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Full Length Research Paper

Synergy of daptomycin with fusidin against invasive systemic infection and septic arthritis induced by type VI group B streptococci in mice

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In view of the emergence of multidrug-resistant group B streptococci (GBS), and its significant clinical impact, there is a necessary need for the development of more effective therapeutic alternatives. Here, we assessed the therapeutic efficacy of daptomycin, a novel lipopeptide antibiotic, in the treatment of type IV GBS-induced invasive systemic infection and septic arthritis in mice. We also evaluated the possible synergy between daptomycin and fusidin to combat GBS disease. Mice infected with type IV GBS and left without drug treatment displayed high incidence of deaths and severe diffuse septic arthritis, associated with excessive production of proinflammatory cytokines (tumor necrosis factor alpha (TNF-α), interleukin-1beta (IL-1β) and interleukin-6 (IL-6)), cyclooxygeanse-2 (COX-2) and prostaglandin E2 (PGE2) in their blood and joints. However, treatment of these GBS-infected mice with daptomycin significantly inhibited the inoculated bacteria to grow in the blood and joints. Daptomycin-treated mice had significantly showed lower mortality rates, less frequent arthritis and lower levels of TNF-α, IL-1β, IL-6, COX-2 and PGE2 than infected untreated animals. More interestingly, a marked *in vivo* synergy between daptomycin and fusidin that completely protected the mice from GBS infection and its associated mortality and serious sequels was clearly observed. In summary, the present study showed that daptomycin is a welcome newcomer antibacterial arsenal to eradicate GBS invasive infection and septic arthritis, in particular when given in combination with other antibacterial agents such as fusidin.

Key words: Group B streptococci, septic arthritis, daptomycin, fusidin, proinflammatory cytokines, cylooxygenase-2, prostaglandin E2.

INTRODUCTION

Daptomycin is a novel lipopeptide antibiotic with unique mechanism of action and excellent bactericidal activity against a wide range of pathogenic gram-positive bacteria, including multidrug-resistant gram-positive cocci (LaPlante and Rybak, 2004; Brauers et al., 2007). Antibacterial activity of daptomycin against many clinical isolates was compared with that of β-lactams, vancomycin, linezolid and quinupristin/dalfopristin. Overall, daptomycin showed greater bactericidal activity, low incidence of bacterial resistance and more safety profile than all other tested antibiotics (Rybak et al., 2000; Pfaller et al., 2007). Furthermore, synergistic interactions between daptomycin and other antimicrobials such as aminoglycosides, β-lactams and rifampicin were also observed (Rand and Houck, 2004; Credito et al., 2007; Figueroa et al., 2009).

Fusidic acid and its salt sodium fusidate (fusidin) are narrow-spectrum antibiotics. The main indication for their systemic use is in treatment of penicillin-resistant staphylococcal infections, including osteomyelitis and endocarditis, in combination with other antibacterials to prevent emergence of resistance (Falck et al., 2006). In addition to its antibacterial activity, fusidin has also powerful immunomodulatory and anti-inflammatory

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properties that are probably related to its capacity to reduce inflammatory cell infiltration and down-regulate the production of proinflammatory cytokines (e.g., TNF-α, IFN-γ, IL-1β and IL-2) (Genovese et al., 1996; Milenkovic et al., 2005). These unique properties of fusidin have been reported against human immunoinflammatory diseases (Nicoletti et al., 1999) and in several experimental animal models of organ-specific inflammatory diseases including sepsis and endotoxic shock (Genovese et al., 1996), acute hepatitis (Nicoletti et al., 1997), acute pancreatitis (Osman et al., 1998), allergic encephalomyelitis (Di Marco et al., 2001), formalin-induced edema (Kilic et al., 2002) and myocarditis (Milenkovic et al., 2005).

Group B streptococci (GBS), or *Streptococcus agalactiae*, have long been known as the most common leading cause of life-threatening bacterial infections in neonates and young infants (Pettersson, 2007). Recently, these microorganisms have also been recognized as an ever-growing cause of serious infections and mortality among older adults (Skoff et al., 2009). Case fatality rate of invasive GBS infections has been reported to be between 21 and 32% in newborns (Lukacs et al., 2004) and can exceed 40% in immunocompromised adult patients (Liakopoulos et al., 2004). Rapidly progressive septic arthritis is an important clinical manifestation of GBS infection (Nolla et al., 2003). Unfortunately, one-half of the patients with GBS septic arthritis do not respond to the current therapy, and thus the disease is associated with substantial irreversible functional complications (Lee et al., 2007). The emergence of multi-drug resistant GBS strains has complicated the clinical scenario of GBS disease (Simoes et al., 2004; Nagano et al., 2008). Moreover, there are no vaccines suitable for the prevention of GBS infections, and consequently identify-cation of alternate drug targets is essential for future therapeutic measures (Rajagopal, 2009). Taken together, these facts reinforce the importance of GBS as a public health concern and raise the critical need for develop-ment of more efficient alternative therapeutic strategies.

At the experimental level, Tissi et al. (1990) have described a model of invasive GBS systemic infection and septic arthritis induced in mice by type IV GBS. In this model, the clinical features of induced GBS disease and its associated deaths and septic arthritis greatly mimic the human situation (Tissi et al., 1990, 1999; Puliti et al., 2002, 2010). In the present study, we successfully established this model and investigated the therapeutic efficacy of daptomycin against GBS-induced high mortalities and severe arthritis in mice. We also assessed whether co-administration of fusidin with daptomycin could result in an *in vivo* synergy against this life-threatening bacterial disease. Results showed that therapy with daptomycin significantly suppressed *in vivo* growth of inoculated GBS and its associated lethal trend and septic arthritis. More interestingly, co-administration of fusidin significantly enhanced daptomycin activity in combating GBS infection and its serious sequels.

MATERIALS AND METHODS

Antibiotics and bacteria

Daptomycin (Cubist Pharmaceuticals, Inc., Lexington, Massachusetts, USA) and sodium fusidate (fusidin) (Sigma, St. Louis, MO, USA) were purchased as powder for reconstitution. Prior to each experiment, all drug preparations were freshly made in accordance with their manufacturers' recommendations. Type IV group B streptococci (GBS; reference strain CNCTC 1/82) was supplied by Czech National Collection of Type Cultures (Prague, Czech Republic). For experimental infection, the bacteria were grown overnight at 37°C and 5% CO_2 in Todd-Hewitt broth (Oxoid Ltd., Basingstoke, Hampshire, England) and then washed and suspended in RPMI 1640 medium at a concentrations of 1×10^7 colony forming units (CFU) per ml as previously described (Tissi et al., 1990; Puliti et al., 2002).

Animals, experimental GBS infection and treatment schedule

This study was approved by the Local Animal Care Committee of Umm Al-Qura University, KSA, and carried out in accordance with the principles of laboratory animal care, formulated by the National Academy of Sciences. A total of 60 adult male albino mice (weight, 25 to 35 g) were housed in autoclaved cages, maintained at a 12 h light/dark cycle in a humidity and temperature-controlled environment with autoclaved food and water *ad libitum*. For induction of GSB infection, mice were intravenously inoculated with 10^7 colony-forming units (CFU) of GBS per mouse in a volume of 1 ml of RPMI 1640 medium. The animals were randomly divided into five groups: Group 1 (n = 10); neither infected nor drug-treated control mice, Group 2 (n = 14); GBS-infected and received no drug treatment, Group 3 (n =12); GBS-infected and treated with daptomycin (6 mg/kg/day; intraperitoneally; i.p., for 6 consecutive days), Group 4 (n = 12); GBS-infected and treated with fusidin (80 mg/kg/day; i.p., for 6 sequential days) and Group 5 (n = 12); GBS-infected and treated with daptomycin + fusidin. The used dosage regimens of daptomycin and fusidin were in accordance with those applied in the clinical field or had been tested in the previous studies (Figueroa et al., 2009; Milenkovic et al., 2005). Seven days after GBS inoculation, the survival mice of each group were sacrificed under ether anesthesia. After that, their blood samples were collected and their affected joints were aseptically removed, weighed and homogenized in sterile RPMI 1640 medium (1 ml/100 mg of joint weight) as previously described (Tissi et al., 1990; Puliti et al., 2002, 2010). Each resultant joint tissue homogenate was divided into two portions: a portion employed for bacterial culturing as mentioned subsequently, while the second part was centrifuged at 2,000 ×g for 10 min and then its supernatant was collected and stored at -20°C until used. Similarly, each collected blood sample was divided into unequal two parts: the small part was directly employed for bacterial culturing, while the large one was centrifuged for 10 min at 4000 rpm and its serum was aspirated and stored at -20°C until used.

Clinical evaluation of arthritis and mortality

All mice were daily and individually monitored for the signs of arthritis and for mortality. Arthritis was defined as visible joint erythema and/or swelling of at least one joint (Tanaka et al., 1996). To evaluate the intensity of developed arthritis, a macroscopic

Table 1. Effect of daptomycin and/or fusidin therapy on type IV group B streptococci (GBS)-induced deaths and arthritis in mice.

Group	n	Mortality rate (%)	Arthritis	
			Incidence (%)	Severity (mean ± SEM)
Normal control	10	0	0	0.0
GBS	14	42.9[a]	78 ± 11[a]	2.7 ± 0.3[a]
GBS + daptomycin	12	8.3[b]	17 ± 2[b]	0.5 ± 0.06[b]
GBS + fusidin	12	25[c]	29 ± 3[c]	1.2 ± 0.02[c]
GBS + daptomycin + fusidin	12	0[d,*]	0[d,*]	0.0[d,*]

Mice were infected with 10^7 colony-forming units of GBS/mouse and then treated or not-treated with daptomycin (6 mg/kg/day) and/or fusidin (80 mg/kg/day) for 6 consecutive days. [a]P <0.001, *P =NS, versus normal control group; [b]P <0.01, [c]P <0.05, [d]P <0.001, versus GBS-untreated group.

clinical scoring system of 0 to 3 points for each limb was used (1point = mild swelling and/or erythema; 2 points = moderate swelling and erythema; 3 points = marked swelling, erythema and/or hardening of the joint) as previously described (Verdrengh and Tarkowski, 1997). The arthritis index was constructed by adding the scores from all 4 limbs for each animal. Finally, the total index of developed arthritis per group was constructed by dividing the total score by the number of animals used in each experimental group.

Determination of bacterial growth

On day 7 after GBS inoculation, appropriate dilutions were made from the collected whole blood samples and joint tissue homogenates of all mice, and then 0.1 ml of each sample was individually plated on blood agar plates. After incubation for 48 h at 37°C, the bacterial colonies were counted and multiplied by 10 to give an estimate of the total number of GBS CFU/ ml of sample (Dubost, et al., 2004).

Assessment of TNF-α, IL-1β, IL-6, COX-2 and PGE2 in the sera and joint tissue homogenates

The levels of TNF-α, IL-1β and IL-6, as well-established proinflammatory cytokines and diagnostic biomarkers during the course of GBS sepsis and septic arthritis (Tissi et al., 1999; Mikamo et al., 2004; Puliti et al., 2010), were measured in the sera samples and joint tissue homogenates with commercial enzyme-linked immunosorbent assay (ELISA) kits purchased from R&D System (R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's recommendations. Results were expressed as picograms per ml (pg/ml) of serum or supernatant from joint homogenates. COX-2 and PGE2 concentrations in the joint tissue homogenates were also determined with commercial ELISA kits (COX-2: IBL International GmbH, Hamburg, Germany and PGE2: BIOTRAK-Amersham-Freiburg, Germany) in accordance with the manufacturers' instructions, and their results were expressed as ng/ml and pg/ml, respectively.

Statistical analyses

Quantitative results of arthritis index (score), number (CFU) of grown GBS and levels of Cox-2, PGE2, TNF-α, IL-1β and IL-6 were presented as arithmetic means ± SEM. Differences among the groups were investigated using one-way analysis of variance (ANOVA) followed by a student's t-test. Differences between incidence of arthritis and survival data for the groups were analyzed by Chi-square test. A P value of < 0.05 was considered statistically significant.

RESULTS

Effect of daptomycin and fusidin therapy on GBS infection-induced deaths and arthritis

Mice infected with 1×10^7 CFU of GBS/mouse and then treated or not treated with daptomycin (6 mg/kg/day) and/or fusidin (80 mg/kg/day) for 6 consecutive days were daily monitored by assessing the survival rates, incidence and severity of induced arthritis up to 7 days post-GBS infection. As shown in Table 1, the cumulative mortality rate at the end of observation period was high (6/14; 42.9%) in GBS-infected and drug-untreated mice. In contrast, treatment of GBS-infected mice with daptomycin or fusidin significantly decreased the mortality rate to 1/12 (8.3%) and 3/12 (25%), respectively. More interestingly, no mortalities were observed in GBS-infected mice simultaneously treated with daptomycin plus fusidin (Table 1). Similarly, the clinical signs of arthritis (joint swelling and redness) were not observed in both normal controls and GBS-infected mice treated with daptomycin plus fusidin throughout the entire experimental period (Table 1). However, mice infected with GBS and left without drug treatment had developed the clinical signs of arthritis in their joints as early as 24 h post-infection (data not shown), and the maximum values of arthritis incidence and severity were reached at day 7 (Table 1). By contrary, therapy with daptomycin or fusidin significantly reduced the incidence and clinical severity of GBS-induced arthritis, and a higher anti-arthritic effect was observed in daptomycin-treated animals compared with those treated with fusidin (Table 1). Taken together, co-administration of daptomycin and fusidin resulted in a favorable synergistic interaction that strongly inhibited GBS infection-induced

Table 2. Effect of daptomycin and/or fusidin therapy on systemic and local bacterial growth and intra-articular production of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) in mice infected with type IV group B streptococci (GBS).

Group	n	GBS (CFU/ml)		COX-2(ng/ml)	PGE2 (pg/ml)
		Blood	Joint homogenate		
Normal control	10	0.0	0.0	0.4 ± 0. 1	9.6 ± 2.3
GBS	8	$2.3 \times 10^5 \pm 0.1 \times 10^{5a}$	$4.1 \times 10^6 \pm 0.1 \times 10^{6a}$	35 ± 9.3^a	560 ± 93^a
GBS +daptomycin	11	$0.7 \times 10^1 \pm 0.2 \times 10^{1b}$	$1.5 \times 10^1 \pm 0.1 \times 10^{1b}$	2.5 ± 0.2^b	45 ± 9.5^b
GBS +fusidin	9	$1.3 \times 10^2 \pm 0.4 \times 10^{2c}$	$2.3 \times 10^2 \pm 0.2 \times 10^{2c}$	9.3 ± 2.7^c	135 ± 21^c
GBS + daptomycin + fusidin	12	$0.0^{d,*}$	$0.1 \times 10^1 \pm 0.01 \times 10^{1d}$	$0.5 \pm 0.03^{d,*}$	$11 \pm 2.6^{d,*}$

Mice were infected with 10^7 colony-forming units of GBS/mouse and then treated or not-treated with daptomycin (6 mg/kg/day) and/or fusidin (80 mg/kg/day) for 6 consecutive days. At day 7, all survival mice were examined. Results are expressed as the mean ± SEM. ^{a}P <0.001, *P =NS, versus normal control group; ^{b}P <0.01, ^{c}P <0.05, ^{d}P <0.001, versus GBS-untreated group.

mortalities and septic arthritis in mice.

Effect of daptomycin and fusidin therapy on *in vivo* bacterial growth

To analyze the antibacterial impact of daptomycin and/or fusidin therapy on the *in vivo* growth rate of inoculatedGBS, the bacterial recovery rate from the blood and joints was examined on day 7 post-infection by using quantitative culturing processes. Data showed that a remarkable GBS burden was isolated from the blood and joints of untreated infected mice. However, when these infected mice were treated with daptomycin plus fusidin, the inoculated GBS were completely eradicated from their blood and nearly undetected in their joints (Table 2). A difference between the bacterial eradication effect of daptomycin and fusidin was also observed, whereas a lower extent of inoculated GBS was isolated from the blood and joints of daptomycin-treated group than of fusidin-treated group (Table 2). GBS elimination from the blood and joints of mice treated with daptomycin and/or fusidin was directly correlated with the survival rates and incidence and severity of arthritis (Table 1). Positive cultures were not observed in the blood and joints of normal control group (Table 2).

Effect of daptomycin and fusidin therapy on GBS infection-induced COX-2 expression and PGE2 production

It is well known that COX-2 is highly induced at the sites of inflammation by various inflammatory stimuli. Thus, COX-2 expression at its protein level was determined in the joints of all animal groups. To further assess the possible role of COX-2 in development of GBS arthritis, the intra-articular production of PGE2; an active secretory product of the enzymatic cascade initiated by COXs, was also assessed. At the end of the experimental period, an intensive amount of either COX-2 or PGE2 was detected in the joints of GBS-infected mice and did not receive any medication, as compared to values of normal control mice (Table 2). In contrast, concurrent treatment of these GBS-infected mice with daptomycin and/or fusidin significantly abrogated the intra-articular COX-2 expression and PGE2 production in a synergistic manner (Table 2).

Effects of daptomycin and fusidin therapy on GBS infection-induced systemic and local production of proinflammatory cytokines

Since the role played by TNF-α, IL-6 and IL-1β on the pathogenesis of GBS sepsis and arthritis is well-defined, the local and systemic concentrations of these proinflammatory cytokines were assayed in all experimental groups at day 7 after GBS inoculation. As compared to normal controls, TNF-α, IL-6 and IL-1β were impressively released into the blood and joints of mice infected with GBS and didn't receive antibiotic therapy (Table 3). On the contrary, treatment of GBS-infected mice with daptomycin significantly reduced both systemic and joint tissue levels of these proinflammatory cytokines, and this suppressor effect was significantly augmented by co-administration of fusidin with daptomycin (Table 3).

DISCUSSION

Despite substantial advances in diagnosis and treatment of human microbial diseases, group B streptococci (GBS) are still a leading cause of perinatal morbidity and mortality and an emerging public health problem in adults. Moreover, the emergence of multidrug-resistant GBS strains has complicated the clinical scenario and raised the critical need for development of more efficient alternative therapeutic strategies (Simoes et al., 2004; Nagano et al., 2008; Rajagopal, 2009). In the present study, the therapeutic efficacy of daptomycin, a novel lipopeptide antibiotic with potent activity against a broad

Table 3. Effect of daptomycin and/or fusidin therapy on production of proinflammatory cytokines in mice infected with type IV group B streptococci (GBS).

Group	n	TNF-α (pg/ml)		IL-6 (pg/ml)		IL-1β (pg/ml)	
		Serum	Joint	Serum	Joint	Serum	Joint
Normal control	10	5.3 ± 0.5	5.8 ± 0.7	3.7 ± 0.2	2.9 ± 0.3	6.3 ± 0.8	4.5 ± 0.4
GBS	8	57 ± 11[a]	83 ± 15[a]	600 ± 75[a]	223 ± 43[a]	260 ± 33[a]	167 ± 13[a]
GBS + daptomycin	11	9.5 ± 1.7[b]	11 ± 2.4[b]	37 ± 6.5[b]	13 ± 2.6[b]	20 ± 2.8[b]	13.5 ± 1.2[b]
GBS + fusidin	9	20 ± 3.5[c]	17 ± 3.3[c]	120 ± 25[c]	55 ± 7.7[c]	43 ± 6.3[c]	47 ± 6.3[c]
GBS + daptomycin + fusidin	12	5.8 ± 0.9[d,*]	6.2 ± 1.1[d,*]	4.0 ± 0.2[d,*]	3.1 ± 0.8[d,*]	7.0 ± 1.2[d,*]	5.1 ± 0.5[d,*]

Mice were infected with 10^7 colony-forming units of GBS/mouse and then treated or not-treated with daptomycin (6 mg/kg/day) and/or fusidin (80 mg/kg/day) for 6 consecutive days. At day 7, all survival mice were killed and the levels of tumour necrosis factor alpha (TNF-α); interleukin-6 (IL-6) and interleukin-1beta (IL-1β), in their sera and joint tissue homogenates were measured. Values are expressed the mean ± SEM. [a]P <0.001, *P =NS, versus normal control group; [b]P <0.001, [c]P <0.01, [d]P <0.001, versus GBS-untreated group.

spectrum of gram-positive bacteria (Figueroa et al., 2009), in the treatment of invasive systemic infection and septic arthritis induced in mice by type IV GBS was assessed. In addition, a possible beneficial anti-GBS interaction between daptomycin and fusidin, a narrow-spectrum antibiotic with unique immunomodulatory and anti-inflammatory properties (Milenkovic et al., 2005), was also investigated. Results showed that therapy with daptomycin significantly suppressed *in vivo* growth of inoculated GBS and its associated deaths and septic arthritis. More interestingly, co-administration of fusidin significantly enhanced daptomycin activity to combat GBS infection and its serious sequels.

In this study, data on bacterial clearance from the blood and joints of GBS-infected mice strongly supported the high *in vivo* efficacy of daptomycin against type IV GBS. Furthermore, co-administration of fusidin with daptomycin resulted in a clear *in vivo* synergy, whereas this combination therapy entirely eradicated the inoculated bacteria and completely prevented GBS-induced deaths and arthritis. These findings are in harmony with the previous facts that daptomycin has successful activity to eliminate other Gram-positive bacterial infections from the blood, muscle, kidney, heart, brain, joints and bone tissues of infected hosts (Oleson et al., 2000; Brauers et al., 2007; García-de-la-Mària et al., 2010). Moreover, both *in vitro* and *in vivo* synergy of daptomycin with other antimicrobials including aminoglycosides, β-lactams and rifampicin were also detected previously (Rand and Houck, 2004; Credito et al., 2007).

In this work, GBS-infected mice treated with daptomycin displayed less frequent and mild degree arthritis than infected untreated animals. This phenomenon was further heightened by co-administration of fusidin, whereas the whole clinical signs of GBS arthritis were entirely not observed in daptomycin/fusidin-treated animals. In consistency with these findings, the efficacy of daptomycin in treatment of bone and joint infections caused by other multidrug resistant gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), that were unresponsive to first-line antibiotics has previously been described (Rice and Mendez-Vigo, 2009). As a clinical fact, GBS is a significant causative agent of septic arthritis in neonates and adults; a disease associated with polyarticular involvement, bacteremia and a substantial case-fatality rate (Nolla et al., 2003). In addition, failure of its antibiotic therapy and delays in surgical drainage leads to progressive synovitis and irreversible destruction of cartilage and bone (Mader et al., 2000). Moreover, the emergence of multidrug-resistant GBS strains has increased the frequency of arthritic recurrence and complicated the clinical scenario (Simoes et al., 2004; Nagano et al., 2008). Therefore, there is a necessary need for development of more efficient therapeutic alternatives. In this regard, therapy with daptomycin plus fusidin can be considered as a promising alternative therapeutic approach.

The murine model of GBS infection and septic arthritis has proven to be beneficial in the elucidation of bacterial and host factors responsible for the development of human GBS disease (Tissi et al., 1990). In this model, a number of inflammatory pathways are initiated that collectively orchestrate the inflammatory and destructive events in the affected joints. Among the proinflammatory cytokines, TNF-α, IL-1β and IL-6 are secreted at high levels from macrophages and autoreactive T cells to play a major role in the pathogenesis of GBS sepsis, mortality and arthritis (Mikamo et al., 2004; Tissi et al., 1999). By pathogenic means, TNF-α and IL-1β are known to contribute directly to tissue damage through induction of the release of tissue-damaging enzymes from synovial cells and articular chondrocytes and through activation of osteoclasts (Van de Loo et al., 1995). In addition, IL-6 participates together with IL-1 in catabolism of connective tissue components at sites of inflammation (Ito et al., 1992) and activates osteoclasts, with a consequent increase in joint damage (Green et al., 1994). In constancy with these concepts, we observed that TNF-α, IL-1β and IL-6 were secreted at high levels in the blood

and joints of GBS-infected untreated mice, and their levels were directly correlated with the severity of GBS disease and its associated mortalities and arthritis. By contrast, the levels of these proinflammatory cytokines were extremely low in infected mice simultaneously treated with daptomycin/fusidin combination therapy. Mechanistically, there are atleast two possibilities by which daptomycin and fusidin had exerted their beneficial inhibitory effects on the production of TNF-α, IL-1β and IL-6. First, they directly killed the causative agent (that is, the inoculated type IV GBS). Second, it has been generally accepted that fusidin possess powerful immunomodulating and anti-inflammatory properties that are probably related to its capacity to reduce inflammatory cell infiltration and down regulate the production of proinflammatory cytokines including TNF-α, IFN-γ, IL-1β and IL-2 (Di Marco et al., 2001; Kilic et al., 2002; Milenkovic et al., 2005).

The discovery of cyclooxygenase (COX) isoforms (COX-1 and COX-2) led to the concepts that the constitutive COX-1 isoform tends to be homeostatic in function, while COX-2 is mainly induced during inflammation and tends to facilitate the inflammatory response (Smith et al., 2000). Prostaglandins (PGs) produced by COX-2 (especially PGE2) are potent inflammatory mediators and are associated with pain and other signs of inflammation (FitzGerald, 2003). In the present study, either COX-2 or PGE2 was intensively expressed in the joints of GBS-infected mice and did not receive any medication, while concurrent treatment of these infected mice with daptomycin and/or fusidin significantly and synergistically abrogated the intra-articular COX-2 expression and PGE2 production. An important question remains to be answered: how daptomycin and fusidin did inhibit COX-2 activity and subsequently the production of PGE2. There is evidence that COX-2 is induced in inflamed tissues by inflammatory chemical mediators including TNF-α and IL-1β (Smith et al., 2000). Accordingly, inhibition of these proinflammatory cytokines, as observed here, might, atleast in part, led to inhibition of COX-2 expression and PGE2 production.

Conclusions

The present study clearly indicates the potential therapeutic efficacy of daptomycin antibiotic against invasive GBS infection, and septic arthritis, and demonstrates its remarkable *in vivo* synergy with fusidin in combating the disease. Co-administration of daptomycin with fusidin greatly suppressed GBS infection at the microbiological, clinical and pathologic levels; 1) has complete eradicating effect on the inoculated bacteria, 2) has full protective effect against GBS-induced high mortality rates and high incidence of severe arthritis, 3) significantly down-regulated COX-2 expression and PGE2 production in the joint tissues and 4) significantly decreased the levels of proinflammatory cytokines (TNF-α, IL-1β and IL-6) in the blood and joints of infected mice. Taken together, daptomycin is a welcome newcomer antibacterial arsenal to eradicate gram-positive cocci including GBS, in particular when given in combination with other antibacterial agents such as fusidin.

REFERENCES

Brauers J, Kreshen M, Menke A, Orland A, Weiher H, Morrissey I (2007). Bactericidal activity of daptomycin, vancomycin, teicoplanin and linezolid against Staphylococcus aureus, *Enterococcus faecalis* and *Enterococcus faecium* using human peak free serum drug concentrations. Int. J. Antimicrob. Agents, 29(3): 322–325.

Credito K, Lin G, Appelbaum PC (2007). Activity of daptomycin alone and in combination with rifampin and gentamicin against Staphylococcus aureus assessed by time-kill methodology. Antimicrob. Agents Chemother., 51(4): 1504–1507.

Di Marco R, Puglisi G, Papaccio G, Nicoletti A, Patti F, Reggio A, Bendtzen K, Nicoletti F (2001). Sodium fusidate (fusidin) ameliorates the course of monophasic experimental allergic encephalomyelitis in the Lewis rat. Mult. Scler., 7(2): 101–104.

Dubost JJ, Soubrier M, De Champs C, Ristori JM, Sauvezie B (2004). Streptococcal septic arthritis in adults. A study of 55 cases with a literature review. Joint Bone Spine, 71(4): 303–311

Falck E, Hautala JT, Karttunen M, Kinnunen PK, Patra M, Saaren-Seppälä H, Vattulainen I, Wiedmer SK, Holopainen JM (2006). Interaction of fusidic acid with lipid membranes: Implications to the mechanism of antibiotic activity. Biophys. J., 91(5): 1787–1799.

Figueroa DA, Mangini E, Amodio-Groton M, Vardianos B, Melchert A, Fana C, Wehbeh W, Urban CM, Segal-Maurer S (2009). Safety of high-dose intravenous daptomycin treatment: three-year cumulative experience in a clinical program. Clin. Infect. Dis., 49(2): 177–180.

FitzGerald GA (2003). COX-2 and beyond: Approaches to prostaglandin inhibition in human disease. Nat. Rev. Drug Discov., 2(11): 879–890.

García-de-la-Mària C, Marco F, Armero Y, Soy D, Moreno A, del Río A, Almela M, Cervera C, Ninot S, Falces C, Mestres CA, Gatell JM, Jiménez de Anta MT, Miró JM (2010). Daptomycin is effective for treatment of experimental endocarditis due to methicillin-resistant and glycopeptide-intermediate *Staphylococcus epidermidis*. Antimicrob. Agents Chemother., 54(7): 2781–2786.

Genovese F, Mancuso G, Cuzzola M, Cusumano V, Nicoletti F, Bendtzen K, Teti G (1996). Improved survival and antagonistic effect of sodium fusidate on tumor necrosis factor alpha in a neonatal mouse model of endotoxin shock. Antimicrob. Agents Chemother., 40(7): 1733–1735.

Green J, Scotland S, Sella Z, Kleeman CR (1994). Interleukin-6 attenuates agonist-mediated calcium mobilization in murine osteoblastic cells. J. Clin. Invest., 93(6): 2340–2350.

Ito A, Itoh Y, Sasaguri Y, Morimatsu M, Mori Y (1992). Effects of interleukin-6 on the metabolism of the connective tissue components in rheumatoid synovial fibroblasts. Arthritis Rheum., 35(10): 1197–1201.

Kilic FS, Erol K, Batu O, Yildrim E, Usluer G (2002). The effects of fusidic acid on the inflammatory response in rats. Pharmacol. Res., 45(4): 265–267.

LaPlante KL, Rybak MJ (2004). Daptomycin - a novel antibiotic against Gram-positive pathogens. Expert Opin. Pharmacother., 5(11): 2321–2331.

Lee HC, Chong YE, Cheng YK (2007). Invasive Streptococcus agalactiae septic arthritis as an initial presentation of tonsillar carcinoma. Singapore Med. J., 48(7): 678–681.

Liakopoulos V, Petinaki E, Bouchlariotou S, Mertens PR, Trakala M, Kourti P, Riehl J, Ikonomov V, Stefanidis I (2004). Group B Streptococcus (*Streptococcus agalactiae*) peritonitis associated with continuous ambulatory peritoneal dialysis (CAPD). Clin. Nephrol., 62(5): 391–396.

Lukacs SL, Schoendorf KC, Schuchat A (2004). Trends in sepsis-related neonatal mortality in the United States, 1985–1998. Pediatr. Infect. Dis J., 23(7): 599–603.
Mader JT, Shirtliff ME, Bergquist S, Calhoun JH (2000). Bone and joint infections in the elderly: practical treatment guidelines. Drugs Aging, 16(1): 67–80.
Mikamo H, Johri AK, Paoletti LC, Madoff LC, Onderdonk AB (2004). Adherence to, invasion by, and cytokine production in response to serotype VIII group B Streptococci. Infect. Immun., 72(8): 4716-4722.
Milenković M, Vucićević D, Milosavljević P, Ranin NA, Vukanić ZS, Colić M (2005). Suppression of experimental autoimmune myocarditis by sodium fusidate (fusidin). Pharmacol. Res., 52(6): 491–496.
Nagano N, Nagano Y, Kimura K, Tamai K, Yanagisawa H, Arakawa Y (2008). Genetic heterogeneity in pbp genes among clinically isolated group B Streptococci with reduced penicillin susceptibility. Antimicrob. Agents Chemother., 52(12): 4258–4267.
Nicoletti F, Beltrami B, Raschi E, Di Marco R, Magro G, Grasso S, Bendtzen K, Fiorelli G, Meroni PL (1997). Protection from concanavalin A (Con-A)-induced T cell dependent hepatic lesions and modulation of cytokine release in mice by sodium fusidate. Clin. Exp. Immunol., 110(3): 479–784.
Nolla JM, Gomez-Vaquero C, Corbella X, Ordonez S, Garcia-Gomez C, Perez A, Cabo J, Valverde J, Ariza J (2003). Group B streptococcus (*Streptococcus agalactiae*) pyogenic arthritis in nonpregnant adults. Medicine (Baltimore), 82(2): 119–128.
Oleson FB, Berman CL, Kirkpatrick JB, Regan KS, Lai JJ, Tally FP (2000). Once-daily dosing in dogs optimizes daptomycin safety. Antimicrob. Agents Chemother., 44(11): 2948–2953.
Osman MO, El-Sefi T, Lausten SB, Jacobsen NO, Larsen CG, Jensen SL (1998). Sodium fusidate and the cytokine response in an experimental model of acute pancreatitis. Br. J. Surg., 85(11): 1487–1492.
Pettersson K (2007). Perinatal infection with Group B Streptococci. Semin Fetal Neonatal Med., 12(3): 193–197.
Pfaller MA, Sader HS, Jones RN (2007). Evaluation of the in vitro activity of daptomycin against 19615 clinical isolates of Gram-positive cocci collected in North American hospitals (2002–2005). Diagn. Microbiol. Infect. Dis., 57(4): 459–465.
Puliti M, Bistoni F, Tissi L (2010). Lack of B7-1 and B7-2 costimulatory molecules modulates the severity of group B Streptococcus-induced arthritis. Microbes Infect., 12(4): 302–308.
Puliti M, von Hunolstein C, Bistoni F, Castronari R, Orefici G, Tissi L (2002). Role of macrophages in experimental group B streptococcal arthritis. Cell Microbiol., 4(10): 691–700.
Rajagopal L (2009). Understanding the regulation of Group B Streptococcal virulence factors. Future Microbiol., 4(2): 201–221.
Rand KH, Houck HJ (2004). Synergy of daptomycin with oxacillin and other beta-lactams against methicillin-resistant Staphylococcus aureus. Antimicrob. Agents Chemother., 48(8): 2871–2875.
Rice DA, Mendez-Vigo L (2009). Daptomycin in bone and joint infections: a review of the literature. Arch. Orthop. Trauma Surg., 129(11): 1495-1504.
Rybak MJ, Hershberger E, Moldovan T, Grucz RG (2000). *In vitro* activities of daptomycin, vancomycin, linezolid, and quinupristin-dalfopristin against staphylococci and enterococci, including vancomycin-intermediate and -resistant strains. Antimicrob. Agents Chemother., 44(4): 1062–1066.
Simoes JA, Aroutcheva AA, Heimler I, Faro S (2004). Antibiotic resistance patterns of Group B Streptococcal clinical isolates. Infect. Dis. Obstet. Gynecol., 12(1): 1–8.
Skoff TH, Farley MM, Petit S, Craig AS, Schaffner W, Gershman K, Harrison LH (2009). Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990-2007. Clin. Infect. Dis., 49(1): 85-92.
Smith WL, De Witt DL, Garavito RM (2000). Cyclooxygenases: structural, cellular, and molecular biology. Annu. Rev. Biochem., 69: 145–182.
Tanaka Y, Otsuka T, Hotokebuchi T, Miyahara H, Nakashima S, Kuga Y, Nemoto H, Niiro H, Niho Y (1996). Effect of IL-10 on collagen-induced arthritis in mice. Inflamm. Res., 45(6): 283–288.
Tissi L, Marconi P, Mosci P, Merletti L, Cornacchione P, Rosati E, Recchia S, von Hunolstein C, Orefici G (1990). Experimental model of type IV Streptococcus agalactiae (group B Streptococcus) infection in mice with early development of septic arthritis. Infect. Immun., 58(9): 3093–3100.
Tissi L, Puliti M, Barluzzi R, Orefici G, von Hunolstein C, Bistoni F (1999). Role of tumor necrosis factor alpha, interleukin-1beta, and interleukin-6 in a mouse model of group B streptococcal arthritis. Infect. Immun., 67(9): 4545–4550.
Van de Loo FA, Joosten L, van Lent PL, Arntz OJ, van den Berg WB (1995). Role of interleukin-1, tumor necrosis factor alpha, and interleukin-6 in cartilage proteoglycan metabolism and destruction. Arthritis Rheum., 38(2): 164–172.
Verdrengh M, Tarkowski A (1997). Role of neutrophils in experimental septicemia and septic arthritis induced by *Staphylococcus aureus*. Infect. Immun., 65(7): 2517–2521.