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Full Length Research Paper

Investigation of different infectious pathways used by *Streptococcus suis* type 2 and other bacteria in mice

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The purpose of this study was to investigate the infectious pathways used by *Streptococcus suis* type 2 and enterohemorrhagic *Escherchia coli* O26 (EHEC O26) in mice. Six-week mice were intraperitoneally injected with mitomycin (0.2 mg/mouse) to decrease the protective immunity. The mice were then challenged with *S. suis* type 2 and EHEC O26 strains through wounded skin, stomach lavaging and intraperitoneal injection. *Enterococcus faecalis* ATCC 29212 and *E. coli* ATCC25922 were used as negative controls. Clinical and microbiological examinations were performed for all the infected mice routinely. The mortality rate of the mice infected with *S. suis* type 2 strain through wounded skin was 60% within 2 - 5 days. The mortality rate of the mice infected with *S. suis* type 2 strain through stomach lavaging was 60% within 7 - 10 days. *S. suis* type 2 can be isolated from the puncturing fluid of heart in the dead mice. Mortality was not observed in mice infected by *S. suis* type 2 and *E. faecalis* ATCC 29212 strains through intraperitoneal injection. However, intraperitoneal injection with EHEC O26 and *E. coli* ATCC25922 caused death of mice within 10 h. We demonstrated an important pathway used by *S. suis* type 2 strain in mouse infection model. Intraperitoneal injection with gram negative bacteria (*E. coli* ATCC25922 and EHEC O26) caused non-specific death in mice. These results provided fundamental bases for investigating the pathogenesis of *S. suis* type 2, designing vaccine and evaluating the efficiency of antimicrobial drugs.

Key words: *Streptococcus suis* type 2, enterohemorrhagic *Escherchia coil* O26, model, mouse.

INTRODUCTION

Streptococcus suis is a zoonotic bacterium causing acute and thermal infectious disease (Higgins et al., 1995). Based on the Epidemic Prevention Law of the Peoples Republic of China, *S. suis* disease is listed as National Class II infectious disease. *S. suis* can cause porcine septicemia, meningitis, arthritis and bronchitis. *S. suis* can also cause septicemia, endocarditis, meningitis or even death in particular population of humans. In the summer of 1998, outbreak of *S. suis* disease happened in Nantong and Taixing of Jiangsu Province in China, leading to the death of thousands of pigs, 25 human infection cases and 14 human mortalities (Zhu et al., 2001). In 2005, Sichuan province reported a total of 204 human cases infected with *S. suis* type 2 (38 mortalities) and 647 pig mortalities (Liu et al., 2005).

Recently, enterohemorrhagic *Escherchia coli* (EHEC) became another severe enteric pathogen. In many countries, *E. coli* O157:H7, *E. coli* O111 and *E. coli* O26 are the main pathogenic bacteria causing epidemics. The diseases caused by these pathogens include hemorrhagic colitis, haemolytic-uraemic syndrome, thrombotic thrombocytopenic purpura or even death (Riley et al., 1983). We isolated *S. suis* type 2 from the blood of one hospitalized patient in July 2005. We also detected strong toxin-producing EHEC O26 from the enteric canal of the same patient. This was the first reported case infected with both EHEC O26 and *S. suis* type 2 in China (Cao et al., 2006). In order to investigate the role of these two bacteria in the pathogenesis, we performed mouse challenge experiment using *S. suis* type 2 and EHEC O26 strains. *Enterococcus faecalis* ATCC 29212 and *E. coli* ATCC25922 were used as

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Table 1. Mortality of the mouse infected with *S. suis* type 2 strain.

Bacterial challenge route	Number of mouse	Number of mouse that died at different days after infection						Number of death
		2	3	5	7	8	10	
Intraperitoneal injection	10	0	0	0	0	0	0	0
Stomach lavaging	10	0	0	0	2	3	1	6
Wounded skin	10	2	2	2	0	0	0	6

negative controls in the animal experiment.

MATERIALS AND METHODS

Bacterial strains

S. suis type 2 strain was isolated previously and stored in our laboratory (Cao et al., 2006). The serotype of the strain was determined to be D group. Through automatic rapid ID32 STREP analysis, this isolate was determined to be *S. suis* type 2. The main biochemical reaction for this strain was -glucosidase (+), methyl-B-D-glucopyranoside (+), -galactosidase (+) and raffinose (+). EHEC O26 was isolated and stored in our laboratory (Gui, 2005). *E. faecalis* ATCC 29212 and *E. coli* ATCC25922 was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

Animals and grouping

Clean level ICR male mice (6-week old) were purchased from Animal Center of Nantong University with a License NO. SCXK (Su 2003-0002). The mice were divided into three groups with 10 animals in each group. Each group of mice was challenged with *S. suis* type 2 strain through stomach lavaging, intraperitoneal injection and wounded skin, respectively. Similar grouping and challenge were performed for EHEC O26, *E. faecalis* ATCC 29212 and *E. coli* ATCC25922 strains. All the mice were intraperitoneally injected with 0.5 ml of mitomycin (0.5 mg/ml) before bacterial challenge. The mice were fed once in every morning and were provided with sufficient water after infection.

Bacterial growth medium and reagents

Columbia blood agar base was used to grow *S. suis* type 2, which displayed small colonies with incomplete hemolysis. EHEC O26 displayed gray, wet and medium-sized colonies on this medium. Mitomycin was purchased from Kyowa Hakko Kirin Co., Ltd (Japan).

Preparation of bacterial suspension

S. suis type 2 was inoculated into Columbia blood agar base and grown in the condition of 35°C and 5% CO_2 for 18 - 24 h. Sterile cotton swab dipped with normal saline was used to wash off the bacteria from the plate. Nine Maxwell units of bacterial suspension were prepared by adding 0.1 ml of the original suspension into 3 ml of normal saline. OD_{600} was measured in a spectrophotometer. EHEC O26, *E. faecalis* ATCC 29212 and *E. coli* ATCC25922 were prepared in a similar way.

Routes and dosage of the bacteria infection

Bacterial suspension (0.3 ml) was used to challenge the mice through stomach lavaging. For intraperitoneal injection, 0.5 ml of bacterial suspension was used. For the infection through wounded skin, the tail of the mice was cut with a scalpel to produce a 1 cm-wide incision. Sterile cotton swab was dipped in the bacterial suspension and daubing on the wounded skin for 1 min.

Clinical and microbiological examinations

The mental behavior, appetite and physical signs of the mice were observed every day. Necropsy as well as microbiological examination was performed once the mouse was dead. The undead mice were sacrificed for microbiological examinations after 21 days of bacterial challenge.

RESULTS

Clinical observations

From the second day of *S. suis* type 2 infection through wounded skin or seventh day of the infection through stomach lavaging, part of the mice exhibited narcoma, anorexia, fluffy hair, followed by quivering, orbiting, limping, quadriplegia, lying rigidly and death. Morbidity occurred in all the ten mice infected with *S. suis* type 2 strain through wounded skin and 6 mice died after 2 - 5 days of infection (Table 1). Morbidity occurred in 8 mice infected with *S. suis* type 2 strain through stomach lavaging and 6 mice died after 7 - 10 days of infection (Table 1). No significant changes were observed in the mice infected with *S. suis* type 2 strain or *E. faecalis* ATCC 29212 through intraperitoneal injection (Table 1 and 2). In contrast, all the 10 mice infected with EHEC O26 or *E. coli* ATCC25922 through intraperitoneal injection died within 10 h (Table 2).

Microbiological examinations

A total of 12 mice were dead after infection with *S. suis* type 2 strain. *S. suis* type 2 was isolated from the heart puncturing fluid of the dead mice. However, the challenging bacteria were not isolated from the undead mice or the mice challenged with control bacteria.

Table 2. Mortality of the mouse infected with EHEC O26, E. *coli* ATCC25922and *E. faecalis* ATCC 29212 strain.

Kind of bacteria	Number of mouse	Number of mouse that died at different hours after infection					Number of death
		2	4	6	8	10	
EHEC O26	10	0	1	2	5	2	10
E. coli ATCC25922	10	0	2	4	2	2	10
E. faecalis ATCC 29212	10	0	0	0	0	0	0

DISCUSSION

In this study, we used an infection model by intraperitoneally injecting mice with mitomycin before bacterial challenging through different pathways (Fujii et al., 1994). Similar to cyclophosphamide, mitomycin can reduce the immunity of mouse by decreasing the concentration of leukocytes and platelets, leading to the increased susceptibility to *S. suis* type 2 infections. With this approach, the symptoms occurred in human and pigs infected with *S. suis* type 2 can be partially reproduced in mouse.

Previous studies showed that morbidity and mortality were not found in the mouse intraperitoneally injected with 18 h culture of *S. suis* type 2 (0.5 ml/mouse), suggesting that mouse is not susceptible to *S. suis* type 2 (Gui, 2005). We showed here that treatment with mitomycin reduced the immunity and increased the mouse susceptibility to *S. suis* type 2 infections. In addition, we found that intraperitoneal injection was not the most effective route to cause *S. suis* type 2 infections in mouse because non-specific death was also observed in the mouse intraperitoneally injected with *E. faecalis* and *E. coli* strains. Furthermore, intraperitoneal injection is not the normal infection route. Thus, intraperitoneal injection is not the recommended method for virulence test in mouse. EHEC O26 strain did not cause mortality in mouse infected through wounded skin, stomach lavaging and intraperitoneal injection, suggesting that EHEC O26 might not be pathogenic to mouse.

Previous studies showed that wounded skin was the main route for *S. suis* type 2 infections. Very minor wound in the skin can cause infections (Huang et al., 2005; IP et al., 2007). However, the conclusions were reasoned by identifying the wounds on the arm and legs of the patients with *S. suis* type 2 infections. We artificially infected mouse through the wounds and the corresponding symptoms that occurred. More importantly, we also isolated bacteria from the infected mouse, which is completely consistent with the Koch's postulate (Yang et al., 2006). Therefore, the current study confirmed the previous speculation that *S. suis* type 2 can cause infections through wounded skin.

In conclusion, this study demonstrated that *S. suis* type 2 can cause infection in mouse through wounded skin and the mortality rate for the 6-week old mouse was higher than 50%. We established *S. suis* type 2 infection model in mouse, which provided fundamental bases for investigating the infection route of *S. suis* type 2, examining the bacterial pathogenesis and evaluating the efficiency of vaccine and antimicrobial agents.

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