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Monoclonal Antibody against Lipooligosaccharide of *Moraxella catarrhalis* Decreases Resistance to Aminopenicillins

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Abstract

The following *Moraxella catarrhalis* strains were used in the experiments: eight beta-lactamase-producing clinical isolates and four isolates including a control strain 353 CCUG without beta-lactamase. The other strains used as controls in this procedure were two isolates of *Staphylococcus aureus* and two of *Escherichia coli*: beta-lactamase producers and non-producers control strains of *S. aureus*: ATTC 29213 and *E. coli* ATCC 25922. All of the microbial strains were tested with ampicillin alone and with ampicillin in combination with murine monoclonal antibody (MAb) 219 against lipooligosaccharide of *M. catarrhalis* in a 96-well plate to determine the minimal inhibitory concentration (MIC). The testing was performed by broth dilution method and minimal bactericidal concentration (MBC) on brain heart infusion agar (BHI).

The effect of the combination of aminopenicillin with MAb219 was manifested as a fourfold reduction of the MIC and the MBC of the tested beta-lactamase producing *M. catarrhalis*. The effect over the control strains and moraxellae without beta-lactamase was either missing or there was a slight reduction of MIC and MBC.

The conclusion of the study is that MAb219 in combination with ampicillin in *in vitro* testing can decrease resistance to aminopenicillins in *M. catarrhalis* after the linking of MAb219 with lipooligosaccharide of the OM in the antigen-antibody complex.

Key words: *Moraxella catarrhalis,* beta-lactamase, lipooligosaccharide, monoclonal antibody

Резюме

В експериментите бяха използвани следните щамове *Moraxella catarrhalis*: осем бета-лактамаза продуциращи клинични изолата и четири, включително контролен щам 353 CCUG без бета-лактамаза. Другите щамовете, използвани като контроли в тази процедура са два изолата на *Staphylococcus aureus* и два *Escherichia coli*: бета-лактамаза продуценти и непроизвеждащи бета-лактамази контролни щамове *S. aureus*: ATTC 29,213 и E. *coli* ATCC 25922. Всички микробни щамове бяха тествани с ампицилин самостоятелно и с ампицилин в комбинация с мише моноклонално антитяло (MAb) 219 срещу липоолигозахарида на *M. catarrhalis* в 96-ямкова плака за определяне на минималната инхибираща концентрация (MIC). Тестването се извършва по мето-да на разреждане на бульон и минимална бактерицидна концентрация (MBC) на мозъчно-сърдечен инфуз (BHI) агар. Ефектът на комбинацията аминопеницин с MAb219 се проявява като четирикратно намаляване на MIC и MBC при изпитваните бета-лактамаза позитивни *M. catarrhalis.* Ефектът върху контролните щамове мораксели без бета-лактамаза беше липса или леко намаление на MIC и MBC. Заключението от проучването е, че MAb219 в комбинация с ампицилин в ин витро тестване може да се намали резистентността към аминопеницилините в *M. catarrhalis* след свързването на MAb219 с външномембранния липоолигозахарид в комплекс антиген-антитяло.

Introduction

Moraxella catarrhalis is a Gram-negative diplococcus that colonizes the mucosa in healthy preschool children and also is considered as an important cause of respiratory tract infections in children and elderly people (Gergova *et al.*, 2013; Hays 2009; Sethi *et al.*, 2007; Verduin *et al.*, 2002; Verhaegh *et al.*, 2011). The permanent expansion of infections caused by this microorganism in the last

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years is caused by two things: - on the one hand, the increased resistance to antimicrobial drugs by producing beta-lactamase (mainly aminopenicillins and first generation cephalosporins), and on the other hand, the resistance to the normal serum bactericidal effect. (Gergova *et al.*, 2009; Theoga *et al.*, 2014; Verhaegh *et al.*, 2011; Wirth *et al.* 2007; Zaleski *et al.* 2000). These interesting facts are a stimulus for new investigations into *M. catarrhalis* pathogenesis and its prevention with an appropriate vaccine (Luke-Marshall *et al.*, 2013; Gergova *et al.*, 2007; Mitov *et al.*, 2010; Ren, 2011; Tan *et al.*, 2007; Su, 2012). In previous studies, the murine monoclonal antibody MAb 219 IgM (Gergova *et al.* 2007) has been produced created and characterized as a bactericidal antibody against *M. catarrhalis*, *H. influenzae* and *H. parainfluenzae* in the presence of a complement.

The aim of this study is to determine the effect of the combination of MAb 219 (against lipooligosaccharide of *M. catarrhalis*) and aminopenicilin to decrease the resistance of beta-lactamase-producing moraxellae.

Material and methods

Eight clinical isolates of *M. catarrhalis*, previously identified as serotype A (Gergova *et al.* 2007) and BRO-1 producers, with *bro1* gene (Gergova *et al.* 2009) were randomly chosen. A control strain *M. catarrhalis* 353 CCUG and three other clinical serotype A isolates without *bro* gene were used.

Ampicillin (Sigma) was applied to determine the minimal inhibitory concentration (MIC) of M. catarrhalis strains by the broth dilution method and minimal bactericidal concentration (MBC) on brain heart infusion agar (BHI) using the agar dilution method, according to the Clinical and Laboratory Standards Institute (CLSI) 2014 criteria (CLSI, 2014).

Murine monoclonal antibody MAb 219 IgM, produced by hybridoma technology bactericidal with complement (C`) only, (Gergova *et al.*, 2007) was used in undiluted and 1/4 to 1/64 diluted ascitic fluid to test the MIC and MBC of *M. catarrhalis* isolates for ampicillin in twofold dilutions by the broth dilution method (Mahon *et al.,* 2007). Two controls with ampicillin alone and with MAb 219 IgM alone for missing suppressing effect were used to each strain (0.5 ml PBS was added versus MAb with ampicillin). The synergistic action of MAb 219 IgM and ampicillin was tested three times.

The strains of *M. catarrhalis* (diluted in 0.5 ml PBS to 0,5 MF) with the antibacterial agent - 0.5ml and 0.5ml MAb 219 on a 96-well polystyrene microtiter plate (Nunc) were incubated at 35ºC for 2 hours. After incubation, 20µl were dropped from each dilution to BHI. After incubation at 35ºC/24 hours of the 96-well plates and petri dishes with BHI, the MIC in the plates and MBC on the agar were determined.

Table 1. Results of MICs and MBCs of tested ***M. catarrhalis*** and control strains

Microbial strain	Ampicillin alone		Amp with MAb219 undiluted		Amp with MAb219 diluted ½		Amp with MAb219 diluted ¼	
	MIC mg/L	MBC mg/L	MIC mg/L	MBC mg/L	MIC mg/L	MBC mg/L	MIC mg/L	MBC mg/L
M. catarrhalis bro 1 (n=4)	8	16	2	4	2	4	4	8
M. catarrhalis bro 1 (n=4)	16	32	4	8	4	8	8	16
M. catarrhalis without *bro* (n=3)	0.25	0.5	0.125	0.25	0.125	0.25	0.25	0.5
M. catarrhalis 353 CCUG	0.125	0.25	0.125	0.25	0.125	0.25	0.25	0.5
S. aureus (n=2)	16	32	16	32	16	32	16	32
S. aureus ATTC 29213	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5
E. coli (n=2)	64	128	64	128	64	128	64	128
E. coli ATCC 25922	0.5	1	0.5	1	0.5	1	0.5	1

Results

The MICs of ampicillin alone of the selected strains with *bro1* gene were in the range of 8-16 mg/L, and the MBCs were in the range of 16-32 mg/L (Table 1). When ampicillin was used in combination with MAb 219 undiluted, the MICs and the MBCs of the tested *M. catarrhalis* isolates decreased four times. After a ¼ dilution of the MAb 219, the effect of the combination decreased. The other strains (without *bro* gene) were sensitive to ampicillin with MIC - 0.25 mg/L and MBC - 0.5 mg/L, respectively. The missing or minor effect of the combination aminopenicillin with MAb was a reduction of the MIC and the MBC (one diluting dilution of the control strains).

Discussion

It is known that ampicillin, like other beta-lactam antimicrobial agents, must use penicillin binding proteins (PBPs) to pass through the bacterial cell wall (Mahon *et al.,* 2007). In *M. catarrhalis* an essential outer membrane porin M35 has already been established, a potential vaccine target that mediates the transport and susceptibility to aminopenicillins (Jetter *et al.*, 2009).

The role of LPS is still being researched as an attractive vaccine candidate such as antigen of the cell wall of *M. catarrhalis* with a unique structure of glycolipids (Gergova *et al.* 2007; Martin *et al.,* 2015). The contribution of the LPS - as a fundamental element of the OM of the Gram-negative bacteria, in the transport through the Gram-negative cell wall is still very unclear. During the last years that process has been studied extensively especially in *Escherichia coli*, *Salmonella enterica, Neisseria meningitides* and *M. catarrhalis* as appropriate subjects (Bos *et al.*, 2007; Clements *et al.*, 2002; Martin *et al.,*2015). The function of LPS is known to be a formidable barrier for hydrophobic molecules. The LPS also mediated rearrangements in the OM surface-exposed loops of few proteins. The so-called Lpt system consists of seven LPS transport proteins (LptA-G) (Bos *et al.,* 2007; Bowyer, 2011; Narita, 2009, Narita, 2011). Molecules participating in the biogenesis of LPS may represent new antimicrobial targets, as demonstrated by the development of antibiotics targeting LpxC, an enzyme involved LPS biogenesis and LptD, an OM protein involved in LPS transport. As already known, LpxC is a target for the bacteriostatic agent chloramphenicol. Mutations in the lpxC gene can lead to increased susceptibility to other antibiotics, due to the changed permeability in the gram-negative OM (Clements *et al.,* 2002). LPS with protection of OMP OprH is linked with aminoglicosides permeability in *P. aeruginosa,* but no data about the participation of LPS in beta-lactams transport (Moore and Flaws, 2011) has been proved.

The results in this study were provided to test the hypothesis that some antibodies against lipopolysaccharide (LPS) of *M. catarrhalis* in the complex with ampicillin reduce the resistance to aminopenicillins.

The cases of *M. catarrhalis* respiratory diseases will increase further in the next years after the introduction of vaccines against Streptococcus pneumoniae and Haemophilus influenzae, because of the biological niche they opened for the spread of *M. catarrhalis* (Gergova *et al.*, 2013; Hays, 2009). The features and the qualities of the LOS of *M. catarrhalis* therefore are examined and options are still being sought for obtaining a vaccine with a part of this immunogenic ingredient (Luke-Marshall *et al*, 2013; Ren, 2011; Tan and Riesbeck, 2007; Su, 2012). It is known that MAb219 against the common epitope for the three serotypes of *M. catarrhalis* LOS is bactericidal in the presence of C` (Gergova *et al.*, 2007), but now it is established that MAb219 can increase susceptibility to aminopenicillins in vitro. This result supports the idea of common epitope from *M. catarrhalis* LOS to run as a prospective vaccine target, which stimulates the production of very useful antibodies. The resistance to aminopenicillins in clinical isolates of *M. catarrhalis* is increased and is now near 100%. The antibodies against A, B, C common epitpe of LOS can recover the susceptibility of *M. catarrhalis* to the most adequate antimicrobial agents for the childhood - aminopenicillins.

Refference

Bos, M. P., J. Tommassen (2011). The LptD chaperone LptE is not directly involved in lipopolysaccharide transport in *Neisseria meningitidis*. *J. Biol. Chem*. **286**: 28688-28696.

Bowyer, A. (2011). Characterization of interactions between LPS transport proteins of the Lpt system. *Biochem. Biophys. Res. Commun.* **404**: 1093-1098.

Brook, I. (2010). β-Lactamase-producing bacteria in upper respiratory tract infections. *Curr. Infect. Dis. Rep*. **12**: 110-117.

Budhani, R. K., J. K. Struthers (1998). Interaction of *Streptococcus pneumoniae* and *Moraxella catarrhalis*: Investigation of the indirect pathogenic role of β-lactamase-producing *Moraxellae* by use of a continuous-culture biofilm system. *Antimicrob. Agents Chemother.* **42**: 2521-2526.

Clements, J. M., F. Coignard, I. Johnson, S. Chandler, S. Palan, A. Waller, J. Wijkmans, M. G. Hunter (2002). Antibacterial Activities and Characterization of Novel

Inhibitors of LpxC. *Antimicrob.* Agents Chemother. **46**: 1793-1799.

Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial susceptibility testing; Twuentieth Informational Supplement**.** (2014). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. *Approved standard M07-A8. M100 - S24*. **34**: 84-142.

Frank, P. M., I. R. Collins, I. Peak, D. Grice, J. C. Wilson, (2015). An Unusual Carbohydrate Conformation is Evident in *Moraxella catarrhalis* Oligosaccharides *Molecules*, **20**: 14234-14253.

Gergova, R., P. Minchev, M. Dikova, S. Gergov, C. Jurova, T. Ilieva, I. Mitov (2013). Bacterial spectrum of etiology of the community-acquired respiratory infections in childhood and antimicrobial resistance (in Bulg.). *Bulg. Med. J.* **2**: 45-54.

Gergova, R., R. Markovska, I. Mitov **(2009).** Antimicrobial resistance and production of beta-lactamases in Bulgarian clinical isolates *Moraxella catarrhalis*, *Ann. Microbiol.* **59:** 169-172.

Gergova, R. T., I. D. Iankov, I. H. Haralambieva, I. G. Mitov (2**007).** Bactericidal monoclonal antibody against *Moraxella catarrhalis* lipooligosaccharide cross-reacts with *Haemophilus spp. Current Microbiol*. **54:** 85-90.

Hays, J. P. (2009). *Moraxella catarrhalis*: A mini review. *J. Pediatric Inf. Dis*. **4:** 211-220.

Jetter, M., N. Heiniger, V. Spaniol, R. Troller, A. Schaller, C. Aebi (2009). Outer membrane porin M35 of *Moraxella catarrhalis* mediates susceptibility to aminopenicillins *BMC Microbiology* **9:**188.

Luke-Marshall, N. R., K. J. Edwards, S. Sauberan, F. St. Michael, E. V. Vinogradov, A. D Cox (2013). Campagnari Characterization of a trifunctional glucosyltransferase essential for *Moraxella catarrhalis* lipooligosaccharide assembly. *Glycobiology* **23**: 1013-1021.

Mahon, C. R., D. C. Lehman, G. Manuselis (2007). Textbook of Diagnostic microbiology, third edition, pp. 303-366.

Mitov, I.G., R.T. Gergova , V. V. Ouzounova-Raykova (2010). Distribution of genes encoding virulence *ompB2*, *ompCD*, *ompE,* beta-lactamase and serotype in pathogenic and colonizing strains *Moraxella catarrhalis. Arch Med. Res.* **41**: 530-535.

Moore, N. M., M. L. Flaws (2011). Antimicrobial Resistance Mechanisms in *Pseudomonas aeruginosa*, *Clin Lab Sci*. **24**: 47-51.

Narita, S. I. (2011). ABC transporters involved in the biogenesis of the outer membrane in Gram-negative bacteria. *Biosci. Biotechnol. Biochem.* **75**: 1044-1054.

Narita, S. I., H. Tokuda (2009). Biochemical characterization of an ABC transporter LptBFGC complex required for the outer membrane sorting of lipopolysaccharides *FEBS Letters* **583**: 2160-2164.

Ren, D. (2011) Mutant lipooligosaccharide-based conjugate vaccine demonstrates a broad-spectrum effectiveness against *Moraxella catarrhalis*. *Vaccine* **29**: 4210-4217.

Sethi, S., R. Sethi, K. Eschberger, P. Lobbins, X. Cai, B. J. Grant, T. F. Murphy (2007). Airway Bacterial Concentrations and Exacerbations of Chronic Obstructive Pulmonary Disease. *Am. J. Respir. Crit. Care Med*. **15**: 356-361.

Su, Y. C. B. Singh, K. Riesbeck (2012). *Moraxella catarrhalis*: from interactions with the host immune system to vaccine development. *Future Microbiol.* **7:** 1073-1100.

Tan, T. T., K. Riesbeck (2007). Current progress of adhesins as vaccine candidates for *Moraxella catarrhalis, Expert Rev. of Vaccines*, **6:** 949-956.

Theoga, R. C. J., E. M.·Shankar, H. A. Rothan, U. A. Rao, (2014). Molecular Characterization of Clinical Isolates of *Moraxella catarrhalis* by Randomly Amplified Polymorphic DNA Fingerprinting. *J. Mol. Microbiol. Biotechnol.* **24**:270-278.

Verduin, C. M., C. Hol, A. Fleer , H. van Dijk, A. van Belkum (2002). *Moraxella catarrhalis*: from emerging to established pathogen. *Clin. Microbiol. Rev.* **15**: 125-144.

Verhaegh, S. J. M. L. Snippe,F. Levy, H. A. Verbrugh,V. W. V. Jaddoe, A. Hofman, H. A. Moll,A. van Belkum, J. P.Hays (2011). Colonization of healthy children by *Moraxella catarrhalis* is characterized by genotype heterogeneity, virulence gene diversity and co-colonization with *Haemophilus influenzae*. *Microbiology* **157**: 169-178.

Wirth T, G. Morelli, B. Kusecek, A. van Belkum, C. van der Schee, A. Meyer, M. Achtman (2007). The rise and spread of a new pathogen: Seroresistant *Moraxella catarrhalis, Genome Res.* **17:** 1647-1656.

Zaleski, A., N. K. Scheffler, P. Densen, F. K. Lee, A. A. Campagnari, B. W. Gibson (2000). Lipooligosaccharide P(k) (Galalpha1-4Galbeta1-4Glc) epitope of *Moraxella catarrhalis* is a factor in resistance to bactericidal activity mediated by normal human serum. *Infect. Immun.* **68**: 5261-5268.