THE EFFECTS OF TIGECYCLINE ON HUMAN OSTEOBLASTS *IN VITRO*

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RESUMO

A osteomielite, infecção no tecido ósseo, é uma doença que afecta gravemente os pacientes, uma vez que destrói grandes quantidades de tecido ósseo. A tigeciclina é um antibiótico recentemente disponível, que pode proporcionar melhores resultados no combate a infecções ósseas por organismos resistentes a outros antibióticos. Não existem na bibliografia dados disponíveis sobre a interacção da tigeciclina com as células ósseas. Neste trabalho foi demonstrado que a tigeciclina em elevadas concentrações tem efeitos nocivos para o tecido ósseo nomeadamente na proliferação dos osteoblastos. Esta informação deve ser ponderada na aplicação de dispositivos de aplicação local do antibiótico. Em baixas concentrações este fenómeno não foi verificado.

PALAVRAS-CHAVE: Osteomielite; Tigeciclina; Osteoblastos; Cultura celular.

ABSTRACT

Osteomyelitis (bone tissue infection), is a very serious disease affecting many patients by destroying large portions of bone tissue. Tigecycline is a recently available antibiotic to overcome bacteria resistance phenomena in bone tissue infections. No information is available regarding its direct effects on bone cells. We have demonstrated deleterious effects of high tigecycline concentrations on viability and proliferation of osteoblasts *in vitro*. This finding should be carefully taken into account when a local antibiotic application is used. However, at lower concentrations tigecycline this effect was not observed.

KEY-WORDS: Osteomyelitis; Tigecycline; Osteoblasts; Cell culture.

1. INTRODUCTION

Osteomyelitis is a very serious disease affecting many patients by destroying large portions of bone tissue, nowadays going through an expansion as a consequence of bacterial infection following immune deficiency caused by HIV/Aids virus and the associated infections, and increasing number of associated infections with multiple antibiotic resistances, namely tuberculosis, due to inappropriate use of antibiotics.

Osteomyelitis and prosthetic joint infections are challenging to treat. The most common treatment regimen involves surgical debridment, or implant removal and systemic antibiotic treatment for at least 4-6 weeks (Houshian e Zawadski *et al.*, 2000; Mader e Shirtliff *et al*., 1999; Mader e Wang *et al.*, 2001). This approach may lead to success, but there are still significant relapse rates. With the objective of increasing local antibiotic concentrations compared to those achieved following oral or systemic administration (Heard e Oloff *et al.*, 1997; Huneault e Lussier *et al.*, 2004; Mohanty e Kumar *et al.*, 2003; Smilack e Flittie *et al*., 1976), several local delivery systems have been developed (del Real e Padilla *et al.*, 2000; Nijhof e Dhert *et al.*, 2000; Suzuki e Tanihara *et al*., 1998; Yagmurlu MF e Korkusuz *et al.*, 1999). Local drug delivery systems are used in orthopedic surgery to prevent occurrence or re-occurrence of prosthetic joint infections and can be used to treat osteomyelitis and implant infections. With the use of local delivery system very high local antibiotic concentrations can be achieved without exposing the patient to toxic systemic levels.

Gram positive organisms are responsible for the majority of bone and joint infections. *Staphylococcus aureus* is the single most common organism causing osteomyelitis and septic arthritis (Sax e Lew, 1999; Waldvogel e Vasey, 1980; Goldenberg, 1998; Hamed e Tam *et al.*, 1996). Coagulase-negative staphylococci (CoNS) are more prevalent in prosthetic joint infection (PJI) followed by *S. aureus* (Brause, 2000). β–Haemolytic streptococci are also responsible for bone infection, e.g. Lancefield group B osteomyelitis in neonates and group A septic arthritis in other age groups. Enterococci are recognized causes of PJI (Raymond e Henry *et al.*, 1995) as are non-haemolytic and viridans streptococci. In contrast, *Streptococcus pneumoniae* is a relatively rare cause of septic arthritis and raises the question of underlying immunosuppression (Oliker e Cunha *et al.*, 1999)as does *Listeria monocytogenes*, a rare cause of PJI (Allberger e Kasten *et al.*,1992; Massarotti e Dinerman, 1990).

Anaerobes may contribute to polymicrobial osteomyelitis in vasculopathic infection such as diabetic foot infection and in septic arthritis following animal bites. Gram negative organisms are responsible for a low proportion of all bone and joint infections although particular patient groups are predisposed to specific Gram negative infections. Prior to the introduction of the Hib vaccine, for example *Haemophilus influenza* was a major cause of septic arthritic joint in children of pre-school age but this is now a much rarer case, while *Neisseria gonorrhoea* may be responsible for septic arthritis in young adults (Darley e MacGowan, 2004).

The choice of antibiotic to be used should be considered carefully in order to overcome resistant bacteria phenomena. Delayed or ineffective treatment causes significant morbidity in terms of pain, loss of function and the need or further surgery and antibiotics. The relatively high failure rate following antibiotic treatment of bone infection is well documented. Risk factors for poor outcome include inadequate initial debridment, the presence of prosthetic material, duration of infection and previous treatment failure. Selection of the most appropriate antibiotic therapy will therefore need to take several facts into account: the organism isolated and its sensitivity profile, penetration into bone and the patient individual tolerance to drugs (Darley e MacGowan, 2004).

In the past decade, increasingly resistante organisms, e.g. methicilin-resistant sthaphylococci (MRSA) and vancomycin-resistant enterococci have been recognized as causes of orthopaedic infection. Vancomycin and Linezolid respectively, are the antibiotics of choice in such infections. Tigecycline, recently available antibiotic is other alternative to overcome bacteria resistance phenomena (Yin e Lazzarini *et al.*, 2005).

Tigecycline is the 9-t-butylglycylamido derivative of minocycline and is the first in the class of glycylcycline antibiotics to be developed. Tigecycline acts by inhibition of protein translation in bacteria by binding to the 30S ribosomal subunit and blocking entry of amino-acyl tRNA molecules into the A site of the ribosome. This prevents incorporation of amino acid residues into elongating peptide chains. Unlike the classical tetracyclines, tigecycline is not affected by any of the known tetracycline resistance determinants. Tigecycline demonstrates activity against a broad range of gram-positive (particularly MRSA and vancomycin resistant species) gram-negative, aerobic, anaerobic, and atypical antibiotic-susceptible and –resistant bacteria (Bradford e Petersen *et al.*, 2005).

Although tigecyclyne is an alternative antibiotic for bone tissue infections there is no data available concerning the direct effects of tigecycline on human bone cells. The objective of this study was to investigate the effects of tigecycline on proliferation of human osteoblasts *in vitro*.

2. **MATERIALS AND METHODS**

2.1. **CELL CULTURE**

Human bone marrow (obtained from surgery procedures) was cultured in α–MEM containing 10% foetal bovine serum (FBS), 2.5µg ml^{-1} fungizone and 50 µg ml^{-1} gentamicine (standard medium) until near confluence (approximately, 10-15 days). Detailed description is fully described elsewhere (Fernandes e Costa *et al.*, 1997, Ferraz e Monteiro *et al.*, 1999). At this stage, adherent cells were enzimatically released (0.04% trypsin and 0.025% collagenase), counted using a hemocytometer and cultured at a density of 10^4 cell cm^{-2}, in 96-well cultured plates, for periods of up to 28 days with standard medium supplemented with ascorbic acid (AA) (50g.ml^{-1}), β-glicerophosphate (βGP) (10mmol.L^{-1}) and dexamethasone (Dex) (10nmol.L^{-1}) without antibiotic (control) and in the presence of 0.1; 1 and 10 µg/ml of tigecycline.

Cultures were characterised at days 3, 7, 14, 21 and 28 for cell growth parameters. Cultures were incubated at 37°C in a humidified atmosphere of 95% and 5% CO_2 and culture medium was changed twice a week; monitoring of the cultures was done daily using phase contrast inverted.

2.2. **CELL VIABILITY/PROLIFERATION**

MTT assay (reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to a purple Formazan product) was used to estimate cell viability/proliferation. Cells were incubated with 0.5mg ml-1 of MTT in the last 4 hours of the culture period tested; the medium was then decanted, Formazan salts were dissolved with dimethyl-sulphoxid and the absorbance was determined at 560nm.

Statistical analysis for MTT data was based on each point represents the mean standard deviation of 8 measurements of each sample.

3. **RESULTS AND DISCUSSION**

Human bone marrow cells were cultured in standard medium in the presence of AA, βGP and Dex for periods up to 28 days under selected experimental conditions (presence of different tigecycline concentrations). All the experiments were performed in the first subculture as previous results showed that serial passage of bone marrow cells results in a progressive loss of the osteoblastic phenotype.

Results concerning the cell proliferation/differentiation behaviour of the cultures growing in the different experimental conditions are shown in Figures 1 and 2. Bone marrow cells proliferated gradually with the incubation time. After a lag phase of approximately on week, bone marrow cells enterd a period of active proliferation until day 14, after that decrease in cell growth was observed.

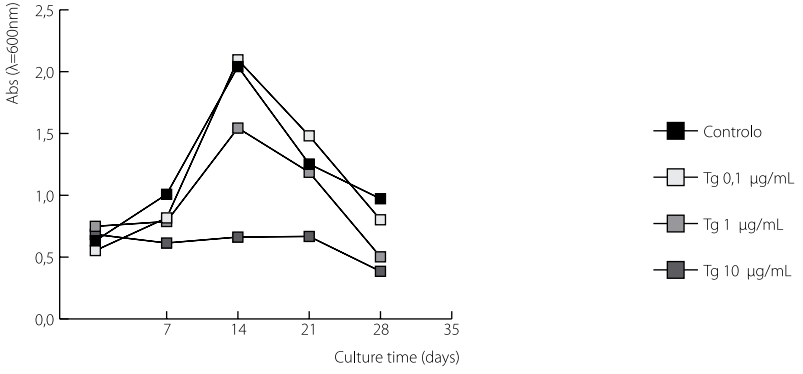

Figure 1. Effects of the presence of 0,1μg.mL^{-1} (□); 1μg.mL^{-1}(■);and 10μg.mL^{-1}(■) tigecyclin on the cell viability/proliferation of human bone marrow cultured cells. Cells cultured in the absence of tigecycline represent control cells (■). The MTT assay was carried out at day 7, 14, 21 and 28 of culture as described in materials and methods. (média ± desvio padrão, n = 6, * p< 0,05 teste t de Student).

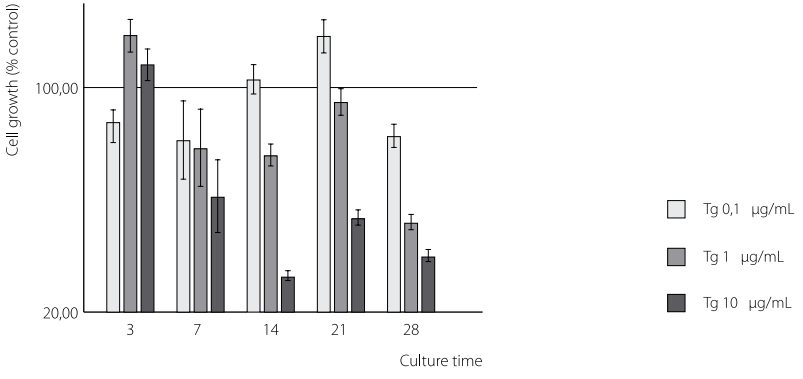

Figure 2. Cell growth relative to control cultures. The bars show the average of MTT values (±SD) for the of 0,1μg.mL^{-1}(close bars); 1μg.mL^{-1}(striped bars);and 10μg.mL^{-1}(open bas), relative to the MTT of the control culture in each experiment, which is defined as 100%.

Cultures grown in the presence of 0.1μg.mL^{-1} tigecycline presented a similar cell growth, being MTT reduction slightly higher than control on day 21. Cultures grown in the presence of 1 μg.mL^{-1} presented lower cell/ viability than control cultures indicating that the presence of this concentration of antibiotic

is slightly toxic to osteoblastic cells. The 10 $\mu g.mL^{-1}$ tigecycline concentration severely affects cell growth clearly indicating a severe toxicity to osteoblastic cells (statistically different from other concentrations).

To our knowledge, the present study has demonstrated the inhibitory effects of high concentration of tigecycline on human osteoblasts for the first time. Our results are in line with previous reports for other antibiotics. Edin *et al.* (Edin e Miclau *et al*., 1996) compared the effects of the antibiotics vancomycin and cefazolin on osteoblastic-like cells. These results are particularly important when thinking of local delivery systems, once that tigecycline levels near bone tissue following systemic application are considerably low. Low tigecycline concentrations revealed no cytotoxic effects.

These preliminary results indicate the necessity of studing not only cell viability/ proliferation but also mineralization. The differentiation of human bone marrow cells into osteoblastic cells can be evaluated using several mineralization markers. Therefore in the future it will be important to evaluate the effect of tigecycline in the differentiation of osteoblastic cells.

4. **CONCLUSION**

Calculated MIC cut-off values for wild-type strains were between 0.11 and 0.96 mg/L for Gram-positive species, and between 0.44 and 8.3 mg/L for Gram-negative species, except for *Pseudomonas aeruginosa*, which had a cut-off value of 450 mg/L, consistent with earlier reports on the lack of activity of tigecycline against this species therefore a wide variety of concentrations may have to be used to overcome an infection. (Kronvall e Karlsson *et al*., 2006).

In conclusion, we have demonstrated deleterious effects of high tigecycline concentrations on viability and proliferation of osteoblasts *in vitro*. This finding should be carefully taken into account when a local antibiotic application is used, and also in some cases cited in the above paragraph. However, at lower concentrations tigecycline this effect was not observed. Further investigation is necessary to determine the impact of this antibiotic on local bone metabolism.

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