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The Synergistic Antibacterial Effects of Chitosan Combined with Rocephin Against Escherichia Coli

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Abstract: In this study, the synergistic antibacterial efficiency of Chitosan and rocephin against *Escherichia coli* (*E. coli*) is investigated. Diameter of inhibition zone (DIZ) measurement show the antibiotic action of Chitosan-rocephin complex was better than the superposition of pure Chitosan and rocephin. The infrared ray (IR) spectrum and ultraviolet-visible (UV-vis) absorbance spectrum indicate that Chitosan is bond with rocephin and form a stable antibacterial group to restrain the growth of *E. coli*.

Keywords: Chitosan; Rocephin; Synergistic antibacterial effects

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Introduction

Since the discovery of penicillin, β-lactam antibiotics have become the most important spectrum of antibacterial agents [1, 2]. However, the function of β-lactam will inevitably reduce because of drug resistance of pathogenic bacteria With the prevalence of microorganisms resistant to antibiotics and the continuing emphasis on health care costs, many researchers have tried to develop new, efficacious, resistance-free and cost-effective antimicrobial reagents. The multiple action antibiotics are considered as ideal candidates to meet such requirements, because synergistic action of multiple antimicrobial agents can reduce the need for high dosages level and minimize side effects [3, 4].

Chitosan, a natural nontoxic biopolymer derived by deacetylation of chitin, has been well studied for its antibacterial activity [5, 6]. Due to its biocompatibility, biodegradability, and bioactivity, Chitosan attracted a great interests in a wide range of biomaterial, pharmaceutical, and medical applications [7-9]. However, little information is available about their applications in multiple antimicrobial agents are still absent in pharmacy.

Here, we used Chitosan to enhance efficacy of rocephin, one of the most widely used antibiotic drugs. They reassembled to achieve much higher antimicrobial effects (1+1>2) than that of pure Chitosan or rocephin. It will provide a novel method to develop new antimicrobial agents.

Experimental Section

Acetic acid was diluted to a 0.02 g/mL aqueous solution and 4 g Chitosan was added to 100 mL of the above solution to form Chitosan solution. The products were bonded with rocephin through ultrasonic oscillations for 30 min and Chitosan-rocephin complex formed.

Single-cell stock suspensions of live *E. coli* BL21 were grown at 37℃ in Luria–Bertani (LB) medium for more than 12 hours and diluted with phosphate buffered solution (PBS) until the absorbency of bacteria cultures rose to 0.04 at 600 nm (A600). LB agar plates were made with four holes digging on its cross. 100ul vari-

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ous concentrations of Chitosan, rocephin and Chitosan-rocephin complex were dropped in the four holes in each LB agar plate separately and hold standing for 1 h to distribute equally. The*E. coli* culture (300 μl) was uniformly spread on as-prepared LB agar plates. The plates were incubated at 37℃ for 24 h and the DIZ were measured.

Results and Discussion

The cooperative antimicrobial activity was examined by comparing the DIZ of *E. coli* treated by various concentrations of antimicrobial agents. The DIZ reflects magnitude of susceptibility of the microorganism and evaluates the effect of antimicrobial agents. As can be seen in the Fig. 1, while the 1 mg/ml Chitosan don't show any effective antibacterial to *E. coli*, the disks impregnated with combination of 10 mg/ml rocephin+1 mg/ml Chitosan achieve a significant large DIZ, almost 40% higher than that of 10 mg/ml rocephin. Thus, compared to pure Chitosan and rocephin, the Chitosan-rocephine complex has reasonably higher cooperative antibacterial efficiency, and ability to generate "1+1>2" antimicrobial effects to *E. coli*.

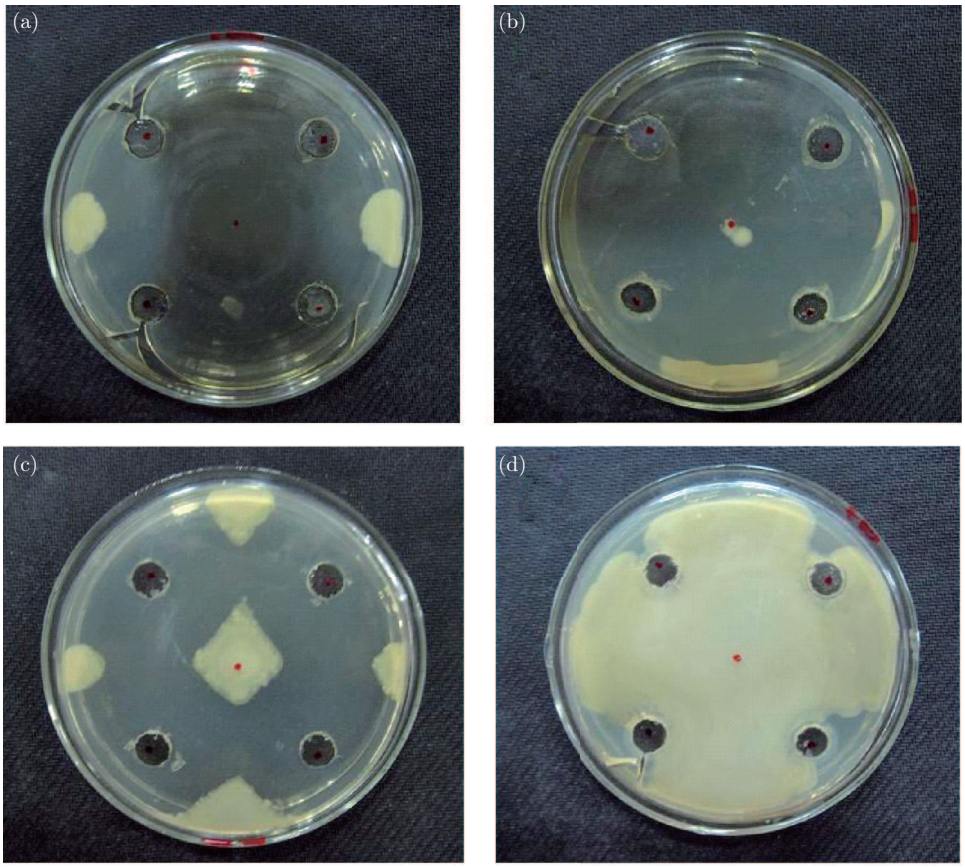

Fig. 1 The DIZ surrounding rocephin, Chitosan and Chitosan-rocephine complex impregnated disks in presence of *E. coli* (a. 20 mg/ml rocephin, b. 10 mg/ml rocephin+1mg/ml Chitosan, c 10 mg/ml rocephin, d. 1 mg/ml Chitosan).

In order to interpret the mechanism of the synergistic antibacterial effect of Chitosan with rocephin, IR and UV spectras were studied. For rocephin, peaks at 1110, 1040 and 706 cm^{-1} are assigned to stretching vibration of C–N and flexural vibrations of =CH in heteroaromatics and $–NH_2$ respectively. In addition, multi-peaks at 1670, 1590 and 1540 cm^{-1} are also found in the spectrum, associated with stretching vibration of heteroaromatics. And peaks at 1650 cm^{-1} and 1313 cm^{-1} which are stretching vibration C=O in polypeptide chain are the characteristic peaks of Chitosan. When Chitosan and rocephin are combined, these characteristic peaks are also exsit in the IR spectra. And there are a new peaks appearing at 2080 cm^{-1}. It is indicated that Chitosan bind with rocephin in the combination.

In the Fig. 3, rocephin has absorption peak at 242 nm and Chitosan has absorption peak at 267 nm. When Chitosan and rocephin are combined, a blue-shift of the peak at 242 nm is observed from spectra. A new peak is presented at 233 nm. This results show that the combination of Chitosan and rocephin is not simply mixed, and there are some reactions between them.

According to the above results, the possible mechanism of cooperative antimicrobial effects against *E. coli*

with Chitosan -rocephin complex is presented as follows: as shown in the Fig. 4, Chitosan is polysaccharide with positively charged. NH_3^+ contained by Chitosan may bind with carboxyl group in the rocephin and

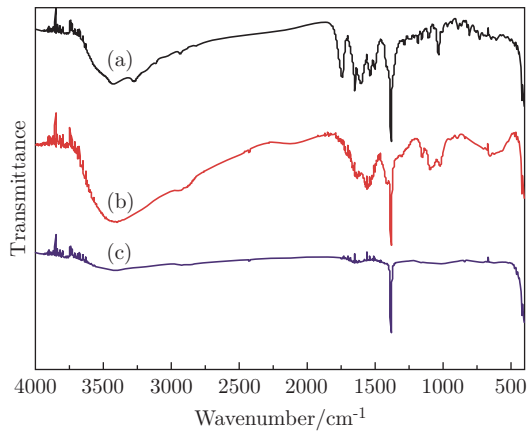

Fig. 2 IR spectra of a. Chitosan- rocephin complex, b. rocephin and c. Chitosan.

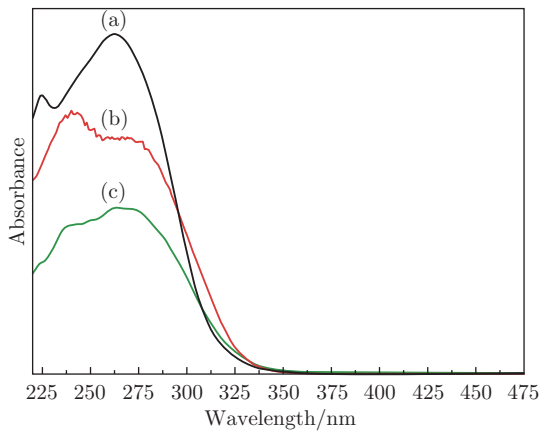

Fig. 3 UV-Vis absorbance spectra of a. Chitosan- rocephin complex, b. rocephin and c. Chitosan.

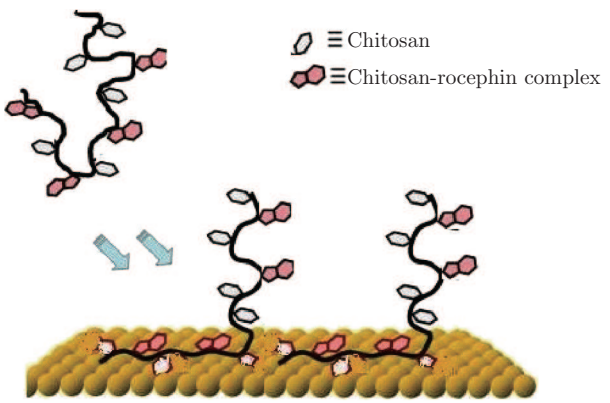

Fig. 4 Schematic diagram of cooperative antimicrobial effects against *E. coli* with Chitosan -rocephin complex.

they can form a stable antibacterial group. Meanwhile, the NH_3^+ on Chitosan can attract with negative ion on the bacterial wall, which help the antibacterial group could assemble on the bacterial will wall more easily. Rocephin could inactivate a bacterium's transpeptidase enzymes and inhibite the forming of bacterial wall. [10, 11] Thus, Chitosan-rocephin complex have higher antibacterial activity than pure rocephin and Chitosan.

Conclusion

In conclusion, the combination of Chitosan and rocephin demonstrated a significant synergistic antibacterial efficiency against *E. coli*. These results provided helpful insights to the development of antimicrobial agents.

Acknowledgements

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