. All data derived from three independent experiments.

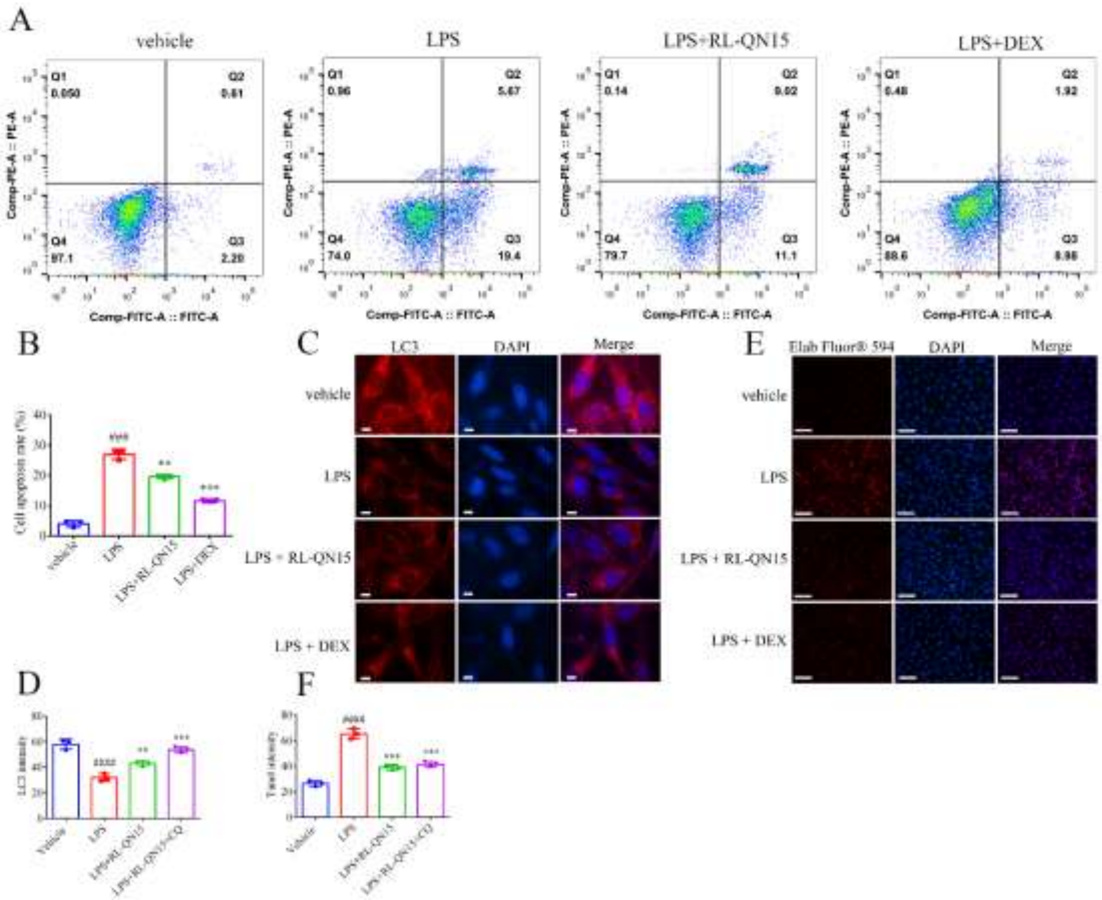


Figure 2. RL-QN15 reduced the apoptosis induced by LPS and promoted the autophagy after the treatment of LPS.

A. Flow cytometry showed that RL-QN15 significantly inhibited apoptosis induced by LPS treatment. B. Data statistics of RL-QN15 inhibiting apoptosis of rat vaginal epithelial cell. C. IF staining revealed the LC3 expression under the different treatment of PBS, LPS, LPS + QL-QN15 and LPS + DEX. D. Statistical representative diagram of LC3 fluorescence intensity. E. IF staining revealed the Tunel fluorescence intensity under the different treatment of PBS, LPS, LPS + QL-QN15 and LPS + DEX. F. Statistical representative diagram of Tunel fluorescence intensity. $^{###}p < 0.001$ and $^{####}p < 0.001$ indicated the statistics significance between vehicle and LPS group. $^{**}p < 0.001$ and $^{***}p < 0.001$ displayed the statistics significance compared to LPS group. All data derived from three independent experiments.

3.2 RL-QN15 Promoted the Regeneration of Vaginal Mucosal Injury in Rat

Given that RL-QN15 demonstrates inhibitory effects on cellular over-apoptosis and promotes autophagy and proliferation in vaginal epithelial cells, these findings suggest its potential therapeutic value for vaginal mucosal repair in rat. To evaluate its translational potential, we further investigated the efficacy of RL-QN15 in promoting vaginal mucosal regeneration using an established rat model of vaginal mucosal injury. As shown in Figure 3A-B, the model group still have defects and thicker vaginal mucosal compared to Vehicle. Interestingly, the treatment of RL-QN15 significantly promoted the recovery of vaginal mucosal and exhibited the thinner vaginal mucosal than model group (Figure 3A-B). The promoting regeneration ability of RL-QN15 against vaginal mucosal is greater than Erythromycin Ointment group, evidencing by more integral mucosal layer and a thinner vaginal mucosa (Figure 3A-B). Moreover, RL-QN15 also suppressed the expression of pro-inflammatory factor IL-6, indicating that RL-QN15 reduced the excessive inflammation in vaginal mucosal injury (Figure 3C). To sum up, RL-QN15 displayed excellent therapeutic effects on vaginal mucosal.

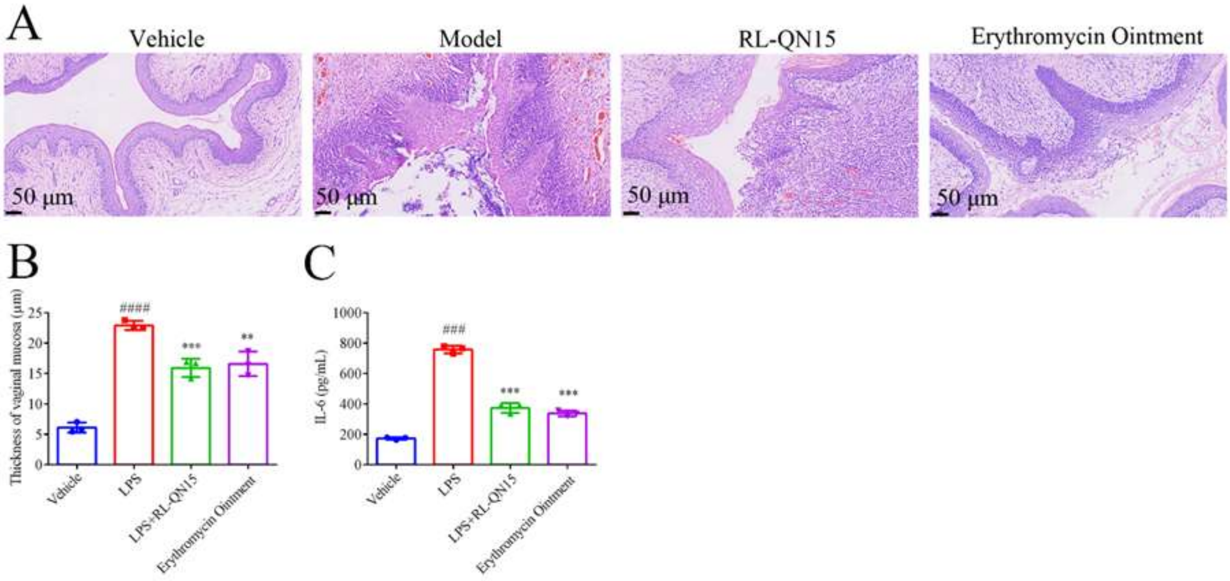


Figure 3. RL-QN15 promoted the vaginal mucosal repair after the treatment of LPS.

A. H&E staining demonstrated changes in rat vaginal mucosa architecture, comparing normal tissue, LPS-induced inflammatory injury, and the subsequent effects of treatment with PBS, RL-QN15, or Erythroycin Ointment. B. Quantification of vaginal mucosal epidermal thickness. C. ELISA assay indicated the expression of IL-6 after different treatment. $^{###}p < 0.001$ and $^{####}p < 0.001$ indicated the statistics significance between vehicle and LPS group. $^{**}p < 0.001$ and $^{***}p < 0.001$ displayed the statistics significance compared to LPS group. All data derived from three independent experiments.

3.3 RL-QN15 Inhibited Apoptosis and Promoted Autophagy to Boost the Regeneration of Vaginal Mucosal Injury

To delineate the therapeutic mechanisms of RL-QN15 in vaginal mucosal repair, we evaluated its dual regulatory effects on autophagy and apoptosis in LPS-induced injury models (Figure 4A). In rat vaginal epidermal cell model, compared to vehicle, LPS suppressed autophagic activity, evidenced by reduced Beclin1 expression (1.8-fold decrease), diminished LC3-II/LC3-I ratio, and elevated p62 accumulation (2.1-fold increase), while concurrently inducing apoptosis via caspase-3 activation (2.1-fold upregulation) and Bax overexpression (3.5-fold) alongside BCL2 downregulation (0.3-fold) (Figure 4B-G). RL-QN15 not only restored autophagic markers ， normalizing Beclin1, increasing LC3-II/LC3-I ratio, and reducing p62 by 40%，but also attenuated apoptotic signaling, suppressing caspase-3 and Bax to 0.7- and 0.6-fold of LPS-group values while elevating BCL2 by 2.0-fold, demonstrating superior efficacy to dexamethasone (Figure 4B-G).

In the *in vivo* model, LPS provoked paradoxical autophagy dysregulation, elevating Beclin1 (1.8-fold) and LC3-II/LC3-I (2.1-fold) yet impairing autophagic flux through p62 accumulation (1.5-fold) (Figure 4H-N). Interestingly, RL-QN15 resolved this dysregulation, reducing Beclin1 and LC3-II/LC3-I to 0.7- and 0.6-fold of LPS levels and suppressing p62 by 40%, indicative of restored lysosomal degradation. Simultaneously, RL-QN15 mitigated apoptosis by downregulating caspase-3 (0.5-fold) and Bax (0.6-fold) while upregulating BCL2 (1.8-fold), correlating with a 35% reduction in necrotic tissue burden (Figure 4H-N). In summary, RL-QN15 coordinates autophagy-apoptosis crosstalk by resolving autophagic stagnation through p62/LC3 axis modulation and suppressing inflammation-driven apoptosis via BCL2-mediated signaling, thereby establishing its role as a multitarget agent for inflammatory mucosal regeneration.

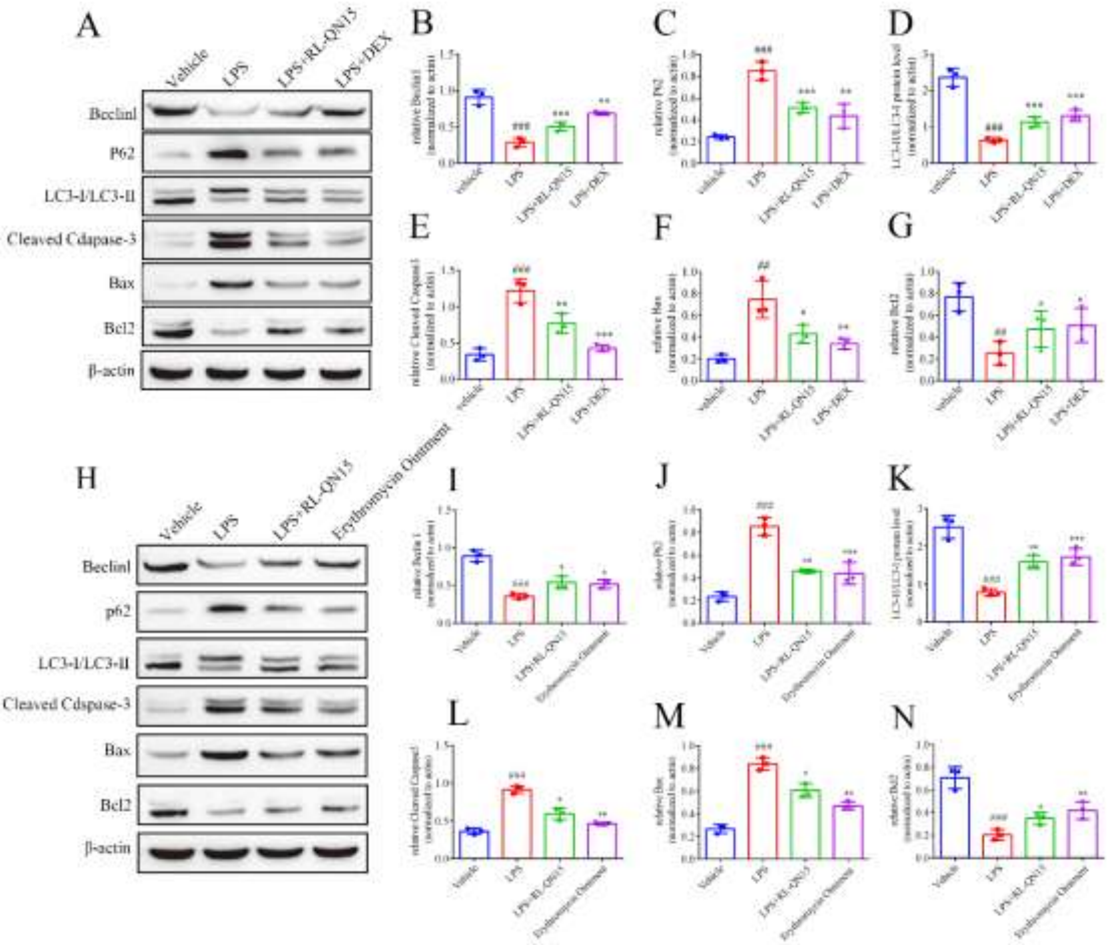


Figure 4. RL-QN15 inhibited apoptosis and promoted autophagy in cellular and animal level in the excessive inflammatory condition induced by LPS.

A. Western blot detected the expression of Beclin1, p62, LC3-I/LC3-II, cleaved caspase 3, Bax and Bcl2 after LPS-induced inflammatory injury, and the subsequent effects of treatment with PBS, RL-QN15, or DEX. B-G. Quantification the expression Beclin1, p62, cleaved caspase 3, Bax and Bcl2, as well as the ratio of LC3-I/LC3-II. H. Western blot showed the expression of Beclin1, p62, LC3-I/LC3-II, cleaved caspase 3, Bax and Bcl2 after LPS-induced inflammatory injury, and the subsequent effects of treatment with PBS, RL-QN15, or Erythroycin Ointment. I-N. Quantification the expression Beclin1, p62, cleaved caspase 3, Bax and Bcl2, as well as the ratio of LC3-I/LC3-II in vaginal mucosal repair in rat. $^{###}p < 0.001$ indicated the statistics significance between vehicle and LPS group. $^{*}p < 0.05$, $^{**}p < 0.001$ and $^{***}p < 0.001$ displayed the statistics significance compared to LPS group. All data derived from three independent experiments.

4 Discussion

Amphibian-derived pro-healing peptides represent a novel intervention strategy for chronic non-healing wounds, and peptide RL-QN15 has exhibited great therapeutic efficacy in acute and chronic skin wounds, oral ulcer mucosal injuries, and pulmonary fibrosis [32, [42]. However, its therapeutic potential for vaginal mucosal injury remained unexplored. In this study, we demonstrated that RL-QN15 exhibits potent therapeutic efficacy for vaginal mucosal injury, surpassing the clinically used erythromycin ointment by modulating autophagy, apoptosis, and inflammatory responses [43]. In conclusion, our results provide the first evidence that RL-QN15 promotes vaginal mucosal repair by mitigating inflammatory damage and enhancing tissue regeneration through autophagy-apoptosis crosstalk modulation, offering new insights into peptide regulatory mechanism and clinical development of peptide-based drugs.

Vaginal mucosa repair is influenced by multiple factors, including mechanical trauma, infection, and hormonal deficiency [44]. Persistent inflammation, suppressed angiogenesis, and microbiome dysbiosis constitute key barriers to this healing process [45]. Current therapeutic strategies mainly employ biomaterials (e.g., hyaluronic acid, collagen hydrogels) to rebuild physical barriers and maintain hydration for epithelial regeneration; growth factors (e.g., EGF, bFGF) to stimulate cellular proliferation and angiogenesis [46]; antimicrobial agents (e.g., erythromycin ointment) targeting infectious complications in atrophic vaginitis [47]; and anti-inflammatory/antioxidant compounds (e.g., vitamin E, curcumin derivatives) mitigating oxidative stress. Although existing therapies alleviate symptoms and promote repair, they exhibit limitations including insufficient long-term efficacy [20, [48, [49], significant interindividual variability, and limited efficacy against multifactorial etiologies. Consequently, developing novel therapeutic agents with superior target specificity and biocompatibility is imperative. Amphibian-derived peptides represent promising candidates for novel targeted therapeutics due to their high biocompatibility, target specificity, bioactivity, and selectivity [31, [50]. However, peptide-based therapeutics remain largely unexplored for vaginal mucosal repair. Here, we demonstrate that the amphibian-derived peptide RL-QN15 significantly suppresses excessive inflammation and promotes mucosal regeneration, thereby accelerating the repair of vaginal mucosal injury and confirming its promising therapeutic efficacy. Notably, RL-QN15 exhibits dual functionality (promoting mucosal regeneration and suppressing inflammation), whereas erythromycin is solely anti-inflammatory. In all, utilizing amphibian-derived peptides (e.g., RL-QN15) as molecular probes to elucidate their therapeutic mechanisms in vaginal mucosal repair will advance understanding of pathological molecular bases and accelerate discovery of novel therapeutic targets and clinical drug development.

The balance between autophagy and apoptosis is essential for vaginal mucosal repair [28, [51]. Under physiological conditions, moderate autophagy promotes epithelial cell survival and regeneration by clearing damaged organelles, supplying energy substrates (including ATP), and reducing oxidative stress [52, [53]. For instance, in estrogen-deficient (e.g., postmenopausal atrophy) or infection-induced injury models, autophagy enhancers (e.g., rapamycin) accelerate barrier repair by upregulating LC3-II/Beclin-1 expression, inhibiting mTOR signaling, and reducing epithelial apoptosis [54, [55]. Conversely, excessive apoptosis-observed in radiation injury or chronic inflammation-disrupts mucosal integrity through caspase-3 activation, elevated Bax/Bcl-2 ratio, and PARP cleavage, thereby impeding re-epithelialization. Our study demonstrates that RL-QN15 significantly promotes vaginal epithelial proliferation and regeneration. At cellular level, RL-QN15 alleviated LPS-induced dysregulation of autophagy (increased LC3-II/LC3-I ratio, p62 accumulation) and suppressed excessive apoptosis (caspase-3/Bax pathway). Notably, we identify RL-QN15 as the first amphibian peptide shown to mitigate inflammatory damage by modulating autophagy-apoptosis crosstalk. *In vivo* validation further confirmed RL-QN15's dual regulation of autophagy (p62/LC3 axis) and apoptosis inhibition (Bcl-2/caspase-3 pathway). These findings suggest RL-QN15 modulates ER stress or upstream kinases (e.g., AMPK/mTOR), warranting further mechanistic investigation.

Collectively, our study identifies the amphibian peptide RL-QN15 as a novel therapy for vaginal mucosal repair, advancing translational development of peptide-based interventions. We demonstrate that RL-QN15 outperforms conventional therapies (e.g., erythromycin) by mediating three regenerative mechanisms: ameliorating pathological inflammation, restoring autophagy-apoptosis balance, and enhancing epithelial proliferation. Its efficacy in mitigating LPS-induced dysregulation (autophagy impairment and caspase-3/Bax-mediated apoptosis) and *in vivo* validation of p62/LC3 axis modulation and Bcl-2/caspase-3 pathway suppression highlight RL-QN15's unique capacity to target multifactorial etiology. As the first documented amphibian peptide to modulate vaginal mucosal autophagy-apoptosis crosstalk, RL-QN15 establishes a blueprint for multitargeted biologics development while revealing novel mechanistic targets (e.g., ER stress, AMPK/mTOR kinases), thereby advancing peptide therapeutics toward clinical translation for complex mucosal pathogenesis.

Conclusion

This study demonstrates that the amphibian-derived peptide RL-QN15 effectively treats vaginal mucosal injuries in rats by mitigating LPS-induced inflammation, restoring autophagy (increased LC3-II/LC3-I, decreased p62), and inhibiting apoptosis (regulated Bax/Bcl-2, Caspase-3). Superior to erythromycin, RL-QN15 promotes mucosal regeneration, revealing a novel mechanism for peptide-based therapeutics.

CRediT Authorship Contribution Statement

Yue Jia: Writing-original draft, Methodology, Investigation. Yuying Pang: Writing-original draft, Investigation, Validatio. Ying Liu: Writing-original draft, Investigation. Zhiling Yan: Data curation. Youqin Ruan: Validatio. Jinwei Liu: Investigation. Yuye Li: Conceptualization. Die Li: Validatio. Linlin Yang: Conceptualization, Funding acquisition. Yan Hu: Writing-review and editing, Methodology, Investigation, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, Prof. Yan Hu, upon reasonable request.

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