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ANTIMICROBIAL PEPTIDE MPX ALLEVIATES THE LETHAL ATTACK OF ESCHERICHIA COLI IN MICE

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Escherichia coli is an important zoonotic pathogen causing intestinal diseases. In recent years, due to the unreasonable use of antibiotics, the drug resistance of bacteria has been increasing, and the proportion of multi-drug resistant strains has also been rising, which directly threatens the health of animals and humans. 80% of E. coli are multi-drug resistant strains, with strong resistance to aminoglycosides, sulfonamides, tetracyclines, and chloramphenicol. E. coli is extremely harmful and difficult to control. Therefore, there is an urgent need to find new antibacterial drugs that against E. coli infection and not easy to develop drug resistance. Antimicrobial peptides are a type of small molecule peptides that can resist the invasion of pathogenic microorganisms into the body. They are an important part of the innate immune system. With their small molecular weight, good water solubility, and resistance to resistance, they are considered the best alternative to antibiotics and become a research hotspot in recent years. The antimicrobial peptide MPX was isolated from wasp venom and had better antibacterial activity against both Gram-positive and Gram-negative bacteria. Studies have found that MPX had better bactericidal activity against E. coli in vitro. However, whether MPX also has better bactericidal activity in mice still unknown. In this study, the results found that E. coli infected mice loss of appetite, diarrhea, and grouping together, while MPX treatment significantly alleviated these symptoms. The results of autopsy found that the intestinal congestion, bleeding, thinning of the intestinal wall, yellow viscous fluid in the intestinal cavity, congestion of the lungs, necrosis in the liver, congestion and bleeding of the spleen, and MPX treatment effectively relieved the above symptoms. The qRT-PCR results found that MPX could increase the mRNA expression of the antibacterial protein TFF3 in the jejunum and colon, and reduce the expression of the antibacterial protein Remlβ and REG3γ in the jejunum and colon. H&E staining results further found that MPX could alleviate the pathological damage of mouse intestines and organs caused by E. coli infection. The above results show that MPX has good bactericidal activity against E. coli infection in mice, providing an important reference for the screening of drugs for the clinical treatment of E. coli infection.

Key words: *antimicrobial peptide MPX, Escherichia coli, mice.*

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Introduction. *Escherichia coli* is gram-negative bacterium and common zoonotic pathogen, which causes many human epidemics. In the United States, more than 100,000 people were infected with EHEC 0157:H7 every year (M, et al., 2019). Studies have reported that the infection of EHEC in pig intestinal tract contents and feces in central of China is high (YM, et al., 2021). The harm of *E. coli* is not only manifested in causing animal diseases and bringing huge economic losses to the breeding industry and animal husbandry, but also a reservoir of drug resistance genes for other pathogenic bacteria, and the drug resistance genes carried by food chain passed to Humans (G, et al., 2021). 80% of *E. coli* are multi-drug resistant strains, with strong resistance to aminoglycosides, sulfonamides, tetracyclines, and chloramphenicol (S, 2021). *E. coli* is extremely harmful and difficult to control. Therefore, there is an urgent need to find new antibacterial drugs that against *E. coli* infection and not easy to develop drug resistance.

Antimicrobial peptides are a type of small molecule peptides that can resist the invasion of pathogenic microorganisms into the body. They are an important part of the innate immune system. With their small molecular

weight, good water solubility, and resistance to resistance, they are considered the best alternative to antibiotics and become a research hotspot in recent years (Santos, et al., 2021). Antimicrobial peptides have various biological functions such as anti-bacterial, anti-virus, anti-parasitic, anti-inflammatory, anti-cancer, improving animal performance and immunity (Al, et al., 2021; Piyadasa, et al., 2021; Xie, et al., 2020; Gong, et al., 2021). MPX was extracted from wasp venom consisted of 14 amino acids and had 4 positive charges which had good bactericidal activity against both Gram-positive and Gram-negative bacteria (X, et al., 2021). Previous studies of our group found that MPX had good bactericidal activity against *E. coli* in vitro. Whether MPX also had good bactericidal activity in vivo still unknown.

Aim. The purpose of this study is to further explore the effect of MPX against *E. coli* infection in vivo.

Materials and methods.

Ethics Statement. BALB/c mice (18-22 g, female) were purchased from Zhengzhou University (Henan Province, China). All animal experiments were approved by the Animal Ethics Committee and were performed in accordance with the guidelines of the Animal Welfare and Research Ethics Committee.

Peptide Synthesis. Antimicrobial peptide MPX (H-INWKGIAAMAKKLL-NH2) was synthesized and purified by Ji er sheng hua (Shanghai, China) at purity greater than 98% and antimicrobial peptide MPX was very soluble in ddH_2O.

Clinical symptoms and observation of necropsy lesions. BALB/c mice were randomly divided into 4 groups, namely control group, *E. coli*, *E. coli* + MPX, *E. coli* + enrofloxacin, and the dose of *E. coli* infected BALB/c mice was $4.5x10^7$ CFU /mice, MPX (20 mg/kg) and Enro (20 mg/kg) were treated by intraperitoneal injection after infection with *E. coli* for 2 h, and treatment was continued for 3 days. Observed the clinical manifestations and necropsy of the mice after *E. coli* infection, took out the mouse lungs, liver, spleen and intestines with scissors and toothless forceps, observed the pathological changes of the mouse intestines and organs, and took pictures.

qRT-PCR. Total RNA extraction kit (Solarbio, China) was used to extract total RNA from mouse jejunum and colon. Jejunum and colon powder was slowly added to 1.5 mL EP, 200 μL chloroform was added to each well, and shaken on a shaker for 15 s, centrifuged at 12000 rpm, 4°C for 10 min, added 500 μL isopropanol and mix well, centrifuged at 12000 rpm, 4°C for 10 min, discard the supernatant, added 1 mL to each tube 75 centrifuge in % ethanol, 12000 rpm, 4°C for 5 min, added 20-30 μL of DEPC water and mix well, then measure the RNA concentration. Reverse transcription kit (Takala, Japan) was used to reverse RNA into cDNA. The primer sequences as shown in Table 1.

H&E staining. After wiping clean with alcohol cotton, the mouse organs and intestines were fixed with 4% paraformaldehyde, paraffin embedded, sectioned, and H&E stained to observe the pathology of the mouse duodenum, ileum, colon and liver, spleen, and lungs. Change, refer to the specific operation steps (He, et al., 2015).

Statistical Analysis. GraphPad Prism 5 data processing software to carry out and difference analysis of experimental results (One-Way ANOVA), P<0.05 means significant difference (marked in the text *P <0.05; **P <0.01; *** P <0.001;#P <0.05; ##P <0.01; ###P <0.001)

Results and discussion.

MPX alleviates the clinical manifestations of mice. Observation of clinical symptoms after infection of *E. coli* in mice was shown in Figure 1A and B: mice infected with *E. coli* alone showed loss of appetite, rapid heartbeat, body tremor, loose hair, bunching up, arched back, anal prolapse, feces clinical manifestations such as irregularities, while MPX treatment significantly alleviated the adverse reactions caused by *E. coli* infection. Mice increased appetite, smooth coat, and the effect was better than enrofloxacin treatment. The control group did not show any adverse reactions.

Table 1

The primer sequences for qRT-PCR

Genes	Sequence
Reg3γ	F:5'-CCCGACACTGGGCTATGAAC-3'
	R:5'-GGTACCACAGTGATTGCCTGA-3'
Relmβ	F:5'–CTGATAGTCCCAGGGAACGC-3'
	R:5'-GTCTGCCAGAAGACGTGACA-3'
TFF3	F:5' -CCTGGTTGCTGGGTCCTCTG-3'
	R:5'-GCCACGGTTGTTACACTGCTC-3'
GAPDH	F:5' -GAGAAACCTGCCAAGTATGATGAC-3'
	R:5'-TAGCCGTATTCATTGTCATACCAG-3'

Figure 1. Observation of clinical symptoms of *E. coli* infection with BALB/c mice (A, B)

MPX alleviates the pathological changes of mice by necropsy. The results of the necropsy were shown in Figure 2, the intestines of mice in the control group were normal, with thick and flexible intestinal walls, and no pathological changes were seen in the liver, spleen, and lungs. Mice infected with *E. coli* had intestinal congestion, hemorrhage, intestinal wall thinning and easy to rupture, the intestinal cavity was filled with yellow viscous liquid, the jejunum was severely congested, and the lungs, liver, and spleen were congested and bleeding. While MPX could effectively alleviate the intestinal inflammatory response and organ pathological damage caused by *E. coli* infection, and its effect was equivalent to that of the antibiotic Enro.

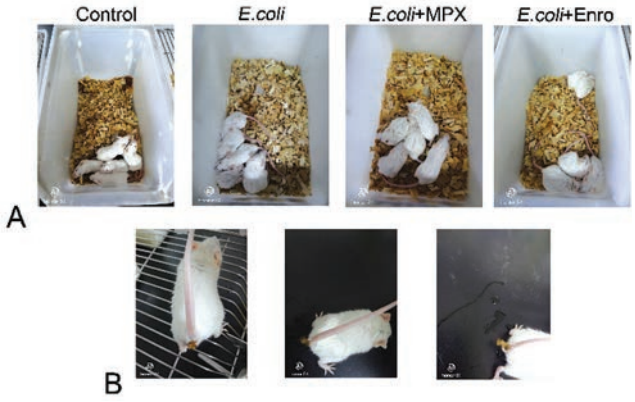

Figure 2. Autopsy results of mouse intestines and organs after *E. coli* infection

MPX increases the expression of intestinal antimicrobial peptide protein. The mRNA expression of intestinal antibacterial related proteins REG3γ, Remlβ, and TFF3 by qRT-PCR. In the jejunum (Figure 3A), compared with the control group, the TFF3 gene expression level in the jejunum of the *E. coli* group was increased (P<0.05); while the TFF3 gene expression in the jejunum of *E. coli*+MPX significantly lower than the *E. coli* group (P <0.05), and no significant difference from the control group. Compared with the control group, the mRNA expression level of Remlβin the jejunum tissue of *E. coli* infected mice was significantly increased (P<0.001). MPX significantly reduced the mRNA expression level of Remlβ, which was equivalent to the effect of Enro; while the expression level of Remlβin mouse colon was not significantly different in other group. In addition, *E. coli* infection leads to increased REG3γ expression in mouse jejunum and colon, and MPX could significantly reduce REG3γ mRNA expression caused by *E. coli* infection.

A: The mRNA expression of TFF3 in mouse jejunum and colon; B: The mRNA expression of Remlβ in mouse jejunum and colon; C: The mRNA expression of REG3γ in mouse jejunum and colon.

MPX relieves intestine pathological damage. Further H&E staining was used to observe the pathological changes of the duodenum, ileum and colon after *E. coli* infection. As shown in Figure 4, the duodenum, ileum, and colon of mice infected with *E. coli* showed intestinal villi shedding, breaking and falling into the intestinal lumen, catarrhal enteritis, degeneration, necrosis, shedding of intestinal mucosal epithelial cells, congestion of the lamina propria and a large number of neutrophil infiltration, showing the pathology of necrotizing enteritis and fibrinous necrotizing enteritis Changes (Figure 4A, B, C). While the pathological changes of each bowel segment were significantly alleviated after treatment with MPX. The intestinal villi of the control were neatly arranged without the above-mentioned pathological changes.

MPX relieves pathological damage of organs in mice. *E. coli*-infected mice developed acute interstitial pneumonia, widened alveolar septum, ruptured alveoli, neutrophil infiltration, and mild lung disease, showing local pulmonary congestion and a small amount of red blood cell and inflammatory cell infiltration (Figure 5A). Symptoms of hemorrhagic splenitis, congestion, local necrosis, small

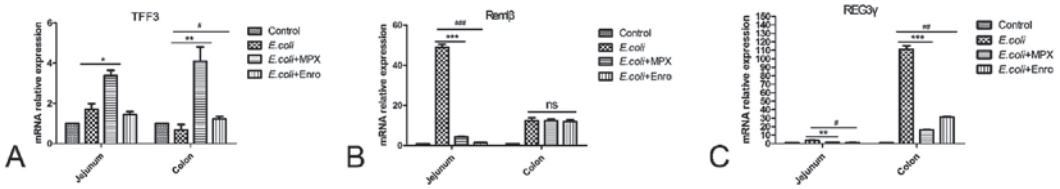

Figure 3. Them RNA expression of antibacterial protein in mouse intestine

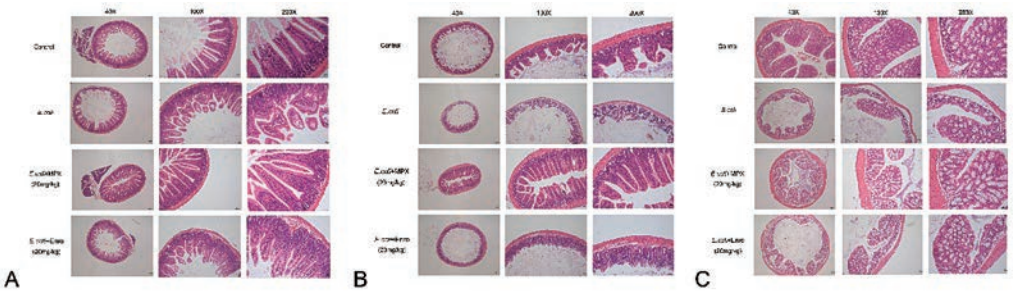

Figure 4. H&E staining of intestines after *E. coli* infection in mice. A: H&E staining of duodenum after *E. coli* infection in mice; B: H&E staining of ileum after *E. coli* infection in mice; C: H&E staining of colon after *E. coli* infection in mice

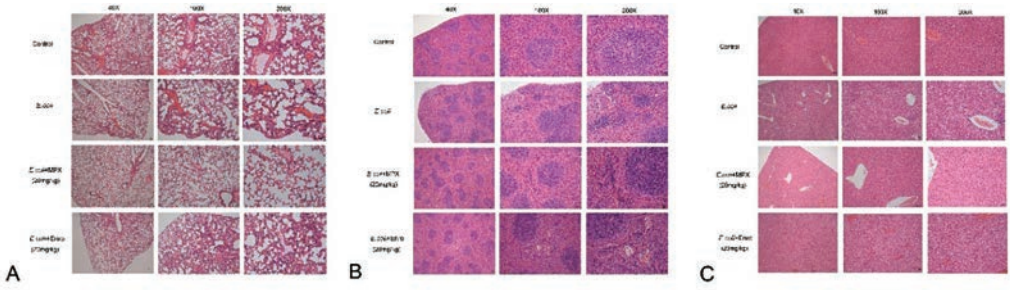

Figure 5. H&E staining of the organs infected with *E. coli* in mice. A: H&E staining of lung infected with *E. coli* in mice; B: H&E staining of spleen infected with *E. coli* in mice; C: H&E staining of liver infected with *E. coli* in mice

splenic corpuscles appear in the spleen, a large number of neutrophil infiltration in the splenic sinus (Figure 5B), degeneration and necrosis of hepatocytes, and acute necrosis in the liver, disintegration of liver cells, congestion, liver congestion, dilation of liver sinusoids, infiltration of red blood cells and neutrophils (Figure 5C). The above symptoms were significantly alleviated after treatment with MPX, indicating that MPX can protect mice against the damage of *E. coli* to the organs.

Discussion. In this study, *E. coli* was used to establish BALB/c mouse infection model, and MPX treatment to evaluate the effect against *E. coli* infection in mice. The clinical symptoms, intestinal and organ necropsy and pathological changes, and the mRNA expression of antibacterial protein in mice were evaluated. The results showed that MPX could alleviate the clinical symptoms of mice caused by *E. coli* infection, relieve the pathological changes of the intestines and organs, and increase the mRNA expression of the antimicrobial protein TFF3. This study evaluated the effect of MPX against *E. coli* in vivo, laying a foundation for the study of MPX in mice, providing a reference of drugs for the treatment of *E. coli* infection.

MPX can alleviate the intestinal damage caused by *E. coli* infection in mice. Intestine is the largest digestion and absorption organ of animal, as well as the most important immune organ of the body. Zhang et al. found that adding antimicrobial peptide plectas in to chicken diets could improve chicken performance, immune function and intestinal health, and increase the length of intestinal villi (Zhang, et al., 2021). Roque-Borda CA et al. found that the antimicrobial peptide Ctx(Ile)-Ha could effectively alleviate intestinal pathological damage (Roque-Borda, et al., 2021). Shang et al. found that the antimicrobial peptide Microcin J25 could alleviate DSS-induced intestinal inflammation and improve intestinal morphology (Shang, et al., 2021). Xiong et al. found that oral antimicrobial peptide-defensin-1 (DEFB1) could improve intestinal function and enhance intestinal barrier function (Xiong, et al., 2021). The results found that MPX could effectively reduce the intestinal damage caused by *E. coli* infection in mice.

Intestine is in direct contact with the external environment and colonizes a large number of microorganisms. Antimicrobial proteins secreted by intestinal epithelial cells play an important role in maintaining the homeostasis of intestinal epithelium and normal microbial flora (Gallo, et al., 2012; Wlodarska, et al., 2010). REG3γ is mainly expressed in the small intestine tissues of mice and humans. In addition, REG3γ also conditionally expressed when pathogen infection or inflammation occurs in the large intestine tissues (Christa, et al., 1996). Study showed that REG3y was almost not expressed in the intestinal tract of sterile mice, and the expression of REG3y was significantly increased after the normal flora was colonized (Cash, et al., 2006). The expression of RemLp is mainly regulated by Th2 cytokines, which plays an important role in the process of innate immunity and host defense (A, et al., 2017). TFF3 is produced by mucous secreting cells, which plays an important role in the function of the intestinal mucus layer and mucosal repair function (Ge, et al., 2015). In this study, the results found that MPX can increase the mRNA expression of the antimicrobial protein TFF3 in the jejunum and colon, and reduce the expression of the antimicrobial protein Remlβ and REG3γ in the jejunum and colon.

In conclusion, MPX can resist the lethal attack of *E. coli* in mice, alleviate the pathological changes of mice intestines and organs, and increase or decrease the mRNA expression of antimicrobial proteins in the jejunum or colon to varying degrees, providing important reference value for clinical drug screening of *E. coli* infection.

Author's contributions. Xueqin Zhao participated in the study design, carried out data analyses, participated and performed measurements, laboratory testing's and wrote this manuscript.

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Антимікробний пептид MPX зменшує летальний ефект Escherichia Coli у мишей

Кишкова паличка є важливим зоонозним збудником, що викликає кишкові захворювання. Останніми роками через необґрунтоване застосування антибіотиків зростає лікарська стійкість бактерій, а також зростає частка мультирезистентних штамів, що безпосередньо загрожує здоров'ю тварин і людини. 80% штамів кишкової палички мають множинну лікарську стійкість до аміноглікозидів, сульфаніламідів, тетрациклінів та хлорамфеніколу. Кишкова паличка надзвичайно шкідлива, і її важко контролювати. Тому існує нагальна потреба у пошуку нових антибактеріальних препаратів, які ефективні проти інфекції кишкової палички і до яких немає резистентності. Антимікробні пептиди – це тип пептидів, які можуть протистояти вторгненню патогенних мікроорганізмів в організм тварини і людини. Вони є важливою частиною вродженої імунної системи. Завдяки невеликій молекулярній масі, гарній розчинності у воді та стійкості до резистентності вони вважаються найкращою альтернативою антибіотикам, і останніми роками стали центром дослідження. Антимікробний пептид MPX був виділений з отрути оси і мав кращу антибактеріальну активність як проти грампозитивних, так і грамнегативних бактерій. Дослідження показали, що MPX має кращу бактеріальну активність проти E. coli in vitro. Проте чи має MPX кращу бактерицидну

активність у мишей, поки невідомо. У цьому дослідженні результати виявили, що кишкова паличка, що інфікувала мишей, викликала втрату апетиту, діарею та скупчення мишей разом, тоді як лікування MPX значно полегшило ці симптоми. Результати розтину виявили кишкову непрохідність, кровотечу, зменшення стінки кишки, жовту в'язку рідину в кишковій порожнині, застійні явища в легенях, некроз у печінці, застій і кровотечу селезінки. Застосування MPX ефективно усуває вищевказані симптоми. Результати qRT-PCR виявили, що MPX може підвищити експресію мРНК антибактеріального білка TFF3 у порожній і товстій кишці і зменшити експресію антибактеріального білка Reglβ і REG3γ в порожній і товстій кишках. Результати забарвлення H&E також показали, що MPX може полегшити патологічні пошкодження кишечника та внутрішніх органів миші, спричинені інфекцією, викликаною E. coli. Наведені вище результати показують, що MPX має хорошу бактерицидну активність проти E. coli у мишей, що є важливим для скринінгу ліків для клінічного лікування інфекції E. coli.

Ключові слова: *антимікробний пептид MPX, Escherichia coli, миші.*