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Is Intraarticular Antibiotic Administration Effective in the Treatment of Methicillin-Resistant *Staphylococcus aureus?*

Je intraartikulární podávání antibiotik efektivní v léčbě infekce způsobené methicillin-rezistentním stafylokokem?

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ABSTRACT

PURPOSE OF THE STUDY

Septic arthritis is an infection of joints caused by a pathogenic microorganism. Septic arthritis has a mortality rate of 11–40% when it's not treated properly. The mortality rate with methicillin-sensitive *Staphylococcus aureus (MSSA)* is 5–7%, while the rate with methicillin-resistant *Staphylococcus aureus (MRSA)* is 13–20%. The aim of this study is to evaluate the effects of intraarticular vancomycin and teicoplanin on joint cartilage in in vivo settings and its utility in routine MRSA treatment.

MATERIALS AND METHODS

In our study, 35 male Sprague-Dawley rats aged 28 days were used. Rats were obtained from the Regenerative and Restorative Medicine Research Center (REMER) of Istanbul Medipol University. Rats were randomly divided into 5 groups each containing 7 rats. Joint injections were administered with isoflurane analgesia every day at 6 am. Three rats (15 rats) from each group were sacrified in seventh day and evaluated immunohistologically to evaluate acute healing in articular cartilage. All remaining rats were sacrificed on day 28 and their knees were evaluated by immunohistochemical examination.

RESULTS

In our study, there were no complications in any rat during injection and the study period. Hematoxylin eosin (H & E) histological staining for evaluating cartilage healing and healing levels did not show statistically significant differences between the groups at first week ($p > 0.05$). Matrix metalloproteinase-13 (MMP-13) staining did not show any statistically significant difference between the groups. ($p > 0.05$).

DISCUSSION

MRSA septic arthritis, diagnosed for the first time in 1960, has recently been responsible for 6-22% of all septic arthritis and is increasing day by day. The use of systemic vancomycin or teicoplanin is the first-line treatment method in *MRSA* septic arthritis. Serum levels reach the desired level, especially with intravenous infusion dose. On the other hand, it has been shown that intraarticular concentration does not reach a sufficient level in studies conducted. The use of intraarticular antibiotics during treatment can lead to more effective and early disease control by turning this negative situation into favor of the patient. As a result, intraarticular vancomycin and teicoplanin maximale tolerable and maintenance doses can be safely used beside surgery and intravenous antibiotics to increase efficacy of treatment, reduction of recurrence rates and reduction of mortality in *MRSA* septic arthritis.

CONCLUSIONS

Intraarticular vancomycin and teicoplanin maximale tolerable and maintenance doses can be safely used beside surgery and intravenous antibiotics to increase efficacy of treatment, reduction of recurrence rates and reduction of mortality in *MRSA* septic arthritis.

Key words: arthritis, infectious; methicillin-resistant *Staphylococcus aureus*; mortality.

INTRODUCTION

Septic arthritis is an infection of joints caused by a pathogenic microorganism. The synovial membrane is the most important structure in the formation of septic arthritis. It doesn't have effective basal membrane and a good vascularity that eliminates the transition of the microorganisms from blood into the knee (14) The incidence is 4–29/100,000 patients/year (11). Cartilage and subchondral bone damage begin within 24–48 hours if appropriate antibiotic therapy is not applied (6). Joint drainage and antibiotic therapy are the basis of treatment (12).

Septic arthritis has a mortality rate of 11–40% without treatment (2, 13). In addition to patient-related risk

factors such as polyarticular involvement, immunodeficiency, and rheumatoid arthritis, the infectious pathogen is also important in increasing the mortality risk (2, 8). In recent years, independent of the risk factors of septic arthritis, 40–50% *Staphylococcus aureus* is responsible for pathogenicity. *Methicillin-resistant S. aureus (MRSA)* is the causative agent in 6–22% of the cases and this rate is increasing day by day (3, 5). The mortality rate with *methicillin-sensitive S. aureus (MSSA)* is 5–7%, while the rate with *MRSA* is 13–20% (1, 15). The main reason for the high mortality rate of *MRSA* is the insufficiency of the applied antibiotics for *MRSA* during the period of culture antibiogram.

The most effective drugs used in the current treatment of *MRSA* are vancomycin and teicoplanin. While both reach the desired serum levels in systemic use, the intraarticular concentration does not reach a sufficient level. Intraarticular vancomycin therapy has been used in infected prosthetic patients after the first revision, and it is known that infection recurrence rates are reduced.

The aim of this study is to evaluate the effects of intraarticular vancomycin and teicoplanin on joint cartilage in *invivo* settings and its utility in routine *MRSA* treatment.

MATERIAL AND METHODS

Animal groups

In our study, 35 male Sprague-Dawley rats aged 28 days were used. Rats were obtained from the Regenerative and Restorative Medicine Research Center (REMER) of Istanbul Medipol University. All rats were followed for 4 weeks, fed *ad libitum* in cages at a room temperature of 25 °C, 12 hours of night/12 hours of day on a circadian rhythm.

Rats were randomly divided into 5 groups each containing 7 rats;

- Intraarticular saline treatment group (control group),
- Group administered 5 mg teicoplanin (400 * 3 mg-adult maximale tolerable dose) (Study group-1),
- Group administered 1.5 mg of teicoplanin (400 mg-adult maintenance dose) (Study group-2),
- Group administered 8.5 mg vancomycin (2000 mg-adult maximale tolerable dose) (Study group-3),
- Group administered 2.5 mg vancomycin (700 mg-adult maintenance dose) (Study group-4).

All knee joint injections were administered under isoflurane sedo-analgesia every day at 6 am. Three rats (15 rats) from each group were sacrified in seventh day and evaluated immunohistologically to determine acute healing in articular cartilage. All remaining rats were sacrificed on day 28 and their knees were evaluated by immunohistochemical examination.

Histological evaluation

Tissues were fixed with 10% formaldehyde and subsequently held in 10% formic acid, Then the articular region sectioned and embedded in paraffin. Sections taken from paraffin blocks were stained with hematoxylin eosin and matrix metalloproteinase-13 (MMP-13) immune staining and added to positive charged lamellas. Stain MMP-13 (collagenase-3) Ab-1 (clone VIIIA2) mouse monoclonal antibody thermo Scientific, Freemont, CA, USA. Formalin fixed sections were pretreated with citrate prior to immunostaining. All the staining process done with automatic machines.

Statistical evaluation

Statistical analysis was performed by using the NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) program. The Kruskal Wallis test was used for the comparison of the non-normal distribution of the parameters and the Mann Whitney U test was used for the determination of the group that caused the difference, as well as descriptive statistical methods (mean, standard deviation, median, frequency, ratio). The results were evaluated in a confidence interval of 95% and a significance level of $p < 0.05$.

RESULTS

In our study, 35 male Sprague-Dawley rats aged 20–24 weeks were used. There were no complications in any rat during injection and during the study period (Fig. 1, 2). Hematoxylin eosin (H & E) histological staining for evaluating cartilage healing and healing levels did not show statistically significant differences between the groups at first week ($p > 0.05$). In the fourth week, H & E staining measurements showed statistically significant difference according to the groups ($p < 0.01$). However, when we evaluated the subgroups, we found a better statistical results with high dose application of the same antibiotic therefore this statistical significance was not found to be clinically meaningful.

At week 1, MMP-13 staining was not statistically significant between the groups ($p > 0,05$). However, in detailed comparisons of groups; The staining levels of the control group were significantly higher than the vancomycin 8.5 mg group ($p < 0.05$), but this fact was not clinically significant. There is not a significant difference between the levels of staining of other groups ($p > 0.05$). At week 4, MMP-13 staining did not show any statistically significant difference between the groups ($p > 0.05$) (Fig. 3, 4).

DISCUSSION

In this study, the effects of different doses of vancomycin and teicoplanin applied to the knee joint on intact joint cartilage were evaluated and similar results were obtained with the control group. Vancomycin and teicoplanin can be safely used intraarticularly as the part of the *MRSA* septic arthritis treatment.

MRSA septic arthritis, diagnosed for the first time in 1960, has recently been responsible for 6–22% of all septic arthritis and is increasing day by day. In a study by Lin et al., *MRSA* septic arthritis was found at a rate as high as 40% (1, 7). In a study by Dubost et al., Up to

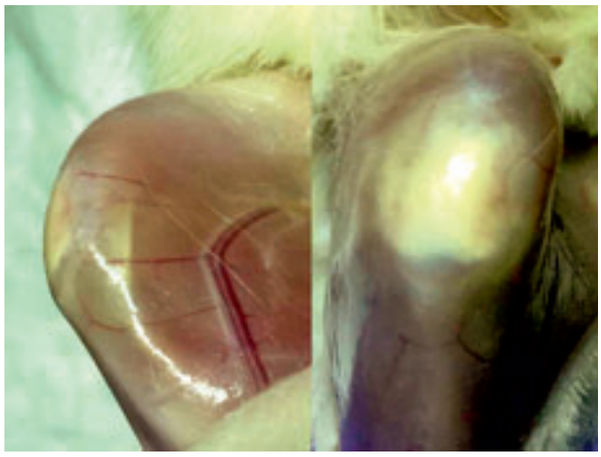

Fig. 1. Joint capsule after the scarification of the rats and normal knee macroscopic view.

Fig. 2. After the removal of the patella , and normal knee cartilage macroscopic view.

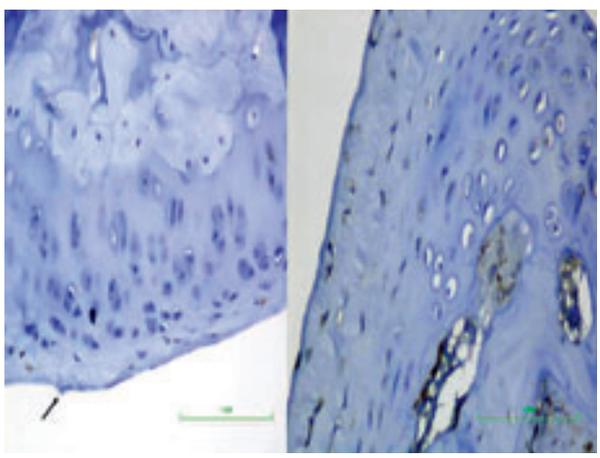

Fig. 3. Similar staining and cell characteristics with MMP-13.

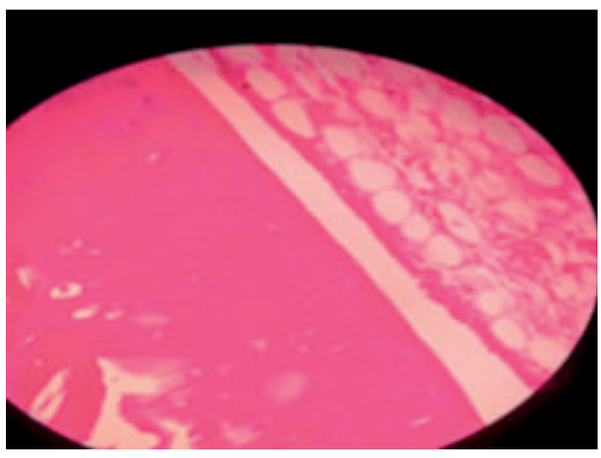

Fig. 4. Normal cartilage property of rat in the study group with H & E staining.

20% of the cases had *MRSA* as agent of septic arthritis (5, 13). Most of the patients suffering from septic arthritis need ıntensıve care and have additional comorbidities. This situation increases the frequency of *MRSA* and makes treatment and healing harder. Minguez et al. showed that 92% of *MRSA* septic arthritis patients, have comorbidities such as diabetes mellitus, cardiomyopathy and hypertension (4). In this respect, when we evaluate *MRSA* treatment antibiotherapy for 4–6 weeks doses there is a risk for organ failure and toxicity of this patient group. Intraarticular antibiotherapy is an important alternative. It can be used in combination with systemic use during the bacteriemic period to provide better systemic and local effects. The use of systemic vancomycin or teicoplanin is the first-line treatment method in *MRSA* septic arthritis (4, 9). Serum levels can reach the desired level, especially at maximale tolerable and maintenance doses. On the other hand, it has been shown that intraarticular concentration does not reach to sufficient level in studies conducted (9). The use of intraarticular antibiotics during treatment can lead to more effective and early disease control by turning this negative situation into favor of the patient. There was no statistically significant difference between the control group and antibiotic applied group in levels of cartilage destruction indicator MMP-13 Besides H & E staining showed that these antibiotics can be safely used intra-articularly by showing healthy cartilage character.

Another important problem of orthopedic surgeries is prosthetic infections (13). During the first two days after antibiotic cement applying after prosthetic infections, drug concentration is high inside and around the joint (15). However, especially after the first week, the drug release level decreases rapidly and after a while the cement becomes a foreign body on where the agents of infection form a biofilm layer and it loses its treatment efficiency (1, 15). On the other hand, intra-articular adminitration is limited to prosthetic infections. It has been shown that 3 weeks of vancomycin administration with Hickmann catheter placed in the knee after single-stage revision in infected knee prosthesis reduces infection recurrence and revision rates (10).

Table 1. Distribution of hematoxylin eosin

Top hematoxylin staining		1th week Teico 1.5 mg	Teico 5 mg	control	Vanco 2.5 mg	Vanco 8.5 mg	4th week Teico 1.5 mg	Teico 5 mg	control	Vanco 2.5 mg	Vanco 8.5 mg
No staining	n	6	6	3	4	5	7	6	7	7	3
	%	100.0%	100.0%	50.0%	66.7%	83.3%	87.5%	75.0%	87.5%	87.5%	37.5%
Staining up to%5	n	0	0	1	2	0	1	2	1	1	2
	%	0.0%	0.0%	16.7%	33.3%	0.0%	12.5%	25.0%	12.5%	12.5%	25.0%
Above %5	n	0	0	2	0	1	0	0	0	0	3
	%	0.0%	0.0%	33.3%	0.0%	16.7%	0.0%	0.0%	0.0%	0.0%	37.5%
[a]p		***0.146***					***0.050****				
[b]Teico 1.5–Teico 5		1.000					0.523				
[b]Teico 1.5–control		0.098					1.000				
[b]Teico 1.5–Vanco 2.5		0.138					1.000				
[b]Teico 1.5–Vanco 8.5		0.317					0.033*				
[b]Teico 5–control		0.058					0.523				
[b]Teico 5–Vanco 2.5		0.138					0.535				
[b]Teico 5–Vanco 8.5		0.317					0.079				
[b]control–Vanco 2.5		0.367					1.000				
[b]control–Vanco 8.5		0.290					0.033*				
[b]Vanco 2.5–Vanco 8.5		0.673					0.033*				

Table 2. MMP-13 staining measurements according to groups.(1th week results; 3 rats both knees(6 sample) were treated, 4th week 4 rats both knees (8 sample) treated). On the 4th week at vancomycin 2.5 mg group 1 rat's pathology result was insufficient (Teico: teicoplanin, Vanco: vancomycin)

MMP-13 staining		1th week Teico 1.5 mg	Teico 5 mg	control	Vanco 2.5 mg	Vanco 8.5 mg	4th week Teico 1.5 mg	Teico 5 mg	control	Vanco 2.5 mg	Vanco 8.5 mg
No staining	n	0	1	0	1	0	1	0	0	1	2
	%	0.0%	16.7%	0.0%	16.7%	0.0%	12.5%	0.0%	0.0%	16.7%	25.0%
Up to 5%	n	4	2	1	2	5	2	6	1	3	2
	%	66.7%	33.3%	16.7%	33.3%	83.3%	25.0%	75.0%	12.5%	50.0%	25.0%
Above 5%	n	2	3	5	3	1	5	2	7	2	4
	%	33.3%	50.0%	83.3%	50.0%	16.7%	62.5%	25.0%	87.5%	33.3%	50.0%
[a]p		***0.329***					***0.181***				
[b]Teico 1.5–Teico 5		0.829					0.289				
[b]Teico 1.5–control		0.093					0.239				
[b]Teico 1.5–Vanco 2.5		0.859					0.357				
[b]Teico 1.5–Vanco 8.5		0.523					0.558				
[b]Teico 5–Control		0.211					0.015*				
[b]Teico 5–Vanco 2.5		1.000					0.879				
[b]Teico 5–Vanco 8.5		0.523					0.817				
[b]control–Vanco 2.5		9.211					0.039*				
[b]control–Vanco 8.5		0.027*					0.095				
[b]Vanco 2.5-Vanco 8.5		0.523					0.782				

One of the important reason for this is the unknown effects of intra articular applied vancomycin and teicoplanin. In this study, invivo intrarticular vancomycin and teicoplanin administration is found to have limited chondrotoxic effects as a result of 28 days of treatment.

CONCLUSIONS

As a result, intraarticular vancomycin and teicoplanin maximale tolerable and maintenance doses can be safely used beside surgery and intravenous antibiotics to increase efficacy of treatment, reduction of recurrence rates and reduction of mortality in *MRSA* septic arthritis.

Abbreviations
H&E: hematoxylin eosin
MMP-13: matrix metalloproteinase-13
ng/ml: nanogram/milliliter

Ethical approval: *This study was unanimously approved by the ethics committee of Medipol University Animal Experiments Local Ethics Committee (İMÜ-HADYEK) with the decision number 11 in 10.02.2016. The study was conducted in accordance with international guidelines.*

Availability of data and material: *Data sharing not applicable to this article as no data sets were generated or analysed during the current study.*

Competing interests:*The authors declare that they have no competing interests*

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