Interleukin-1 receptor antagonist gene polymorphism in patients with multidrug-resistant Acinetobacter baumannii-associated pneumonia

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Abstract:
OBJECTIVE: Multidrug-resistant Acinetobacter baumannii (MDRAB)-associated pneumonia has been a common disease and a therapeutic problem in hospitals. Interleukin-1 receptor antagonist (IL-1ra) has been considered a required role for host immune defense in pneumonia disease. The aim of this study was to investigate whether the variable nucleotide tandem repeat polymorphism of the IL-1ra gene was associated with MDRAB-related pneumonia.

METHODS: Sixty-six pneumonia patients were enrolled in the study: 36 subjects had MDRAB-related pneumonia and 30 controls had non-MDRAB pneumonia. Polymerase chain reaction, restriction fragment length polymorphism, and agarose gel electrophoresis techniques were used to determine the IL-1ra genotype.

RESULTS: The frequencies of the IL-1ra genotype in the MDRAB-related pneumonia cases were A1/A1, 0.889 and A1/A2, 0.111; the frequencies of the IL-1ra genotype in the controls were A1/A1, 0.333 and A1/A2, 0.667. A statistically significant difference was determined (P<0.05). We also observed an increase in the frequency of IL-1ra A1 allele in the MDRAB-related pneumonia group. A statistically significant difference was determined (P<0.05).

CONCLUSIONS: We suggested that IL-1ra polymorphism was associated with the risk of MDRAB-related pneumonia.

Key words: Acinetobacter baumannii, IL-1ra, pneumonia, polymorphism

Acinetobacter baumannii, a Gram-negative strain with a character of multidrug resistance, grew well in the aerobic phase.[1] The strain was easy to find in hospitals worldwide, especially in intensive care units (ICU), and contributed to severe clinical infections, such as pneumonia, urinary tract infections, and nosocomial septicemia.[2] An outbreak of nosocomial infections caused by multiresistance A. baumannii (MDRAB) prompted the first investigations, in the USA, in 1991.[3] To date, many MDRAB-related pneumonia cases have been reported.[4-6] Because of the rapid development of resistance to multiple antibiotics, A. baumannii needs to be controlled more strictly and the mechanism through which it becomes resistant to the drugs needs to be clearly illustrated.

Cytokines play an important role in a host's defense against microbial infection.[7] For example, when a host is challenged with bacterial components, such as porins, fimbrial proteins, protein A, peptidoglycan, exotoxin, and superantigens, its defenses induce a cascade of signal transduction on the expression of cytokines.[8,9] The production of pro-inflammatory cytokines such as IL-1 alpha (IL-1α), IL-1 beta (IL-1β), and tumor necrosis factor-α that are affected by the development of microbial infections have been described.[10,11] In addition, some of the pro-inflammatory responses were also regulated by cytokines. For example, the Interleukin-1 receptor antagonist (IL-1ra) could avoid the uncontrolled pro-inflammatory responses to diminish extensive immunopathology.[12] When the cells were stimulated by treatment with lipopolysaccharide, the variable nucleotide tandem repeat (VNTR) polymorphisms of the IL-1ra gene were associated with susceptibility to bacterial pneumonia.[13] Functional polymorphisms in cytokine genes that linked to inflammatory responses[14-16] and infectious disease responses[17-20] have been discussed.

Patwari et al.[21] reported that the VNTR polymorphism in IL-1ra’s intron 2 was associated with respiratory injury in children who had community-acquired pneumonia. However, few reports investigated the association between IL-1ra polymorphisms and MDRAB-associated pneumonia.

A. baumannii-associated pneumonia increased significantly and mortality rates were high among ICU patients. It is unlikely that both MDRAB- and
non-MDRAB-related pneumonia patients were in the same area. In the present study, we investigated the association between IL-1ra polymorphism and susceptibility to MDRAB-related pneumonia.

Methods

Samples collection

The MDRAB- and non-MDRAB-related pneumonia patients were recruited from the Antai Tian-Sheng Memorial Hospital, in Donggang, Pingtung County, Taiwan, between March and October 2009. All 66 participants enrolled in the study had pneumonia: 36 cases had MDRAB-related pneumonia and 30 controls had non-MDRAB pneumonia. They all signed an informed consent for this study. The plan for the study was accepted and supported by the ethics committee of Antai Tian-Sheng Memorial Hospital. All specimens were collected and stored at −20°C until DNA extraction.

DNA extraction

The study used the DNeasy™ Kit (Qiagen, U.S.A.) to perform DNA extraction, following the manufacturer’s recommendations. Briefly, the blood was digested with 0.5 mg/ml proteinase K in 400 µl cell lysis solution for 24 hours, at 55°C until the blood was completely lysed. After adding 200 µl absolute ethanol to the lysed sample, the mixture was transferred into the DNeasy mini column and centrifuged for 1 minute at 8 000 rpm. The DNeasy mini column was washed with 500 µl washing buffer and centrifuged for 1 minute at 8 000 rpm. Finally, the DNA was eluted in a clean 1.5-ml micro-centrifuge tube.

IL-1ra gene polymorphism

The polymorphic region within the IL-1ra gene’s second intron, which contains a VNTR of 86 bp, was amplified by Polymerase chain reaction (PCR) and the primers were as follows: Sense, 5’ CTC AGC AAC ACT CCT AT 3’; antisense, 5’ TTC TGG TCT GCA GGT AA 3’. A Perkin-Elmer 9600 thermal cycler (Applied Biosystems) and polypropylene thin wall tubes, no. 179501 (Biozym), were used to perform amplification. The parameters were an initial denaturation at 94°C for 5 minutes, followed by 45 cycles of denaturation at 94°C for 1 minute, annealing at 56°C for 1 minute, and elongation at 72°C for 1 minute. The final elongation was at 72°C for 5 minutes, followed by cooling to 4°C. The PCR products of 412 bp (A1=four repeats of the 86 bp region), 240 bp (A2=two repeats), 498 bp (A3=five repeats), 326 bp (A4=three repeats), 584 bp (A5=six repeats), and 756 bp (A6=eight repeats) were analyzed by electrophoresis on a standard 2% agarose gel stained with 0.1% ethidium bromide.

Table 1: The frequencies of IL-1ra genotype of pneumonia patients in MDRAB-related subjects and controls

| Gene | Genotype | MDRAB-related subjects (n=36) (%) | Controls (n=30) (%) | χ² (P<0.05) | Odds ratio (95%CI) | Statistical analysis |
|------|----------|-------------------------------|-------------------|-------------|---------------------|---------------------|
| IL-1ra | A1/A1 | 32 (88.9) | 10 (33.3) | 7.407 (0.006) | 0.062 (0.007 - 0.590) | 0.005* |
|       | A1/A2 | 4 (11.1) | 20 (66.7) |             |                     |                     |

* = <0.05

Table 2: The frequencies of IL-1ra allele in pneumonia patients, both MDRAB-related subjects and controls

| Gene | Allele | MDRAB-related subjects (n=36) (%) | Controls (n=30) (%) | χ² (P<0.05) | Odds ratio (95%CI) | Statistical analysis |
|------|--------|-------------------------------|-------------------|-------------|---------------------|---------------------|
| IL-1ra | A1 | 68 (94.5) | 40 (66.7) | 6.349 (0.012) | 0.118 (0.018 - 0.759) | 0.001* |
|       | A2 | 4 (5.5) | 20 (33.3) |             |                     |                     |

* = <0.05

Results

The frequent distributions of IL-1ra genotypes and alleles in MDRAB-cases and controls are shown in Tables 1 and 2, respectively.

The frequencies of IL-1ra A1/A2 and A1/A1 genotypes in the MDRAB-associated patients were 0.111 and 0.889, respectively, and in the controls were 0.667 and 0.333, respectively. The frequencies of A1/A1 genotype in the MDRAB-associated patients were higher than those of the control group, and these differences were statistically significant.

In addition, the frequencies of IL-1ra A2 alleles in the MDRAB-associated patients were 0.055 and 0.945, respectively, and in the control group, they were 0.333 and 0.667, respectively. The frequencies of A1 allele in the MDRAB-associated patients were higher than those of the control group, and these differences were also statistically significant.

Discussion

The study investigated A. baumannii infections with three kinds of clinical characters: Antibiotic resistance, cross-infection, and inducible resistance. Among the microbial infections, MDRAB is one of the most severe nosocomial pathogens and causes cross-infections in hospitals. Tien et al. reported that infections in the injured soldiers contracted while in the military field hospitals were caused by A. baumannii. These isolations were resistant to numerous classes of antimicrobials, including the carbapenems. Previous studies have also reported nosocomial infections caused by A. baumannii, in neurosurgery and neonatal ICUs. In addition, antibiotic-resistant bacterial nosocomial infections were also the main pathogen in ICU. Most bacterial infections, such as A. baumannii, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus pyogenes, were isolated from ICU. Among the...
is a potential regulating element that might affect the production of the interleukin-1 family system. In coding or non-coding regions of the IL-1ra gene, there might be either a single-base pair substitution of one nucleotide for another or a variable nucleotide of repeats of a short, repetitive DNA sequence. These variations may influence the rate of gene transcription, the stability of the messenger RNA, or the quantity and activity of the resulting protein. Thus, the possession of specific alleles might influence the susceptibility to or severity of a number of disorders.[16]

This work studied the distribution of the 86-bp VNTR in the IL-1ra gene’s intron 2. The frequencies of the IL-1ra genotype were associated with the occurrence of MDRAB-related pneumonia in the hospital. Furthermore, the results showed that the frequency of allele 1 in IL-1ra polymorphism in MDRAB-related pneumonia cases was significantly higher than in the control group. This was the first study to investigate the association between IL-1ra polymorphism and MDRAB-related pneumonia. The results revealed that IL-1ra polymorphism might be associated with the risk of MDRAB-related pneumonia. Our study had some limitations, such as the small size of the group and the ethnic differences among its subjects, but the difference was statistically significant. In conclusion, we suggest that IL-1ra polymorphism is associated with patients who have MDRAB-related pneumonia.

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