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Experimental Study / *Deneysel Çalışma*

In Vitro Activity of Tigecycline Against Brucella spp.

Tigesiklinin Brucella spp'ye Karşı İn Vitro Aktivitesi

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Objectives: There are no published data regarding efficacy of tigecycline in brucellosis and in vitro data are scarce. We compared the in vitro activity of tigecycline to that of trimethoprim-sulfamethoxazole, rifampicin, tetracycline, streptomycin and ciprofloxacin against *Brucella* spp.

Study Design: In vitro activities of tigecycline, trimethoprim-sulfamethoxazole, rifampicin, tetracycline, streptomycin and ciprofloxacin were evaluated against 96 strains of *Brucella* spp. Minimal inhibitory concentrations (MIC) were determined by the E-test method.

Results: Tigecycline had low MIC_{50} and MIC_{90} values against all Brucella isolates; the highest MIC observed was 0.19 μg/mL.

Conclusion: Tigecycline had low MICs against Brucella spp. including tetracycline-resistant isolates and its use in therapy should be confirmed by clinical studies.

Key words: Tigecycline; *Brucella* spp.

Amaç: Brucelloz tedavisinde tigesiklinin in vitro etkinliği ile ilgili bilgi çok fazla bulunmamaktadır. Çalışmamızda *Brucella* cinsi mikroorganizmalara karşı trimetoprim-sülfametaksazol, rifampisin, tetrasiklin, streptomisin ve siprofloksasin ile tigesiklinin in vitro aktivitesinin karşılaştırılması amaçlanmıştır.

Çalışma Planı: *Brucella* cinsi 96 izolata karşı tigesiklin, trimetoprim-sülfametaksazol, rifampisin, tetrasiklin, streptomisin ve siprofloksasinin in vitro aktivitesi belirlenmiştir. Minimal inhibitör konsantrasyon (MİK) değerleri E-test yöntemiyle saptanmıştır.

Bulgular: Tüm *Brucella* izolatlarına karşı en düşük $MİK_{50}$ ve $MİK_{90}$ değerleri tigesiklinde belirlenmiştir. Tigesiklinin en yüksek MİK değeri 0.19 μg/mL olarak saptanmıştır.

Sonuç: Tetrasikline dirençli olan izolatlar da dahil olmak üzere *Brucella* izolatlarına karşı en düşük MİK değeri tigesiklin ile belirlenmiştir. Tigesiklinin tedavide kullanımı klinik çalışmalar ile onaylanmalıdır.

Anahtar sözcükler: Tigesiklin, *Brucella* spp.

Brucellosis is a widespread and important zoonosis with serious implications for human and animal health. The disease is endemic in the Mediterranean Basin, the Middle East, India, and Central and South America and is an important public health problem in Turkey.[1-4] Due to their being intracellular pathogens, successful therapy can only be achieved by combination regimens that can effectively penetrate the macrophages. One of the most effective treatments is the combination of doxycycline with streptomycin or rifampicin.[1,4] Both have certain disad-

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vantages such as the long duration of treatment, toxicity and side effects. Tigecycline is a novel glycylcycline derivative of a tetracycline, minocycline.[5] It has been demonstrated to exhibit in vitro activity against aerobic and anaerobic Gram-positive and Gram-negative microorganisms.

MATERIALS AND METHODS

Brucella spp. isolates (n=96) were collected between 1991-2006 from blood and bone marrow cultures of patients with acute brucellosis admitted to Hacettepe University Hospital, Ankara, Turkey. The organisms were identified to genus level by conventional methods, stored at –70 ºC and subcultured twice before the susceptibility tests. All tests were carried out in a Class II biological safety cabinet. Minimal inhibitory concentrations (MIC) for tigecycline, trimethoprim-sulfamethoxazole (TMPSMX), rifampicin, tetracycline, streptomycin and ciprofloxacin were determined by E-test (AB Biodisk, Sweden) method on Mueller-Hinton agar (Oxoid, Basingstoke, UK) supplemented with 5% sheep blood and interpreted after 48 hours of incubation at 5% CO_2. CLSI breakpoints were employed for the results.[6] *Staphylococcus aureus* ATCC 29213 was used as the quality control strain for susceptibility testing.

RESULTS

A total of 96 strains of *Brucella* spp. were studied. In vitro activities of tigecycline, TMPSMX, rifampicin, tetracycline, streptomycin and ciprofloxacin against these isolates were evaluated. The MIC_{50}, MIC_{90} values of the antimicrobial agents are shown in the Table 1.

Tigecycline had low MICs against all *Brucella* isolates; the highest MIC observed was 0.19 μg/mL. Three isolates were resistant to TMP/SMX, tetracycline and streptomycin and had MICs >32 μg/mL for ciprofloxacin. MICs of tigecycline were 0.064-0.094 μg/mL for these isolates.

DISCUSSION

Brucellosis is a zoonotic disease which can be treated with combination of antimicrobial agents such as doxycycline, rifampicin, ciprofloxacin, tetracycline and streptomycin. However, the best regimen for the treatment is not clearly determined. Complete eradication of the microorganism is difficult to achieve and relapses are common.[7]

Several in vitro tests have been employed to determine the most effective antimicrobial agents in vitro against *Brucella* spp.[1,3,4,7] As the interpretive criteria for *Brucella* spp. were not available until recently for some agents, results of in vitro studies were given as MIC_{50} and MIC_{90} values. Rubinstein et al.[4] have reported MIC_{90} values of 3.1, 6.3, 4.0 and 0.8 μg/mL respectively for streptomycin, TMP/SMX, rifampicin and ciprofloxacin. Akova et al.[1] reported MIC_{90} values of 2.0 μg/mL for all three agents; streptomycin, rifampicin and ciprofloxacin, while Garcia-Rodriguez et al.[8] have reported the MIC_{90} values for streptomycin, 4.0 μg/mL; TMP/SMX, 4.0 μg/mL and rifampicin 1.0 μg/mL. MIC_{90} value for ciprofloxacin was 0.5 μg/mL in another study.[9]

Table 1. MIC Range, MIC_{50} and MIC_{90} values and the rates of resistance against antimicrobial agents

Antimicrobial agents	MIC (μg/ml)			Resistance (%)
	Range	MIC_{50}	MIC_{90}	
Tigecycline	0.016-0.19	0.047	0.094	ND[a]
Trimethoprim-sulfamethoxazole	0.006->32	0.032	0.125	3.1
Rifampicin	0.125->32	1	2	ND
Tetracycline	0.016-12	0.047	0.125	3.1
Streptomycin	0.125-256	1	2	8.3
Ciprofloxacin	0.032->32	0.19	0.38	ND

ND[a]: Not determined as there are no CLSI breakpoints.

Our results for streptomycin are in the range of results of similar studies. MIC_{90} value of TMP/SMX is 0.125 μg/mL and resistance is 3.1%, which is lower compared to other studies. MIC_{90} values of rifampicin and ciprofloxacin are also in accordance with similar studies. Resistance rates could be given for TMP/SMX, tetracycline and streptomycin according to CLSI[6] in our study and were highest for streptomycin (8.3%).

Tigecycline is a new antimicrobial agent which has been shown to be effective in vitro against aerobic and anaerobic Gram-positive and Gram-negative microorganisms.[5] Its activity against *Brucella* spp. have been investigated in several studies.[1,3,4,7-10] As resistance breakpoints are not available for this agent in *Brucella* spp., in vitro efficacies can be compared using MIC_{90} values. MIC_{90} value for tigecycline is 0.09 μg/mL in our study. Dizbay et al.[5] reported 0.094 μg/mL and Turan et al.[11] reported 0.125 μg/mL MIC_{90} values for this agent. In all three studies, including ours, E-test methodology have been employed and the results are very similar.

There are very little published data regarding the effect of tigecycline against *Brucella* spp in Turkey. These results indicate that tigecycline has good in vitro activity against *Brucella* spp., including isolates resistant to other agents. Tigecycline has not been affected in vitro by the mechanism of resistance which causes tetracyline resistance. Its efficacy should be confirmed by clinical studies.

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