Clinical Clues / *Klinik İpuçları*

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How Should the Inoculum Effect and its Clinical Interpretation be in the Treatment of Invasive Group A Streptococcal Infection?

İnvaziv Grup A Streptokok Enfeksiyonu Tedavisinde, İnokülüm Etkisi ve Klinik Yorumlanması Nasıl Olmalıdır?

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Question: *In general, we know that combinations of bactericidal (like penicillin/cephalosporin) and bacteriostatic (like clindamycin, macrolide) antibiotics can show antagonistic properties. It is recommended to give clindamycin in addition to penicillin (or cephalosporins) even if it is susceptible in invasive group A streptococcal infection. Could you tell us about the mechanism of this and its role in treatment?* **Gülsüm Mammadlı, MD.**

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Answer (Fatma Dilşad Aksoy, MD; Mustafa Kemal Hacımustafaoğlu, MD)

Introduction and general information: As it is known, bactericidal drugs are most effective especially against actively dividing bacteria. Bacteriostatic drugs, on the other hand, generally inhibit bacterial metabolism and inhibit their growth. As a general information, the combination of bactericide + bacteriostatic drug; may reduce the potential effect of the bactericidal drug, leading to a general decrease in efficacy. Therefore, such combinations are generally considered antagonistic (1). However, this general situation may not be fully valid for some clinical situations (such as invasive group A streptococcal infections) and for some etiologic agents (such as Group A streptococci; GAS; *Streptococcus pyogenes*, sometimes for staphylococci). In addition, specificially, the combination of penicillin (bactericide) and clindamycin (bacteriostatic) does not reveal significant additive, synergistic or antagonistic effects in-vitro (2,3).

The inoculum effect (IE) is generally defined as a significant increase in the minimal inhibitory concentration (MIC) against bacteria in the presence of much more bacteria in the environment, under laboratory conditions (4). In the literature various criteria can be used to define IE. Generally, the presence of viable bacteria concentration of 10 times or more than the standard bacterial density, or an increase in MIC, or a decrease in killing rates over time, are considered among the evaluation criteria of IE (3). IE is mainly defined as a decrease in the effect of antibiotics under laboratory conditions, but this also has clinical implications. In other words, under the conditions where the IE effect occurs; clinical failure may occur with an antibiotic (such as penicillin or cephalosporin) that is normally effective against the causative agent (such as GAS). In this context, IE can be defined as a decrease in anti-

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biotic effect in the presence of increased bacterial density (in vitro or in vivo) (5). If the bacterial density in the environment is much higher than expected, the bacterial population may continue to survive even if the presence of normal standard dose antibiotic concentrations (5). So, the presence of IE is undesirable as they may cause false resistance thought, increase clinical failure and mortality with given standard antibiotic doses, and lead to the formation of resistant pathogens later on (5).

The effect of IE is generally more pronounced with penicillin and other beta-lactam antibiotics. IE is generally less common for antibiotics such as aminoglycoside, quinolone, imipenem, chloramphenicol, and clindamycin (4). Although some antibiotics show IE, they can provide eradication of infection when given in appropriate doses (such as in higher doses). In such cases, due to the increased MIC values due to IE, treatment with antibiotics (such as cephalosporin or aztrenoam), high-dose (in a way to provide an antibiotic concentration of 200-400 times instead of 5-20 times of the standard MIC value) may be required (6). The risk of IE is higher if the number of bacteria in the medium is higher (for example, $>10^6$ CFU/mL) than the standard (is usually considered as 10^5 CFU/ml) (6). In experimental GAS infection, in the presence of high inoculum, bolus administration of penicillin was found to be more effective in bacterial killing compared to continuous infusion (3). Likewise, in the same laboratory-based study, clindamycin and trimethoprim-sulfamethoxazole provided more effective killing in samples with high bacterial density (3).

IE was first demonstrated by Eagle in 1952 in experimental GAS myositis in mice. For this reason, it can also be called the "Eagle effect" by some authors (7). In his study, penicillin was effective in GAS myositis when there were a low number of bacteria at the infection site or at the early stage of the disease. But it was found to be ineffective when given in the presence of high numbers of bacteria in the infected muscle or in the advanced fulminant infection period. Eagle attributed this to the effect of the inoculum (physiologic state of organism of the bacterium), and suggested that the sensitivity of penicillin was lost/decreased when there is high bacterial inoculum (7,8). Likewise, this has been demonstrated in other experimental studies, and bacteriostatic antibiotics such as clindamycin and erythromycin were found to be more effective than bactericidal penicillin in the presence of high bacterial inoculum (8,9).

Bacterial high inoculum and accompanying stationary phase growth features can be seen in in clinical conditions such as invasive skin and soft tissue infections (necrotizan faciitis), endocarditis, meningitis, osteomyelitis, abscess and other deep tissue infections and are associated with poor clinical course (8). In deep-seated and high-density bacterial infections of gram-positive and gram-negative bacteria, bacterial generation times (time to divide into 2 daughter bacteria) can be 60 times longer than in normal in vitro growth, and these slow-growing bacteria become increasingly tolerant of beta-lactam antibiotics (8). Some studies revealed that, there may be changes in penicillin binding proteins (PBP) on the surface of bacteria (such as GAS, *Escherichia coli*, *Haemophilus influenza*) when they pass from exponential phase growth to stationary phase growth (8,10,11). It has been shown that there is a decrease in PBP 1 and PBP 4 levels in experimental GAS myositis during the stationary growth phase. In addition, in the presence of a large number of bacteria in the medium, the binding affinity of penicillin to PBPs decreased in the stationary phase. In this context, it can be thought that the effect of ceftriaxone and other beta-lactam antibiotics will decrease as well as penicillin (8). Compared to penicillin and ceftriaxone in this model, although the in vitro activity of clindamycin was lower, a better in vivo therapeutic was observed (8). This suggests that the antibacterial effect of clindamycin, as a bacteriostatic protein synthesis inhibitor, is not affected by PBP expression or affinity (8). Clindamycin may also decrease M protein synthesis or bacterial exotoxins of GAS (8,12,13). In addition, clindamycin can increase host phagocytosis and intracellular killing (14).

Among the causes of antibiotic treatment failure in GAS infection; it has been suggested that changes in antibiotic susceptibility may play a role when bacteria are in different population densities and reproduction phases. This event is described as IE as mentioned above (3). In case of increased bacterial density or treatment delay, IE becomes more evident (3,8). In parallel, there may be an increase in MIC levels in the presence of increased inoculum (3). IE can also be observed in *Staphylococcus aureus* infections, and in such a case, a better clinical outcome can be achieved with vancomycin + clindamycin dual therapy (3).

GAS is extremely sensitive to beta-lactam antibiotics, which are bactericidal. However, clinical failures may occur with penicillin treatment alone, especially in patients with invasive GAS infection where more organisms may be present (13,15). Experimental infection studies have shown an association between penicillin monotherapy and treatment failure in the setting of high bacterial inoculum (7-9,16,17). In general, beta-lactam antibiotics are believed to be most effective against rapidly growing bacteria. Efficacy is probably highest, especially in the early stages of infection and when there are no excessive bacteria in the environment. However, the rate of bacterial growth/division slows down relatively after organisms multiply very rapidly initially, and the concentrations of organisms in the medium increase causing high inoculum. Then, the bactericidal beta-lactam antibiotic effect is relatively

reduced. This situation may be more pronounced especially in the setting of deep-seated infections (such as necrotizing skin and soft tissue infections) (7).

Although clinical trials are lacking, evidence from observational studies suggests that combination treatment with a beta-lactam (cell wall-inhibiting antibiotic) plus clindamycin (protein synthesis-inhibiting) is superior to beta-lactam alone for the treatment of invasive GAS infection (2,18). Some of the studies supporting this issue, can be summarized as follows. In a retrospective study, clinical improvement in the first 24 hours in children with invasive GAS infection (n= 56), was found to be 14% of children receiving only a cell wall-inhibiting antibiotic (e.g. beta-lactams) and 84% of children with additional clindamycin supplementation (15). In another study, in a retrospective study of 84 adult patients with severe invasive GAS infection (e.g. streptococcal toxic shock syndrome, necrotizing fasciitis, septic shock, cellulitis with hypotension), the addition of clindamycin to beta-lactam therapy was associated with reduced mortality (15% vs. 39%) (19). In another retrospective study, mortality was evaluated in patients with invasive GAS infection (n= 1079); while mortality was 11% in those who received only beta-lactam antibiotics, it was found to be lower (6.5%) in those who received additional clindamycin (20).

The potential advantageous mechanisms of clindamycin in the treatment of invasive GAS infection can be summarized as follows: The efficacy of clindamycin is not affected by inoculum size or growth stage, clindamycin suppresses bacterial toxin production and thus reduces clinical adverse events, clindamycin has a long postantibiotic effect compared to beta-lactam antibiotics (2,8,12,21-23). For these reasons, clindamycin should be used in combination with a beta-lactam antibiotic in the treatment of invasive GAS infection or streptococcal TSS. This combination does not show antagonistic properties, but rather contributes to the simultaneous killing of bacteria in different growth phases and early clinical improvement. However, in non-invasive GAS infections that are common in the community (such as GAS tonsilopharyngitis, simple streptococcal impetigo), since IE is not expected, only penicillin treatment is given and it is sufficient, there is no need to add clindamycin.

However, because of clindamycin is not bactericidal and GAS resistance to clindamycin increases in some regions, it should not be used as a single agent (24,25). An increasing number of GAS isolates (such as 15%) with constitutive or inducible resistance to clindamycin and other macrolide-lincosamide-streptogramin B (MLS) antibiotics have been identified in the United States. Again, in Europe, an increasing rate of structural or inducible resistant GAS isolates have been identified against clindamycin and MLS antibiotics (24, 26-28). In China, clindamycin resistance was found to be higher (as 94%) in GAS isolates (29).

In invasive GAS infection, treatment should be started empirically, but treatment should be continued according to culture results. In suspected invasive GAS infection, treatment should be started without delay, and it should be kept in mind that IE may occur even if treatment is delayed within hours. Clinical pictures of the patients such as sepsis and streptococcal toxic shock syndrome (TSS) cannot be clinically differentiated from sepsis syndromes due to other pathogens. Therefore, empirical therapy should be broad-spectrum, covering not only GAS but also *S. aureus* (including methicillin-resistant *S. aureus*) and gram-negative bacilli. Then, treatment is guided by culture results. In a patient with suspected streptococcal TSS, clindamycin + vancomycin + (a penicillin/beta-lactamase containing antibiotic such as carbapenem or piperacillin/tazobactam) may be appropriate initially (2). If carbapenems cannot be tolerated, fluoroquinolones can be used instead. After the diagnosis of streptococcal TSS is confirmed, clindamycin + penicillin G is given. For patients with beta-lactam hypersensitivity (in the absence of anaphylaxis), alternatives to penicillin include cefotaxime or ceftriaxone. Vancomycin or daptomycin may be considered as penicillin alternatives for patients with a history of anaphylaxis to beta-lactams. In the presence of clindamycin-resistant GAS, penicillin + linezolid can be given (2). Like clindamycin, linezolid is a protein synthesis inhibitor, suppresses toxin production, and has a long post-antibiotic effect (30-32). Unlike clindamycin, linezolid passes well into the cerebrospinal fluid (CSF/blood ratio is approximately 60-70%) (33). Therefore, the combination of penicillin (or 3rd generation cephalosporin) + linezolid is a rational approach in invasive GAS infections involving the central nervous system.

Combination therapy with penicillin and clindamycin in invasive streptococcal infection due to GAS susceptible to clindamycin, should be maintained until patients are clinically and haemodynamically stable for at least 48 to 72 hours (in the absence of necrotizing fasciitis, when the child is fever-free, clinically well, and other manifestations of shock or toxic shock syndrome). Then, after clinically and haemodynamically stablization penicillin monotherapy can be continued (2).

Surgical evaluation and surgical exploration and resection of necrotic tissue, if necessary, are important in the patient (18).

Patients with GAS bacteremia are treated for at least 14 days. In patients with severe soft tissue infections (such as necrotizing fasciitis), the duration of treatment depends on the patient's clinical response. Treatment is usually continued for 14 days from the last positive culture obtained during surgical

debridement. In general, there are no clinical studies addressing the optimal duration of antibiotic therapy in GAS bacteremia or invasive infections, and the duration of antibiotic therapy should be individualized (2).

In conclusion: IE is generally defined as a significant increase in the minimal inhibitory concentration (MIC) against bacteria in the presence of normally much more bacteria in the environment under laboratory conditions. In conditions where IE has the effect of IE (such as invasive GAS infection); Clinical failure may occur with a normally susceptible bactericidal antibiotic (such as penicillin or cephalosporin). To prevent this, clindamycin (a bacteriostatic agent) must be added in addition to the bactericidal penicillin (or beta-lactam antibiotic).

As a general information, the combination of bactericide + bacteriostatic drug; generally considered antagonistic. However, in some situations, this is not the case. Clindamycin should be used in combination with a beta-lactam antibiotic in the treatment of invasive GAS infections. This combination does not show antagonistic properties, but rather contributes to the simultaneous killing of bacteria in different growth phases and early clinical improvement. However, in non-invasive GAS infections that are common in the community (such as GAS tonsilopharyngitis, simple streptococcal impetigo), since IE is not expected, only penicillin treatment is given and it is sufficient, there is no need to add clindamycin.

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