Original Article

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

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Submission: 29-08-11
Accepted: 04-01-12

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 multidrug-resistant *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

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DOI: 10.4103/1817-1737.94523

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 rmp. 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′ TCC 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.

Statistical analysis

Analysis of variance (ANOVA) was performed to test for genotypic differences between the groups. Allele frequencies were compared by the χ^2 test for small sample size. The *P* value, odds ratios, and 95% confidence interval were calculated. A value of less than 0.05 was defined to be of statistical significance and was accepted as $P<0.05$.

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 and A1 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*.[22] 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.[23,24] 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

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

Gene	Genotype	Pneumonia patients		χ^2 (sig)	Odds radio (95%CI)	Statistical analysis
		MDRAB-related subjects (*n*=36) (%)	Controls (*n*=30) (%)			
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	Pneumonia patients		χ^2 (sig)	Odds radio (95%CI)	Statistical analysis
		MDRAB-related subjects (*n*=36) (%)	Controls (*n*=30) (%)			
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

isolations, *A. baumannii* was more resistant to a larger numbers of antibiotics.[25] For example, *A. baumannii* was associated with urinary tract infections, respiratory tract infections, septicemia, bacteremia, meningitis, and wound infections.

IL-1 was among the pro-inflammatory cytokines that contributed to the inflammation and immune response. Activated monocytes and macrophages, as well as T and B cells and NK cells, secrete IL-1, which can affect the activation of T cells and the differentiation of B cells. The IL-1 family consists of IL-1 alpha (IL-1α), IL-1 beta (IL-1β), and IL-1ra. Both IL-1α and IL-1β were the most potent pro-inflammatory cytokines, and they bonded to the IL-1 receptor (IL-1R) on the cell surface. This way, they were able to initiate a cascade of signal transduction to affect the activation of macrophages and neutrophils. In contrast, IL-1ra also bonded to the IL-1R, but the IL-1ra was a competitive inhibitor that attenuated a signal transduction. Romero and Tartakovsky[26] reported that pretreatment with IL-1ra could prevent the pre-term parturition induced by IL-1 in mice experiments. Lynch *et al*.[27] found that the expression was fundamentally different between women and men, with the IL-1ra levels in women being higher. And, the result was also found that IL-1ra might inhibit IL-1 activity or up regulate IL-1 gene expression. McIntyre *et al*.[28] reported that the initiation and termination of the pro-inflammatory response could be mediated by the relative concentrations of IL-1β and IL-1ra.

Tarlow *et al*.[29] reported that the polymorphic site of the IL-1ra gene's intron 2 contains three protein-binding sites: An α-interferon silencer A, a β-interferon silencer A, and an acute-phase response element. These sites, with potential regulating effects, 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 of polymorphic genes would 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.

Acknowledgments

We thank all individuals who agreed to participate in this study. We thank the ethics committee of the Antai Tian-Sheng Memorial Hospital in Donggang, Pingtung for supporting us to collect samples with genotype analysis and giving us certificate of Institutional Review Board approval. Finally, we thank the Asia-Pacific Biotech Developing, Inc. in Kaohsiung for assisting the biotechnology.

References

1. Chang PY, Hsueh PR, Wu PS, Chan PC, Yang TT, Lu CY, *et al*. Multidrug-resistant *Acinetobacter baumannii* isolates inpediatric patients of a university hospital in Taiwan. J Microbiol Immunmol Infect 2007;40:406-10.
2. Cisneros JM., Rodriguez-Bano J. Nosocomial bacteremia dueto *Acinetobacter baumannii*: Epidemiology, clinical features and treatment. Clin Microbiol Infect 2000;8:687-93.
3. Go ES, Urban C, Burns J, Kreiswirth B, Eisner W, Mariano N, *et al*. Clinical and molecular epidemiology of *Acinetobacter* infections sensitive only to polymyxin B and sulbactam. Lancet 1994;344:1329-32.
4. Reid GE, Grim SA, Aldeza CA, Janda WM, Clark NM. Rapid development of *Acinetobacter baumannii* resistance to tigecycline. Pharmacotherapy 2007;27:1198-201.
5. Sebeny PJ, Riddle MS, Petersen K. *Acinetobacter baumannii* skin and soft-tissue infection associated with war trauma. Clin Infect Dis 2008;47:444-9.
6. Touati A, Achour W, Cherif A, Hmida HB, Afif FB, Jabnoun S, *et al*. Outbreak of *Acinetobacter baumannii* in a neonatal intensive care unit: Antimicrobial susceptibility and genotyping analysis. Ann Epidemiol 2009;19:372-8.
7. Renckens R, Roelofs JJ, Knapp S, de Vos AF, Florquin S, Van Der Poll T. The acute-phase response and serum amyloid A inhibit the inflammatory response to *Acinetobacter baumannii* Pneumonia. J Infect Dis 2006;193:187-95.
8. Henderson B, Poole S, Wilson M. Bacterial modulins: A novel class of virulence factors which cause host tissue pathology by inducing cytokine synthesis. Microbiol Rev 1996;60:316-41.
9. Lee JS, Lee JC, Lee CM, Jung ID, Jeong YI, Seong EY, *et al*. Outer membrane protein A of *Acinetobacter baumannii* induces differentiation of CD4+ T cells toward a Th1 polarizing phenotype through the activation of dendritic cells. Biochem Pharmacol 2007;74:86-97.
10. Hurme M, Helminen M. Resistance to human cytomegalovirus infection may be influenced by genetic polymorphisms of the tumor necrosis factor-alpha and interleukin-1 receptor antagonist genes. Scand J Infect Dis 1998;30:447-9.
11. van Deventer SJ. Cytokine and cytokine receptor polymorphisms in infectious disease. Intensive Care Med 2000;26:98-102.
12. Nicklin MJ, Weith A, Duff GW. A physical map of the region encompassing the human interleukin-1 alpha, interleukin-1 beta, and interleukin-1 receptor antagonist genes. Genomics 1994;19:382-4.
13. Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion *in vitro*. Eur J Clin Invest 1992;22:396-402.
14. Laine ML, Farre MA, Crusius JB, van Winkelhoff AJ, Pena AS. The mouthwash: A non-invasive sampling method to study cytokine gene polymorphisms. J Periodontol 2000;71:1315-8.
15. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, *et al*. The role of interleukin-1 polymorphisms in the pathogenesis of gastric cancer. Nature 2001;412:99.
16. Witkin SS, Gerber S, Ledger WJ. Influence of interleukin-1 receptor antagonist gene polymorphism on disease. Clin Infect Dis 2002;34:204-9.
17. Jeremias J, Giraldo P, Durrant S, Ribeiro-Filho A, Witkin SS. Relationship between *Ureaplasma urealyticum* vaginal colonization and polymorphism in the interleukin-1 receptor antagonist gene. J Infect Dis 1999;180:912-4.

18. Wilkinson RJ, Patel P, Llewelyn M, Hirsch CS, Pasvol G, Snounou G, *et al*. Influence of polymorphism in the genes for the interleukin (IL)-1 receptor antagonist and IL-1beta on tuberculosis. J Exp Med 1999;189:1863-74.
19. Read RC, Camp NJ, di Giovine FS, Borrow R, Kaczmarski EB, Chaudhary AG, *et al*. An interleukin-1 genotype is associated with fatal outcome of meningococcal disease. J Infect Dis 2000;182:1557-60.
20. van der Schee C, Sluiters HJ, van der Meijden WI, van Beek P, Peerbooms P, Verbrugh H, *et al*. Host and pathogen interaction during vaginal infection by *Trichomonas vaginalis* and *Mycoplasma hominis* or *Ureaplasma urealyticum*. J Microbiol Methods 2001;45:61-7.
21. Patwari PP, O'Cain P, Goodman DM, Smith M, Krushkal J, Liu C, *et al*. Interleukin-1 receptor antagonist intron 2 variable number of tandem repeats polymorphism and respiratory failure in children with community-acquired pneumonia. Pediatr Crit Care Med 2008;9:553-9.
22. Tien HC, Battad A, Bryce EA, Fuller J, Mulvey M, Bernard K, *et al*. Multi-drug resistant Acinetobacter infections in critically injured Canadian forces soldiers. BMC Infect Dis 2007;7:95.
23. Gulati S, Kapil A, Das B, Dwivedi SN, Mahapatra AK. Nosocomial infections due to *Acinetobacter baumannii* in a neurosurgery ICU. Neurol India 2001;49:134-7.
24. Mittal N, Nair D, Gupta N, Rawat D, Kabra S, Kumar S, *et al*. Outbreak of Acinetobacter spp septicemia in a neonatal ICU. Southeast Asian J Trop Med Public Health 2003;34:365-6.
25. Patwardhan RB, Dhakephalkar PK, Niphadkar KB, Chopade BA. A study on nosocomial pathogens in ICU with special reference to multiresistant *Acinetobacter baumannii* harbouring multiple plasmids. Indian J Med Res 2008;128:178-87.
26. Romero R, Tartakovsky B. The natural interleukin-1 receptor antagonist prevents interleukin-1-induced preterm delivery in mice. Am J Obstet Gynecol 1992;167:1041-5.
27. Lynch EA, Dinarello CA, Cannon JG. Gender differences in IL-1 alpha, IL-1 beta, and IL-1 receptor antagonist secretion from mononuclear cells and urinary excretion. J Immunol 1994;153:300-6.
28. McIntyre KW, Stepan GJ, Kolinsky KD, Benjamin WR, Plocinski JM, Kaffka KL, *et al*. Inhibition of interleukin 1 (IL-1) binding and bioactivity *in vitro* and modulation of acute inflammation *in vivo* by IL-1 receptor antagonist and anti-IL-1 receptor monoclonal antibody. J Exp Med 1991;173:931-9.
29. Tarlow JK, Blakemore AI, Lennard A, Solari R, Hughes HN, Steinkasserer A, *et al*. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet 1993;91:403-4.

How to cite this article: Hsu M, Lu Y, Hsu Y, Liu W, Wu W. Interleukin-1 receptor antagonist gene polymorphism in patients with multidrug-resistant Acinetobacter baumannii-associated pneumonia. Ann Thorac Med 2012;7:74-7.

Source of Support: Nil, **Conflict of Interest:** None declared.