



In vitro bacteriostatic effects of Polymyxin B combined with Propofol medium and long chain fat emulsion injection against *Escherichia coli*

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Background: Polymyxins is a class of cyclic polypeptide antibiotics with strong antibacterial activity against Gram-negative bacteria. However, bacteria become resistant to Polymyxins. Thus, Polymyxin B (PMB) in combination with other antimicrobials may be a better choice in clinic. This study aimed to evaluate the synergistic bacteriostatic effect of PMB combined with Propofol medium and long chain fat emulsion injection against *Escherichia coli* *in vitro*.

Methods: The Minimal Inhibitory Concentration of Polymyxin B combined with Propofol medium and long chain fat emulsion injection and two drugs used alone against *Escherichia coli* were detected with the Kirby-Bauer disk diffusion (K-B) method, and the diameter of the inhibition zone was calculated to evaluate bacteriostatic effects.

Results: Different concentrations of PMB all had obvious bacteriostatic effects on *Escherichia coli*, while different concentrations of Propofol medium and long chain fat emulsion injection had no bacteriostatic effects on *Escherichia coli*. The bacteriostatic effect of the combination of PMB with Propofol medium and long chain fat emulsion injection against *Escherichia coli* was synergistic, and no effects of uncorrelated and antagonism were observed in this combination.

Conclusions: PMB combined with Propofol medium and long chain fat emulsion injection can improve the bacteriostatic effect for *Escherichia coli* *in vitro*.

Keywords: K-B method; Polymyxin B (PMB); Propofol medium and long chain fat emulsion injection; bacteriostatic effect; *Escherichia coli*

Submitted Feb 23, 2021. Accepted for publication Apr 21, 2021.

doi: 10.21037/apm-21-751

View this article at: <http://dx.doi.org/10.21037/apm-21-751>

Introduction

Antibiotics are regarded as “miracle drugs” for the treatment of bacterial infection, but the cheaper availability of antibiotics has become common all over the world (1). Inappropriate and uncontrolled use of antibiotics finally leads to bacterial resistance, which is a major problem in the fight against life-threatening bacterial infections (1,2). *Escherichia coli* is a Gram-negative bacterium, which belongs to ESCAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter species*) group of pathogens, and causes serious infection (3). Nowadays, the growth rate of multi-drug resistance among Enterobacteriaceae is faster than previously thought, leading to a high global mortality (4) and bringing a significant medicine challenge. However, the development of new antibiotics, particularly those anti-Gram-negative bacteria agents, moves along at a slow pace. In the past decade, only dozens of new antimicrobials have been produced. Among these antimicrobial, except for tigecycline, only polymyxins still supports high antibacterial activity for multi-drug resistant (MDR) Gram-negative bacteria (5). Data (6) shows Polymyxins is a class of cyclic polypeptide antibiotics with strong antibacterial activity against Gram-negative bacteria. However, bacteria are becoming resistant to the last resort of Polymyxins, so that it limits the options of clinical treatment for serious infections (4). Thus, to effectively control serious infections caused by MDR Gram-negative bacteria, the combination of antibiotics may be a better choice. It has been demonstrated that polymyxins-based combined with several antibiotics has synergy against MDR *Acinetobacter baumannii*, MDR *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, carbapenemase-producing *K. pneumoniae et al. in vitro* (7-9). However, few studies have evaluated the activity of polymyxin B combinations against *Escherichia coli*. Polymyxin B (PMB) in combination with other antimicrobials is indeed considered a reasonable and safe treatment possibility for MDR Gram-negative pathogens (10) and endotoxin shock (11).

Polymyxins is an old drug that belongs to polypeptides, including Polymyxin A, B, C, D and E. Polymyxins exerts their bacteriostatic effect by damaging the membrane of the bacterial cell. Polymyxins is selectively targeted at the three-domain lipopolysaccharide (lipid A, oligosaccharide, and O-antigen), which is the main component of the outer membrane of Gram-negative bacteria (12,13). This lipopolysaccharide (LPS) has several negative charges with

which polymyxins can bind by electrostatic interactions. This binding competitively replaces membrane-stabilizing ions, such as Ca^{2+} and Mg^{2+} , thereby destabilizing the lipopolysaccharide layer and allowing the introduction of the polymyxins hydrophobic chain into the hydrophobic domain (lipid A). As a result, the permeability of the outer membrane increases and the polymyxins penetrates into the periplasmic space. In the periplasmic space, polymyxin forms contacts between the outer and internal membranes and promotes the fast and selective exchange of anionic phospholipids. This, in turn, causes an osmotic imbalance and ultimately the death of the bacteria (14,15). PMB, one of the clinical available polymyxins, plays an increasingly important role in clinics because it is a “last-line” of defense against Gram-negative ‘superbugs’ (16). Simultaneously, the other data (17) also show the combination of PMB with lysophosphatidylcholine (LPC) can cause the bacterial membrane porous, leading to the degradation of the folding protein regulator *dank* and finally cause the bacterial cell membrane to collapse, further resulting in bacterial death.

Propofol (2, 6-diisopropyl phenol), as a general intravenous anesthetic, is widely used in clinical practice because this highly lipophilic agent has a fast onset and short, minimal side effects and predictable duration of action due to its rapid penetration of the blood-brain barrier and distribution to the CNS (18). More than that, Propofol is a potent antioxidant, has anti-inflammatory properties. As a consequence of these properties propofol is being increasingly used in critically ill septic patients (19). Since propofol is water insoluble and has high liposolubility, it is necessary to add emulsifiers including lecithin to prepare fat emulsions for clinical application (20), then Propofol medium and long chain fat emulsion injection has arose at the historic moment. As a new type of drug-loaded fat emulsion injection, it consists of propofol, soybean oil, egg yolk lecithin and glycerol (21). Owing to partial hydrolysis of egg yolk lecithin may occur during placement, Propofol medium and long chain fat emulsion injection contains LPC and lysophosphatidylethanolamine (LPE), *et al.* (22). As noted above, the combination of PMB with LPC can finally result in MDR Gram-negative bacterial death. Therefore, a bold assumption is put forward that whether PMB combined with Propofol medium and long chain fat emulsion injection has synergistic bacteriostatic effect against *Escherichia coli in vitro*.

The purpose of this study was to observe the inhibitory effect of PMB alone or PMB combined with Propofol medium and long chain fat emulsion injection on *Escherichia*

coli and to determine whether the combination of the two drugs synergically expanded the bacteriostatic effect of a single drug to supply a certain theoretical basis for clinical rational drug use. We present the following article in accordance with the MDAR reporting checklist (available at <http://dx.doi.org/10.21037/apm-21-751>).

Methods

Test strain

The standard strain of *Escherichia coli* (ATCC25922) was derived from ATCC in the United States.

Drugs and reagents

PMB (National Medicine Permission Number H31022631) was purchased from Shanghai Shangyao First Biochemical Pharmaceutical Co., Ltd; Propofol medium and long chain fat emulsion injection (National Medicine Permission Number H20143355) was purchased from Guangdong Jiabo Pharmaceutical Co., Ltd; Phosphate Buffered Saline (PBS): 7.9 g NaCl, 0.2 g KCl, 1.44 g Na₂HPO₄, and 1.8 g K₂HPO₄ were weighed and dissolved in 800 mL distilled water, then HCl was used to adjust the pH value of the solution to 6.0, finally distilled water was added to adjust the volume to 1L; Dimethyl Sulfoxide (DMSO) was purchased from Shanghai Titan Scientific Co., Ltd.

Preparation of standard concentration of bacteria liquid

First, the standard strains of *Escherichia coli* were inoculated and cultured in a nutrient agar medium (23). Second, two colonies were inoculated in a 10-mL liquid medium; then, the processed liquid medium was incubated and shaken at 37 °C for 8 hours. Last, the harvested bacterial liquid was diluted with the liquid medium at 1:9, and the diluted bacterial liquid was inoculated as soon as possible (within 15 minutes).

Preparation of stock solution of PMB

The stock solution of PMB (50 mg/mL) was prepared with pH 6.0 of PBS.

Preparation of stock solution of Propofol medium and long chain fat emulsion injection

The stock solution of Propofol medium and long chain fat emulsion injection (10 mg/mL) was prepared with 0.1% DMSO and the pH 6.0 of PBS.

Procedures for the determination of minimum inhibitory concentration (MIC)

The circular filter paper of 6 mm was sterilized by ultraviolet for 1 hour and stored aseptically. The K-B method was finally used, referring to the related literature (24). The specific methods were as follows.

Dilution of stock solution of PMB

The stock solution of PMB (50 mg/mL) was diluted with pH 6.0 of PBS. According to the breaking point concentrations of sensitivity and drug resistance of Polymyxins that Clinical and Laboratory Standards Institute (CLSI) recommend were ≤ 2 mg/L and ≥ 4 mg/L, respectively (25), the concentrations of PMB were set. Firstly, nine 1.5 mL Eppendorf (EP) tubes were taken out, and each tube was filled with a PMB solution of 0.1 mL. One tube was taken out for later use, and others were frozen in the refrigerator at subzero 20 °C. Secondly, 900 μ L PBS was added into the spare tube to reach the concentration of 5 mg/mL. Thirdly, after dilution ratio, six new 1.5 mL EP tubes were taken out and added into different capacities of PBS successively. Lastly, the concentrations of PMB, in turn, were 1 mg/mL, 10, 5, 1, 0.5, 0.1 μ g/mL.

Dilution of stock solution of Propofol medium and long chain fat emulsion injection

The stock solution of Propofol medium and long chain fat emulsion injection (10 mg/mL) was dissolved and diluted with 0.1% DMSO and pH 6.0 of PBS. Considering LPC has membrane toxicity (26) and British Pharmacopoeia (2013) shows that the limit of LPC is not more than 2 mg/mL, so the concentration of LPC cannot too high. First, nine 1.5 mL EP tubes were taken out, and each tube was filled with Propofol medium and long chain fat emulsion injection of 1ml. One tube was taken out for later use, and others were stored in the refrigerator at 4 °C. Second, five new 1.5 mL EP tubes were taken out, then Propofol medium and long chain fat emulsion injection of 10 μ L was taken out from the spare tube, combined with 1% DMSO of 100 μ L and 890 μ L PBS to make the concentration of Propofol 100 μ g/mL. Third, the same amount of 1% DMSO of 100 μ L and different PBS capacities were added into the remaining four tubes from the dilution ratio. Last, the propofol concentrations were 50, 10, 5, 1 μ g/mL.

Specific details of the experiment

All experiments were conducted by using the K-B method. The details of one test were: First, the aseptic dried circular filter paper was soaked in different concentrations (10, 5, 1, 0.5, 0.1 μ g/mL) of PMB and different concentrations (100, 50, 10, 5, 1 μ g/mL) of Propofol

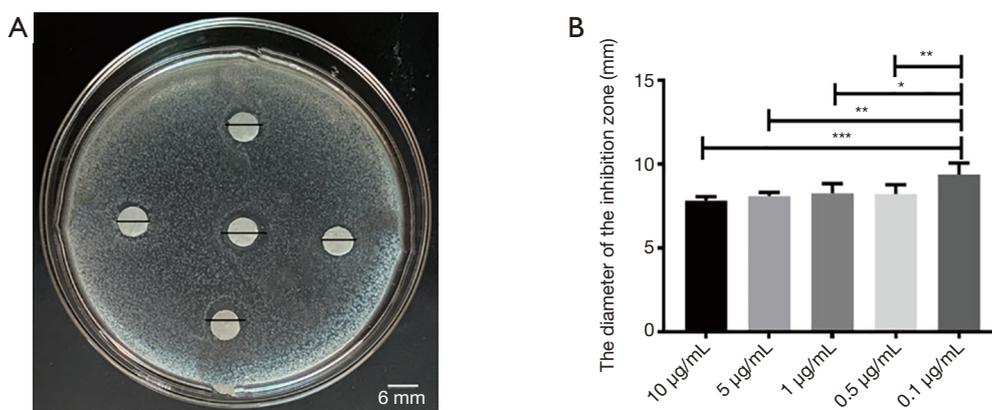


Figure 1 The bacteriostatic results of PMB. (A) Specific graphical representation of bacteriostasis of PMB: The diameter of inhibitory zone; (B) comparative analysis of the diameters of inhibitory zone produced by different concentrations of PMB. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. PMB, Polymyxin B.

medium and long chain fat emulsion injection for 1 hour. Second, the bacterial liquid was coated evenly onto glass dishes, and these circular papers were put on them. Last, these glass dishes were inverted in the refrigerator at 4 °C for 2 hours, then incubated inverted at 37 °C for 18 hours. The other test details were: the MIC of PMB, which the above tests had shown was mixed evenly with different Propofol medium and long chain fat emulsion injection concentrations. First, the aseptic dried circular filter paper was soaked in miscible liquid for 1 hour, Second, the bacterial liquid was coated evenly onto glass dishes, and these circular papers were put on them; Third, these glass dishes were inverted in the refrigerator at 4 °C for 2 hours and then inverted in an incubator at 37 °C for 18 hours. It is worth noting the bacterial liquid must be evenly coated onto each glass dish, and the tweezers must be completely burned on the alcohol lamp when the circular paper was placed each time.

The judgment of results

All glass dishes were cultured for 18 hours, and then the diameters of the inhibitory zone were measured to determine the outcomes. The lowest drug concentration with the largest inhibitory zone diameter was the MIC of PMB and PMB combined with Propofol medium and long chain fat emulsion injection. All experiments were repeated six times.

The processing and analysis of data

Using GraphPad Prism 7 for statistical analysis, the inhibitory zone's diameters with different drug concentrations in the

same group and the inhibitory zone's diameter with different drugs were evaluated by *t*-test. Data were considered statistically different at a *P* value of 0.05 or less.

Results

The bacteriostatic results of PMB

Using the K-B method to evaluate the bacteriostatic effect of PMB, the results were shown in *Figure 1*. Different concentrations of PMB all had bacteriostatic effects in *Figure 1A*. The diameter of an inhibitory zone with different PMB concentrations was evaluated by a *t*-test; it was found the differences only between the concentration (0.1 µg/mL) and others (10, 5, 1, 0.5 µg/mL) were statistically significant in *Figure 1B*.

The bacteriostatic results of Propofol medium and long chain fat emulsion injection

Using the K-B method to evaluate the bacteriostatic effect of Propofol medium and long chain fat emulsion injection, the results were shown in *Figure 2*. Different concentrations of Propofol medium and long chain fat emulsion injection did not have bacteriostatic effects in *Figure 2A*. The diameters of the inhibitory zone with different concentrations of Propofol medium and long chain fat emulsion injection were evaluated by *t*-test; it was found that all differences were not statistically significant in *Figure 2B*.

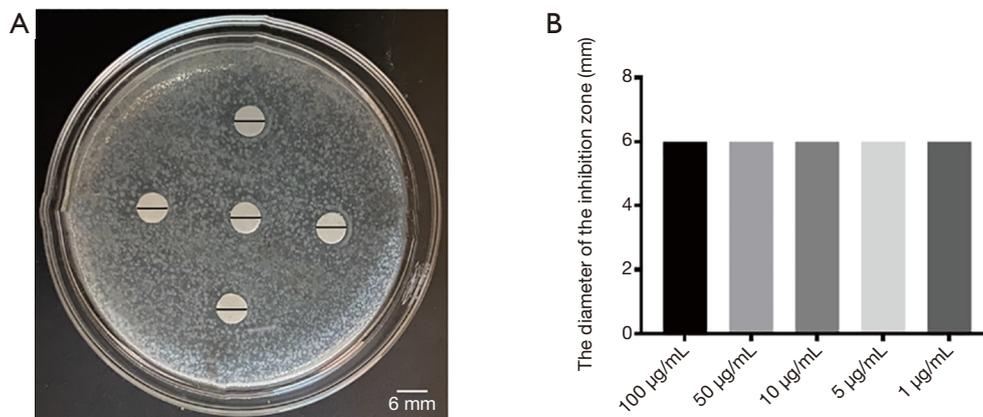


Figure 2 The bacteriostatic results of Propofol medium and long chain fat emulsion injection. (A) Specific graphical representation of bacteriostasis of Propofol medium and long chain fat emulsion injection: The diameter of inhibitory zone; (B) comparative analysis of the diameters of inhibitory zone produced by different concentrations of Propofol medium and long chain fat emulsion injection.

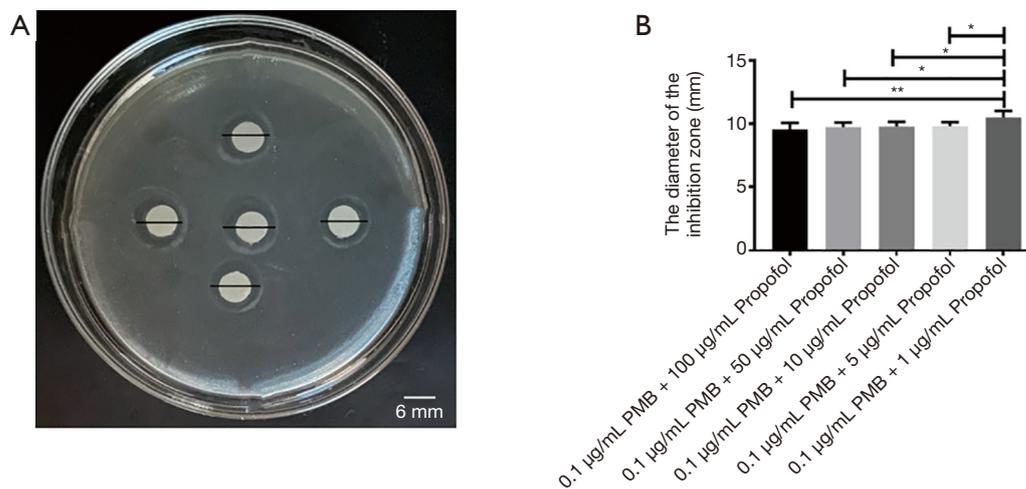


Figure 3 The bacteriostatic results of PMB (0.1 µg/mL) combined with different concentrations of Propofol. (A) Specific graphical representation of bacteriostasis of PMB (0.1 µg/mL) combined with different concentrations of Propofol: the diameter of inhibitory zone; (B) comparative analysis of the diameters of inhibitory zone produced by PMB (0.1 µg/mL) combined with different concentrations of Propofol. *, $P < 0.05$; **, $P < 0.01$. PMB, Polymyxin B; Propofol, Propofol medium and long chain fat emulsion injection.

The bacteriostatic results of PMB (0.1 µg/mL) combined with different concentrations of Propofol medium and long chain fat emulsion injection

Using the K-B method to evaluate the bacteriostatic effect of PMB combined with Propofol medium and long chain fat emulsion injection, the results were shown in *Figure 3*. The PMB (0.1 µg/mL) combined with different concentrations of Propofol medium and long chain fat emulsion injection all had bacteriostatic effects in *Figure 3A*.

The inhibitory zone diameter with the PMB (0.1 µg/mL) combined with different concentrations of Propofol medium and long chain fat emulsion injection was tested by *t*-test. It was found the difference only between the concentration [the combination of PMB (0.1 µg/mL) with Propofol (1 µg/mL)] and others [the combination of PMB (0.1 µg/mL) with Propofol (100 µg/mL), the combination of PMB (0.1 µg/mL) with Propofol (50 µg/mL), the combination of PMB (0.1 µg/mL) with Propofol (10 µg/mL), the combination of PMB (0.1 µg/mL) with Propofol

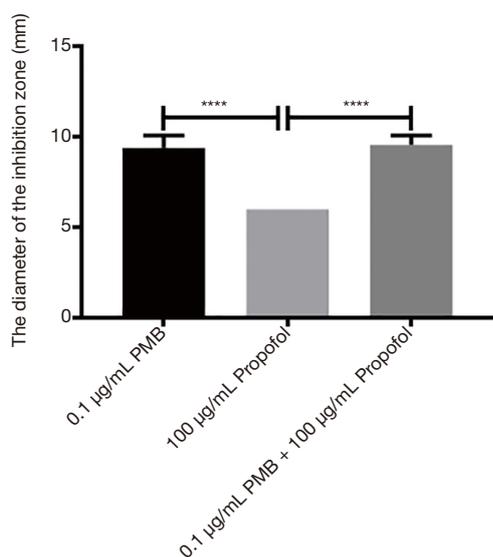


Figure 4 Comparative analysis of the diameters of inhibitory zone of PMB (0.1 µg/mL), Propofol (100 µg/mL) and the combination of two drugs. ****, $P < 0.0001$. PMB, Polymyxin B; Propofol, Propofol medium and long chain fat emulsion injection.

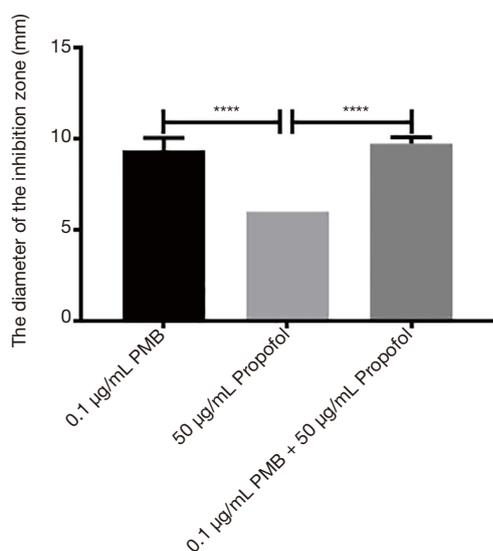


Figure 5 Comparative analysis of the diameters of inhibitory zone of PMB (0.1 µg/mL), Propofol (50 µg/mL) and the combination of two drugs. ****, $P < 0.0001$. PMB, Polymyxin B; Propofol, Propofol medium and long chain fat emulsion injection.

(5 µg/mL)] were statistically significant in *Figure 3B*.

The analysis of the diameter of the inhibitory zone of PMB (0.1 µg/mL), propofol (100 µg/mL), and the combination of two drugs

It was found the differences between propofol (100 µg/mL) and PMB (0.1 µg/mL) or the combination of PMB (0.1 µg/mL) with propofol (100 µg/mL) were statistically significant in the diameter's analysis of an inhibitory zone by *t*-test. However, the difference between PMB (0.1 µg/mL) and the combination of PMB (0.1 µg/mL) with propofol (100 µg/mL) was not statistically significant in *Figure 4*.

The analysis of the diameter of the inhibitory zone of PMB (0.1 µg/mL), propofol (50 µg/mL), and the combination of two drugs

It was found the differences between propofol (50 µg/mL) and PMB (0.1 µg/mL) or the combination of PMB (0.1 µg/mL) with propofol (50 µg/mL) were statistically significant in the diameter's analysis of an inhibitory zone by *t*-test. However, the difference between PMB (0.1 µg/mL) and the combination of PMB (0.1 µg/mL) with propofol (50 µg/mL) was not statistically significant in *Figure 5*.

The analysis of the diameters of the inhibitory zone of PMB (0.1 µg/mL), propofol (10 µg/mL), and the combination of two drugs

It was found the differences between propofol (10 µg/mL) and PMB (0.1 µg/mL) or the combination of PMB (0.1 µg/mL) with propofol (10 µg/mL) were statistically significant in the diameter's analysis of an inhibitory zone by *t*-test. However, the difference between PMB (0.1 µg/mL) and the combination of PMB (0.1 µg/mL) with propofol (10 µg/mL) was not statistically significant in *Figure 6*.

The analysis of the diameters of the inhibitory zone of PMB (0.1 µg/mL), propofol (5 µg/mL), and the combination of two drugs

It was found the differences between propofol (5 µg/mL) and

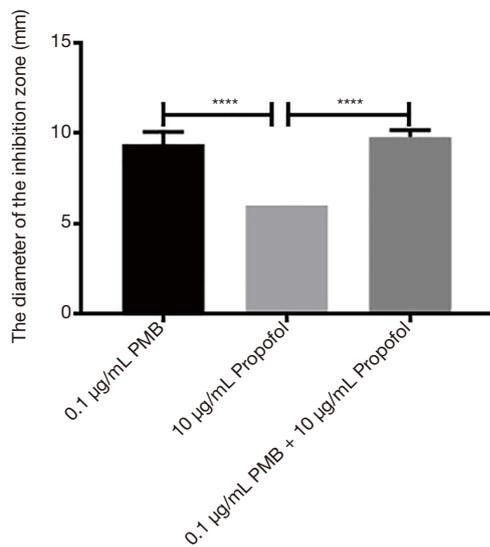


Figure 6 Comparative analysis of the diameters of inhibitory zone of PMB (0.1 µg/mL), Propofol (10 µg/mL) and the combination of two drugs. ****, $P < 0.0001$. PMB, Polymyxin B; Propofol, Propofol medium and long chain fat emulsion injection.

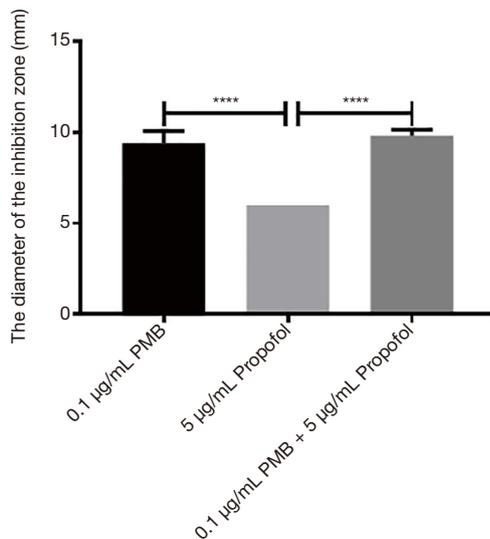


Figure 7 Comparative analysis of the diameters of inhibitory zone of PMB (0.1 µg/mL), Propofol (5 µg/mL) and the combination of two drugs. ****, $P < 0.0001$. PMB, Polymyxin B; Propofol, Propofol medium and long chain fat emulsion injection.

PMB (0.1 µg/mL) or the combination of PMB (0.1 µg/mL) with propofol (5 µg/mL) were statistically significant in the diameter's analysis of an inhibitory zone by *t*-test.

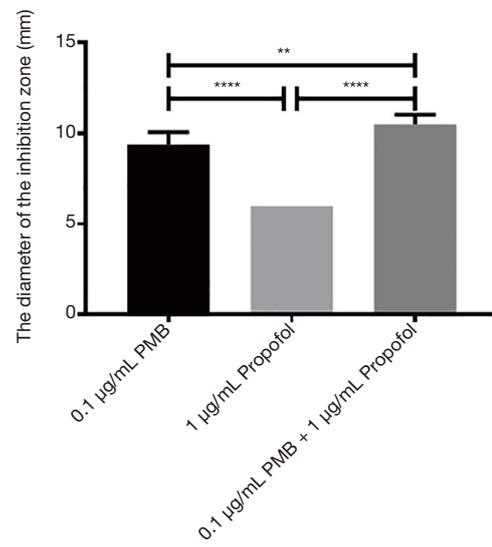


Figure 8 Comparative analysis of the diameters of inhibitory zone of PMB (0.1 µg/mL), Propofol (1 µg/mL) and the combination of two drugs. **, $P < 0.01$; ****, $P < 0.0001$. PMB, Polymyxin B; Propofol, Propofol medium and long chain fat emulsion injection.

However, the difference between PMB (0.1 µg/mL) and the combination of PMB (0.1 µg/mL) with propofol (5 µg/mL) was not statistically significant in *Figure 7*.

The analysis of the diameter of the inhibitory zone of PMB (0.1 µg/mL), propofol (1 µg/mL), and the combination of two drugs

It was found the differences between propofol (1 µg/mL) and PMB (0.1 µg/mL) or the combination of PMB (0.1 µg/mL) with propofol (1 µg/mL) were statistically significant in the diameter's analysis of an inhibitory zone by *t*-test. Meanwhile, the difference between PMB (0.1 µg/mL) and the combination of PMB (0.1 µg/mL) with propofol (1 µg/mL) was statistically significant in *Figure 8*.

Discussion

Nowadays, despite advances in emergency and critical care medicine, the number of Sepsis cases is increasing dramatically, and the incidence of Septic shock is also gradually rising. Furthermore, the mortality rate of septic shock is still at an elevated level; thus, more and more attention has been paid to the treatment of sepsis all over the world (27). It is currently considered sepsis results from a series of inflammatory mediators that cause a systemic

inflammatory response to be out of control, and endotoxin is the main factor of the septic systemic inflammatory response (28). As we all know, endotoxin is a component of the cell wall of Gram-negative bacilli (GNB) and a toxin component, constantly released in the process of bacterial reproduction. Besides its toxicity, free endotoxin is also a strong stimulating factor that can stimulate various cells to produce a series of inflammatory mediators. These inflammatory mediators can interact to form a network reaction known as “Waterfall Reaction” (29). The above reactions make the inflammatory response expand infinitely until it gets out of control, resulting in a systemic inflammatory response characterized by the body cells’ self-destruction. PMB can interact with lipid A, the outer membrane lipopolysaccharide (LPS) component of Gram-negative bacteria, neutralize its endotoxin activity, and antagonize its biological effects endotoxin (30). Besides its routine hypnotic and sedative anesthetic efficacy, Propofol medium and long chain fat emulsion injection has gradually been found to have anti-inflammatory effects. At present, it has been proved propofol can exert anti-inflammatory and cytoprotective effects by restraining many aspects of the inflammatory response, including inhibiting the release of inflammatory cytokines, increasing the expression of antioxidant proteins, preventing the aggregation of neutrophils, improving microcirculation, reducing the synthesis of NO and so on (19). Tyner *et al.* (31) found that propofol’s intravenous injection could significantly promote skin flaps’ survival in the rat model; this discovery’s possible mechanism is propofol inhibited the inflammatory response caused by neutrophils around the skin flaps. This successful experimental study suggests propofol functions, including preventing oxidation, inhibiting neutrophils’ over-activation, and restraining the inflammatory response, have clinical application value.

Due to the combination of PMB with Propofol medium and long chain fat emulsion injection is novel and brand-new, no literature has researched. In this study, we have already evaluated the synergistic bacteriostatic effect of PMB combined with Propofol medium and long chain fat emulsion injection against *Escherichia coli* *in vitro*. Then, we can continue to evaluate the synergistic bacteriostatic effect of two drugs against *Escherichia coli* *in vivo*. Also, we should continue to study and explore the application and related mechanisms of PMB combined with Propofol medium and long chain fat emulsion injection in Sepsis to supply innovative ideas for the anti-inflammatory correlation of systemic inflammatory response syndrome

(SIRS), caused by Sepsis.

Conclusions

In this study, the Minimal Inhibitory Concentration of PMB combined with Propofol medium and long chain fat emulsion injection and two drugs used alone against *Escherichia coli* were detected with K-B method, and the diameters of the inhibition zone were calculated to evaluate bacteriostatic effects. The results were: the MIC of PMB against *Escherichia coli* was 0.1 µg/mL, which had the largest diameter of the inhibitory zone, and Propofol medium and long chain fat emulsion injection does not have an obvious bacteriostatic effect on *Escherichia coli*. The MIC of PMB combined with Propofol medium and long chain fat emulsion injection against *Escherichia coli* was PMB (0.1 µg/mL) combined with Propofol medium and long chain fat emulsion injection (1 µg/mL), which had the largest diameter of the inhibitory zone. Therefore, whether PMB alone or PMB combined with Propofol medium and long chain fat emulsion injection have a suitable bacteriostatic effect on common clinical Gram-negative Enterobacillus *in vitro*. Meanwhile, it also showed the bacteriostatic effect of the combination of PMB with Propofol medium and long chain fat emulsion injection against *Escherichia coli* was synergistic, and no effect of uncorrelated and antagonism was observed in this combination. Thus, the combination of the two drugs can synergistically expand the bacteriostatic effect of a single drug. Relevant literature (22) indicates that Propofol medium and long chain fat emulsion injection contains LPC, and related data (17) shows PMB with LPC finally causes the bacterial cell membrane collapse, further resulting in bacterial death. We can consider the basic mechanism of this experiment as described above. Therefore, PMB combined with Propofol medium and long chain fat emulsion injection can treat Gram-negative Enterobacter infection in clinical practice. However, it is worth noting some reports have suggested polymyxins can damage the kidney and nervous system of the human body (32), so significant doses of long-term use should be avoided so as not to affect the basic physiological functions of the human body.

Acknowledgments

Funding: This study was supported by the National Natural Science Foundation of China Youth (81801893), the Nantong Clinical Medicine Research Center (HS2019005

and HS2020001), and the Nantong scientific projects (MS12020006 and MS12020017).

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at <http://dx.doi.org/10.21037/apm-21-751>

Data Sharing Statement: Available at <http://dx.doi.org/10.21037/apm-21-751>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/apm-21-751>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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(English Language Editor: J. Chapnick)

Cite this article as: Qi L, Wang Y, Chen B, Ren Y, Mao G, Liang G, Liu W, Zhou Y, Huang Z, Sun K, Jiang H. *In vitro* bacteriostatic effects of Polymyxin B combined with Propofol medium and long chain fat emulsion injection against *Escherichia coli*. *Ann Palliat Med* 2021;10(4):4687-4696. doi: 10.21037/apm-21-751