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Experimental study on synergism antibacterial action of Er Yanning *in vitro*

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ABSTRACT

Objective: To observe synergistic antibacterial effect of Er yanning *in vitro*. **Methods:** The Er Yanning NO1 (the main component of rheum officinale), No2 (main component of Scutellaria baicalensis, phellodendron bark) and Er yanning NO1-NO2 compatibility antibacterial activity were detected against anaerobic bacteria and aerobic bacteria. **Results:** No 1 - anaerobic obviously, No 2 on aerobic bacteria, Er yanning 1-2 compatibility has the dual role of anaerobic bacteria and aerobic bacteria resistance, FIC index showed that additive effect on *Bacteroides fragilis* and *Escherichia coli*, respectively 83-82%, 54% of *Pseudomonas aeruginosa*, synergistic effect of 48%. **Conclusions:** Er Yanning No 1 and No 2 compatibility has dual affect in antibacterial anaerobic bacteria and aerobic bacteria

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KEYWORDS

Chronic suppurative otitis media;
Er yanning;
Aerobe anaerobe;
FIC index.

INTRODUCTION

Since 1963 Pasteur proposed the name of anaerobic bacteria, anaerobic bacteriology began to rise in this century 70 time, modern microbiology has been a significant progress, it reveals a previously unknown or known not much microbial world. With the improve of the testing means, Clinical understanding of anaerobic infection also unceasingly thorough. We have observed in chronic suppurative otitis media by anaerobic and aerobic bacteria mixed infection. So the preparation of the Er yanning were observed clinically preliminary achieved satisfactory results. In order to explore the

compatibility of rationality further, this paper carried out on the Er yanning *in vitro* synergistic antibacterial, report as follows:

MATERIALS AND METHODS

Medicines

Er yanning ear drops 1 (selection of medicinal rhubarb *Rheumofficinale* Baill solution, 1ml containing 1 g crude drug, and then diluted crude rhubarb 20 mg per ml. Er yanning ear drops 2 (no Rhubarb, *Scutellaria*, *Phellodendron* composed mainly by Decoction), provided by the Er yanning research group.

Bacteria

10 strains of *Bacteroides fragilis* (B.fragilis, B.f), 8 strains of clinical isolates, 2 strains of standard strains B.fATcc2588, B.ffmp14. 20 strains of *Escherichia coli*, 5 strains of standard strain Atcc25922, others clinical isolates. 20 strains of *Pseudomonas aeruginosa*, 6 strains of standard strain, Atcc27853, other clinical isolates.

Culture medium

Basis of anaerobic bacteria culture medium, using the CDC anaerobic bacteria, For sensitive drug aerobic bacteria by MH medium.

Bacteria liquid preparation

According to Congmin Chen of Shanghai Medical University, who compiled the book anaerobic bacteria and infection^[1]. CDC anaerobic blood lipid composition, the composition of mixed solution, cooling, adjusting PH to autoclave at 7.4, 121°C for 15 minutes, cooling to 50°C and add sterile defibrinated sheep blood or rabbit blood 500 ml, pour plate after mixing.

MIC

Using the micro dilution method

The determination of MBC

dilute the cultured bacteria to 10⁶CFU/ml. Take 23.3 ml into 4 parts. One part plus 0.2 ml culture medium as control. The other three were respectively added into the diluted Er yanning drops No 1 and No 20.2ml, make the minimum inhibitory concentration of the last 4MBC. A copy of each of 5 tubes, quickly add sterilized liquid paraffin to cooling, regular sampling in 37°C water soluble,, 0-10⁶ times dilution according to the concentration of different, 10 μ L in Petri dish, 37°C anaerobic culture for 48 hours, as the colony count

Combined drug sensitivity test

measure Er yanning No 1 and No 2 single and combined against 10 strains of B using the checkerboard method in U shaped microporous polystyrene, F and 20 strains of *Escherichia coli* and *Pseudomonas aeruginosa* antimicrobial activity, antibacterial activity to calculate FIC and FIC index.

RESULTS

1) Anaerobe *Bacteroides fragilis* in determination of

MIC:Er yanning ear drops No 1 and No 2 single on b.fragilis TABLE 1 antibacterial activity in vitro

TABLE 1 : Antibacterial activity of Er yanning ear drops No 1 and No 2 on *Bacteroides fragilis* in vitro

Medicine	Bacterial strain	Durg level (μ g/ml)		
		MIC range	MIC50	MIC90
NO 2	10	116-253	>253	>244
NO 1	10	0.26-3.9	2.0	4.0

2) Er yanning ear drops No 1 and No 2 of 40 strains of aerobic bacteria in vitro antibacterial activity TABLE 2

TABLE 2 : The antibacterial activity of the Er Yanning 1 and 2 against 40 strains of aerobic bacteria in vitro

medicine	Bacterial strain	Medicine level (μ g/ml)		
		MIC range	MIC50	MIC90
NO 2	<i>Escherichia coli</i> (20)	0.23-62	2	14
	<i>Pseudomonas aeruginosa</i> (20)	1-25	8	123
NO 1	<i>Escherichia coli</i> (20)	16-1022	126	1022
	<i>Pseudomonas aeruginosa</i> (20)	33-1022	254	510

3) Er yanning ear drops No 1 and No 2 combined respectively in 10 strains of anaerobic bacteria and 40 strains of aerobes synergistic antibacterial effect in TABLE 3

TABLE 3 : Antibacterial activity of Er yanning ear drops No 1 and No 2 combined application of in vitro

medicine	Bacteria strain	Bacteria FIC Index		
		strain	FIC	Number(%)
		≤ 0.5	0.5-2	≥ 2
M-G	<i>Bacteroides fragilis</i> (10)	2(20)	8(80)	0(0)
M-G	<i>Escherichia coli</i> (20)	1(7)	16(82)	3(11)
M-G	<i>Pseudomonas aeruginosa</i> (20)	9(48)	11(52)	0(0)

M:Er yanning NO1; G:Er yanning NO2

$$\text{FIC index} = \frac{\text{Drug A MIC value after combination}}{\text{Drug A MIC value before combination}} \div \frac{\text{Drug B MIC value after combination}}{\text{Drug B MIC value before combination}}$$

FIC index ≤ 0.5 coordination 0.5-1 :addition 1-2: independence >2: antagonism

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DISCUSSION

At present, universality and importance of clinical anaerobe infection has gradually aroused the attention of medical field at home and abroad, studies of chronic suppurative otitis media, bacterial infection of the common features of most is aerobic and anaerobic bacteria mixed infection, so we should consider both sides when we choose drug. Accordingly, our study based on the traditional Chinese medicine compound Er yanning ear drops were preliminary application and some experimental observations obtained satisfactory curative effect, but the compatibility mechanism and cooperative relationship has not been explored.

In this paper, on the basis of the previous work, experimental study, antibacterial Er yanning ear drops and the results show that, alone Er yanning ear drops 1 on anaerobic bacterium *Bacteroides fragilis* sensitive. The MIC₅₀ and MIC₉₀ were 2 µg and 4 µg/ml. And single application of ear drops 2 ineffective against *Bacteroides fragilis*, its MIC₅₀ and MIC₉₀ were ≥ 253 and ≥ 244 , but the aerobic bacteria *Escherichia coli* and *Pseudomonas aeruginosa* has better antibacterial activity, respectively MIC₅₀ and MIC₉₀ 2-14 µg/ml, MIC₅₀ and MIC₉₀ of *Pseudomonas aeruginosa* 8-123 µg/ml, Er yanning ear drops 1, almost no obvious inhibitory effect on *Escherichia coli*, the MIC₅₀ and MIC₉₀ 126-1022 µg/ml, of *Pseudomonas aeruginosa* to MIC₅₀ and MIC₉₀ 254-510 µg/ml.

Er yanning ear drops No 1 and No 2 combined results show, on *Escherichia coli* antimicrobial effect obviously enhanced, addition rate was 82%, 7% for the synergistic effect, after the union of *Pseudomonas aeruginosa* antimicrobial effects increase rate is 54%, the synergy 48%, no antagonism, observation of combined of *Bacteroides rod* bacteria addition rate 87%, synergistic effect was 20%, no antagonism.

Through the above the Er yanning ear drops and synergistic antibacterial effect of, that Er yanning ear drops reasonable compatibility, local application with chronic suppurative otitis media, which provides scientific basis for the use of Chinese medicine treatment of chronic suppurative otitis media.

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