·Clinical Research·

Pathogen characteristics and its sensitivity against antimicrobial agents in fatal bacterial granuloma after eyelid trauma *in vitro*

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Abstract

• AIM: To understand the pathogen characteristics and its sensitivity against antimicrobial agents in fatal bacterial granuloma after eyelid trauma (FBGT) *in vitro*, and to provide laboratory evidence for diagnosis.

• METHODS: The FBGT pathogens were isolaated and cultured with reformed rabbit-brain anaerobic enriched broth (RRAB), and identified by ATB/API 20A system. The minimum inhibiting concentration (MIC) was determined by anaerobic broth dilution method.

• RESULTS: A total of 22 strains of pathogen were separated from 21 patients with FBGT and identified as *Propionibacterium acnes* (PA) by ATB/API 20A system. The MIC of ciprofloxacin for 22 PA strains was 0.0625-0.5mg/L, the MIC of penicillin, ampicillin, ampicillin/sulbactam, cefoperazone, lincomycin, and imipenem/cilastatin were 0.125-0.5mg/L, the MIC of ticarcillin/clavulanic acid was 0.250-1.000mg/L, and the MIC of metronidazole was 64-256mg/L. The pathogen of FBGT was strictly anaerobic PA, which growed slowly and better in nutritious RRAB broth. All PA were resistant to metronidazole, but susceptive to other routine antimicrobial agents, such as penicillin, ampicillin and lincomycin.

• CONCLUSION: FBGT should not be treated with metronidazole. Clinicians should choose combined use of drugs or operation to treat FBGT according to patients' individual condition and the results of drug sensitivity test.

• KEYWORDS: Fatal bacteria granuloma after trauma; propionibacterium Acnes; antimicrobial agents; minimum inhibiting concentration

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INTRODUCTION

Fatal bacterial granuloma after trauma (FBGT) is a chronic fatal disease. Gao et al from the Department of Dermatology in Xijing Hospital found under the transmission electron microscope that bacterium is the pathogen of this disease, and they named this disease as a new one-FBGT [1-6]. In order to successively observe the growth characteristics of isolated FBGT pathogen and the *in vitro* antibacterial activity of commonly used antimicrobial agents, the growth characteristics of existing 22 strains of pathogen and the *in vitro* antibacterial activity of antimicrobial agents were analyzed to provide laboratory evidence for the clinical diagnosis and treatment of this disease.

MATERIALS AND METHODS

Materials From the year 2000 to present, 22 strains of *Propionibacterium acnes* (PA) have been successively isolated from 21 FBGT patients, and thereafter, the same pathogens were both isolated from one patient who was hospitalized twice with an interval of one year. The PA NCTC737 was provided by the Department of Dermatology in Xijing Hospital. Antimicrobial agents tested were Penicillin (PEN), ampicillin (AMP), ticarcillin/clavulanic acid (TIC), ampicillin/sulbactam (ACA), cefoperazone (CPZ), lincomycin (LIN), metronidazole (MET), imipenem/cilastatin (IMP) and ciprofloxacin (CIP).

The anaerobic broth and anaerobic agar mediums were purchased from UK Oxoid Limited; the reformed rabbit-brain anaerobic enriched broth (RRAB) [6]: 1) Ingredients: 3g of multi-peptone, 2g of trypticase peptone, 0.1g of hydrochloric acid of L-cysteine, 0.3g of magnesium sulfate, 0.6g of yeast extract, 0.5g of sodium chloride, 0.6g of glucose, 0.6mL of Tween-80, 0.3mL of 10g/L zinc sulfate and 2.0mL of 10g/L phytohemagglutinin (PHA).

Table 1 ***In vitro*** **sensitivity of PA against antimicrobial agents** (mg/L)

Strain	PEN	AMP	TIC	ACA	CPF	LIN	MET	IMP	CIP
1	0.125	0.125	0.500	0.250	0.250	0.125	64.000	0.125	0.250
2	0.125	0.125	0.500	0.250	0.250	0.125	128.000	0.125	0.125
3	0.125	0.125	0.500	0.250	0.250	0.125	64.000	0.125	0.063
4	0.125	0.125	0.500	0.250	0.250	0.125	64.000	0.125	0.125
5	0.125	0.125	1.000	0.500	0.250	0.125	128.000	0.500	0.250
6	0.500	0.500	0.500	0.250	0.250	0.125	256.000	0.125	0.250
7	0.250	0.125	0.500	0.500	0.250	0.125	128.000	0.250	0.125
8	0.125	0.125	0.125	0.250	0.500	0.250	256.000	0.125	0.250
9	0.125	0.250	1.000	0.125	0.125	0.250	256.000	0.125	0.500
10	0.250	0.125	0.500	0.125	0.250	0.500	128.000	0.500	0.500
11	0.250	0.250	1.000	0.125	0.125	0.125	256.000	0.250	0.250
12	0.250	0.250	1.000	0.250	0.125	0.250	128.000	0.250	0.500
13	0.500	0.125	0.250	0.125	0.125	0.250	64.000	0.250	0.250
14	0.125	0.500	0.500	0.125	0.250	0.250	128.000	0.125	0.125
15	0.125	0.125	0.500	0.250	0.250	0.500	64.000	0.125	0.500
16	0.500	0.250	0.500	0.250	0.250	0.500	128.000	0.250	0.250
17	0.250	0.250	1.000	0.500	0.125	0.125	128.000	0.250	0.250
18	0.500	0.500	1.000	0.125	0.500	0.250	256.000	0.125	0.500
19	0.500	0.250	0.500	0.500	0.250	0.250	128.000	0.250	0.250
20	0.250	0.250	1.000	0.250	0.250	0.500	256.000	0.125	0.500
21	0.250	0.500	1.000	0.250	0.500	0.500	256.000	0.125	0.250
22	0.250	0.500	1.000	0.500	0.250	0.250	256.000	0.125	0.500
23	0.500	0.250	0.000	0.250	0.250	0.250	128.000	0.250	0.500

Strain 4 and 5 are the same pathogens isolated from one patient with an interval of one year; Strain 15 is the standard strain NCTC737

100-150g of pig brain tissues were ready for use; 2) Method of preparation: after the above ingredients were dissolved in 100mL of distilled water, the fresh pig brain solution (removal of meninges, fat, blood vessels and fascia and other tissues) was added which has been filtered with 200mL of sterile saline homogenate, adjusting to pH7.2 and subpackage into the large test tube (15mL), and after high-pressure steam sterilization, it was stored at 4℃ ready for use.

Methods

Isolation and identification of pathogen After strict disinfection of affected areas of skin, the tissues under the damaged skin of the patients were taken during the surgery, and appropriate amount of physiological saline was added. The tissues were put into RRNB enriched broth medium after homogenized aseptically, and then put into a strict anaerobic environment. After 72 hours, the turbidity, color changes, and odor of the enriched broth were observed everyday. Once it was found by smear that there were small gram-positive irregular bacilli growing, the pathogenic bacteria were isolated from the anaerobic blood plate, and after obtaining the pure strains, ATB/API 20A system (bought from French Bio Merieux) was used to identify to the species. The parallel control experiment with PA's standard strain NCTC737 was conducted.

Determination of *in vitro* antibacterial activity The broth dilution method was used to determine the *in vitro* antibacterial activity of 9 kinds of commonly used antimicrobial agents against 23 strains of PA (including a standard strain), which was conducted according to the requirements in the literature [7].

RESULTS

Pathogen identification A total of 22 strains of pathogen (one patient was hospitalized twice with an interval of one year, and the same pathogen was isolated twice from him) were isolated from 21 patients successively with the RRNB medium. The pathogen isolated for the first time was unable to grow in neither solid nor ordinary broth, but grew well after cultured for 1-2 weeks in RRNB, and after the successful isolation and culture, they grew on anaerobic blood agar plates (needing 3-4 days). The bacteria were identified as PA by ATB/API 20A system, with the identification probability of more than 99% by conducting the parallel experiment with PA's standard strain NCTC737. The results and identification probability of biochemical reactions were basically consistent.

Sensitivity to antimicrobial gents of the pathogen The test results of *in vitro* sensitivity to 9 kinds of antimicrobial agents was different (Table 1) and their MIC values were different too (Table 2).

DISCUSSION

FBGT has been basically established as an independent disease [6,8-10], and it has the following features: 1) More than half of the patients are children; 2) The eyelid, face and forehead had trauma before; 3) The skin has progressive patches without ulceration; 4) New patches may appear near

Table 2 MIC of antimicrobial agents against 22 strains of PA (mg/L)

Agent	MIC range	MIC_{50}	MIC_{90}
PEN	0.125-0.500	0.125	0.500
AMP	0.125-0.500	0.125	0.500
TIC	0.250-1.000	0.250	1.000
ACA	0.125-0.500	0.250	0.500
CPF	0.125-0.500	0.125	0.500
LIN	0.125-0.500	0.125	0.500
MET	64.000-256.000	128.000	256.000
IMP	0.125-0.500	0.125	0.2500
CIP	0.0625-0.500	0.250	0.500

or away from the skin damage; 5) Pathology indicates the inflammatory granuloma; 6) The patients suffer disturbance of consciousness and severe headache in the late stage, but without significant meningeal irritation signs; 7) Almost all the patients died within 1.5-4 years; 8) Glucocorticoid can make the skin damage temporarily shrink, but it significantly accelerates death[3-5].

Fatal features of pathogens: 1) The post-traumatic skin damages occur in the eyelid and face, and all the patients died of intracranial secondary infection [4]; 2) The electron microscopy can confirm the existence of the bacilli [1,2] in cells in the damaged skin tissues; 3) This pathogen has high nutritional requirement and is lipophilic. It grows well in RRNB enriched broth (for 1-2 weeks), while does not grow in ordinary anaerobic broth and glucose broth; 4) It requires strict anaerobic environment, and grows extremely slow in initial culture, so it should be strictly aseptically operated to avoid contamination, otherwise its growth will be very difficult.

The 23 strains are resistant to metronidazole, so metronidazole should not be used clinically to treat FBGT patients. The *in vitro* antibacterial sensitivity to ciprofloxacin is the highest, and those with the second highest sensitivity are penicillin, ampicillin, ampicillin/ sulbactam, lincomycin, cefoperazone and imipenem/ cilastatin, while the one with the third highest activity is ticarcillin/clavulanic acid. As this pathogen needs to be treated with long-term application of antimicrobial agents, in order to prevent drug resistance, clinicians can take the MIC testing as a reference to reasonably choose combined use of different types of antimicrobial agents. Curing of this disease needs to emphasize early diagnosis and treatment, and when necessary, to invite Department of Surgery to assist the surgical treatment, especially that the limited damages need to timely remove the focus through surgery; it may also consider to use the agents which can improve the patients' immunity, such as α interferon *etc*.

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