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Research Article

Assessment of The Antimicrobial Effect of Manuka Honey in The Implant-Related Spinal Infections in Rats

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Summary

Aim: Implant-related infections in spinal surgical procedures are still an important problem despite all the precautions. Various methods and medicinal products have been applied in order to prevent implant-related infections. "Manuka honey" is a type of honey which has been approved by FDA as a medicinal product. "Manuka honey" has bactericidal and bacteriostatic effects on "methicillin resistant Staphylococcus aureus" which is the most frequently isolated bacteria. The purpose of this study is to evaluate whether as an inexpensive and natural product, honey would prevent infection growth on rats in the implant related infection model.

Method: Rats were divided into 3 groups, each of which containing 8 rats:namely, the control, infection and treatment groups. In the 1st group, there was no bacterial proliferation. Planted standard MRSA strain has been detected on rats belonging to the groups 2 and 3. On the vertebral column and implants, bacterial growth was greater in the 2nd group than the 3rd group. Also more bacterial colony growth has been detected in 2nd group as compared to 3rd group. While this difference was deemed significant in implant, the same did not apply to vertebral column.

Result: Manuka honey could not completely eradicate the MRSA infection; however, it did decrease the intensity of the infection. If manuka honey, through constituting a different model, could be used in infections regularly, more objective and promising results would be revealed. Moreover, we consider that experimenting "manuka honey" on different bacteria including other Staph. aureus strains would be appropriate through constituting different models.

Key words: Implant infection, manuka honey, spine, Staphylococcus aureus

Deneysel İmplant İlişkili Spinal Enfeksiyon Modelinde Manuka Honeyin Antibakteriyel Etkisinin Değerlendirilmesi

Özet

Amaç: Spinal cerrahi prosedürlerde implant ilişkili enfeksiyonlar, tüm önlemlere rağmen hala önemli problemlerden biridir. İmplant ilişkili enfeksiyonları önlemeye yönelik bir çok ilaç ve yöntem uygulanmıştır. Manuka honey, ilaç formu FDA tarafından onaylanmış bal türüdür. Manuka honeyin, implant ilişkili enfeksiyonlarda en sık izole edilen bakteri olan metisilin rezistan stafilokokus aureusa karşı bakterisit ve bakteriostatik etkileri vardır. Bu çalışmadaki amacımız, implant ilişkili spinal enfeksiyon modelinde, ucuz ve doğal bir ürün olan balın sıçanlarda enfeksiyon gelişimini önleyici etkisinin olup olmadığını değerlendirmektir.

Yöntem: Sıçanlar sekizerli 3 gruba ayrıldı. 1. grup (kontrol grubu); sadece spinal implant uygulanan grup, 2. grup (enfeksiyon grubu): spinal implantla beraber bakteri ekilen grup. 3. grup (tedavi grubu): spinal implant ve enfeksiyona ilave manuka honey uygulanan grup. Grup 1'deki sıçanların hiç birinde doku, implant ve kan kültürlerinde bakteriyel üreme olmadı. Grup 2 ve 3'teki sıçanların tamamında ekimi yapılan standart MRSA suşu üredi. Gruplar birbirleri arasında enfeksiyon yoğunluğu açısından karşılaştırıldı. Grup 2'deki vertebral kolon ve implantlar üzerinde üreyen bakteri sayısının grup 3'e kıyasla daha şiddetli olduğu, üreyen bakteri koloni sayısının kantitatif olarak daha fazla olduğu tespit edildi. Bu fark, implant üzerinde istatistiksel olarak anlamlı bulunurken, vertebral kolonda anlamlı bulunmadı.
Sonuç: Manuka honey bu modelde, MRSA enfeksiyonunu tam olarak önleyememiş ancak, enfeksiyonun şiddetini belirgin olarak azaltmıştır. Manuka honeyin implant ilişkili enfeksiyonlarda farklı bir model oluşturularak, düzenli aralıklarla kullanılabilmesi durumunda, daha objektif ve umut verici sonuçlar ortaya konabilir. Ayrıca Manuka honeyin, farklı modeller oluşturularak, farklı bakterilerde ve diğer stp aureus suşlarında da denenmesinin uygun olacağı kanaatindeyiz.

Anahtar Kelimeler: Bal, implant enfeksiyonu, omurga, stafilokokus aureus

INTRODUCTION

Implant-related infections (IRIs) still pose a serious challenge in spinal surgery today. While the rate of infections in spinal surgeries such as common disc surgeries or laminectomy is around 1 %; this rate increases up to 2,1 % - 8 % during implant applications[1,7,10]. The factors that increase the risk of IRIs include dead spaces in surgical site, foreign body reaction, necrotic tissue, and the long duration of the surgery[20]. Clinicians have utilized various local or systemic elements in order to prevent IRIs[15]. Current literature puts forward the antimicrobial and healing effect of manuka honey, a standard type of honey approved by FDA as a drug, and this had gathered attention on MH[5,13]. Existing research has proven that MH is effective towards many bacteria including methicillin-resistant Staph. Aureus[2,6,12]. However, these superior effects of MH have not been tried in spinal surgery procedures and clinical applications. No researches suggesting the use of MH for protection of spinal surgery against IRIs have been found out during the literature review. Starting from this point, this research has been conducted in order to analyze the utility of MH in prevention of implant related spinal infections.

MATERIAL AND METHODS

This research was carried out in Çukurova University Medical School, Experimental Animals Laboratory. The honey used in the research was the one produced by the Manuka Honey bees (Leptospermum scoparium) originating from New Zealand. As for the implant-related infections, the model produced by Ofluoglu and Ark was used[16].

Preparation of Bacteria

Standard strains of Staph. aureus (MRSA ATCC 33591), a bacteria resistant to methicillin, was selected for the study in order to create an infection. Bacteria suspensions were prepared in phosphate buffered saline (PBS) in line with MacFarland haze 6 standard, out of MRSA test strains produced by overnight incubation, in the premises of Çukurova University, Department of Medical Microbiology.

Surgical Procedure

Wistar Albino type 24 rats, around 10 – 12 weeks old, each weighing approximately 200 – 250 grams were used in the research. General anesthesia was applied through intraperitoneal 50mg/kg Ketamine hydrochloride (Ketalar, Parke-Davis, Eczacıbaşı, İstanbul) and 10 mg/kg

Xylazine hydrochloride (Rompun). After the rat was fixed on the operation table, the operation site was brushed with povidone iodine scrub (MEDİCA brush; 4% chlorhexidine soap, MEDİCA BV, Netherlands) for ten minutes and was disinfected with povidone iodine (POVİOD; 10 % polyvinyl pyrrolidone iodine complex, Saba, Turkey) solution. Operation site was covered with sterile drapes. Level "L1"was observed. Afterwards, a 3-cm skin incision was performed on the midline through the spinous processes. Paraspinous muscles of the space were peeled off with blunt dissection. The experimental group was divided into three:

• Group 1 (Control): After exposing the facet joint, the screw socket was prepared with a sterile injection needle with a deviation o about 10 degrees towards the lateral. The 3-mm sterile micro screw was propagated to the socket prepared. Only implants (titanium micro screws) were applied to the rats in this group (Figure 1).

• Group 2 (MRSA Group) (n=8): The implants which were dipped for 5 minutes in the MRSA suspension MacFarland haze 6 tube were placed to the rats in this group. No medication was administered to this group either for prophylaxis or medical treatment.

• Group 3 (Treatment group) (n=8): Same implants as the Group 2 were applied to this group however these implants were also dipped for 5 minutes in manuka honey in a beaker after being dipped in the MRSA solution (Table 1).

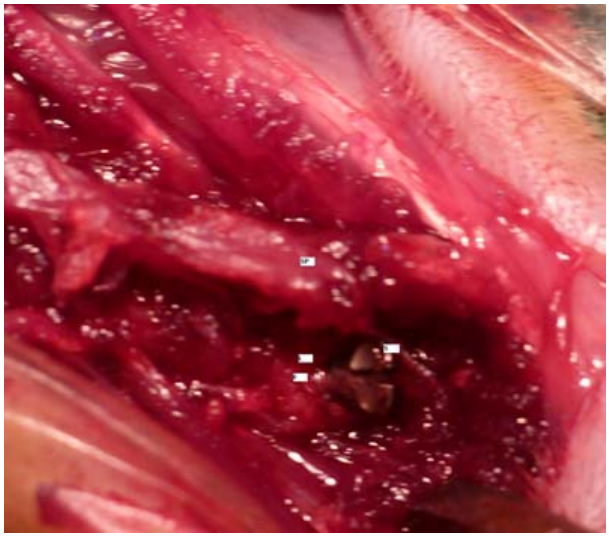

Figure 1: *Titanium micro screw inner facet joint is showed in the figure 1. sp:spinous process, s:screw, l:lamina, f:facet joint.*

Table 1: Colony counts of incubated bacteria.

No	Group 1	Group 2	Group 3
1	0	10^6	10^6
2	0	10^6	10^6
3	0	10^6	10^6
4	0	10^6	10^6
5	0	10^6	10^6
6	0	10^6	10^6
7	0	10^6	10^6
8	0	10^6	10^6

Experimental animals were kept alive for two weeks. At the end of this period, first of all, 5 ml of blood was taken from each rat and these blood samples were planted into broth medium each of them containing 20 ml TSB. Later on the rats were sacrificed by injecting (75-100 mg/kg) Thiopental Sodium (Pentothal Sodium, Abbott, Italy). Relevant columns were extracted as en bloc. Some of the rats were excluded from the study in which dural rupture, nerve root injury or post operative neurological deficit were observed. These rats were replaced by the new ones. Occurrence of bacteria growth and the rates of reproduction were cross-checked and assessed and the results were statistically compared.

Microbiological Examination

Bone, muscle and fascia specimens were gathered from each experimental rat and were brought to the laboratory latest in two hours in tarred falcon tubes containing 10 ml sterile serum 4+-8º C. The specimens brought to the laboratory were reweighed and the 10-ml sample weights were recorded. Later on, the specimen tissues were mechanically broken into pieces with sharp glass rods and were homogenized by vortexing. With the aim of colony counting, serial dilutions were applied to the specimens and the triptych strains were spread (each dilutions on every 5 plates) following the homogenization. Planted broth medium were left for incubation for 24 hours at 37º C. At the end of this period, the average colony numbers were gathered from the countable plates (colony growth between 10 and 100) and from the plates the bacteria growth number of which remains within the standard deviation (of which number of columns are within ± 10 % of the average), the bacterial counts in the tissues were quantitatively (CFU/gr) determined. In order to determine the bacterial growth on the titanium screws, these screws were taken out and vortexed in 1 ml TSB. Later on, the implants were taken out of the tubes. Dilutions were prepared with PBS from the TBS broth in the tubes according to basis log 10 and just like in the tissue specimens, extracts from the dilutions were planted into the TSA broth medium. No bacteria growth other than Staph. aureus was observed in the planted broth medium. In a similar way, no growth was observed either on the blood cultures taken from the rats before sacrifice, or from the cultures which were kept in hold after TSB application, or in the passages made to broth on 3rd, 7th and 15th days of the experiment.

RESULTS

Bacteria growth occurred neither on the implants, tissues nor in the blood cultures in the rats belonging to the Group 1. However, the planted MRSA strain reproduced in all the rats of the Groups 2 and 3 (Figure 2). The bacterial colony counts in Groups 2 and 3 were recorded separately for vertebral columns and implants (Table 2,3).

Statistical Evaluation

Data were evaluated through Fischer's exact test. The groups were compared against each other according to the intensity of infection. On the vertebral columns and implants, bacterial growth was greater in the 2nd Group as compared to the 3rd Group. Also more bacterial column growth has been detected in Group 2 in comparison to the Group 3. While this difference was deemed significant in implants, the same didn't apply in vertebral column (Table 4,5).

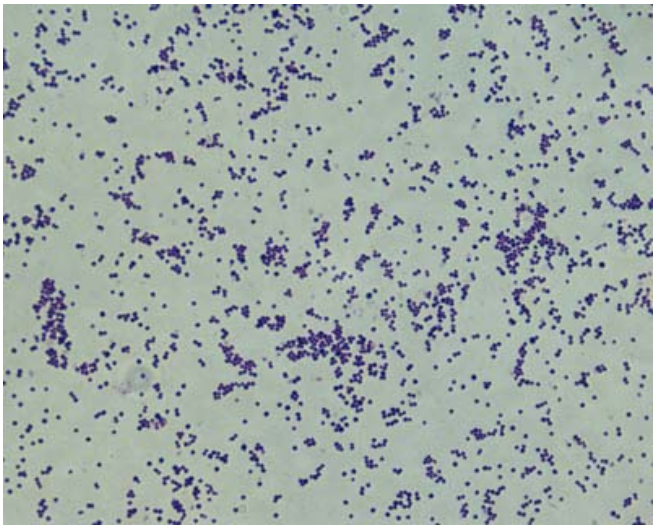

Figure 2: *Bacterial colonizations are showed in the light microscopy.*

Table 2: Bacterial proliferation on the vertebral column

No	Inoculated colony counts	Group 1	Group 2	Group 3
1	10^6	0	$5,1x10^7$	$5,4x10^5$
2	10^6	0	$3,6x10^7$	$3,4x10^6$
3	10^6	0	$2,3x10^6$	$2,8x10^6$
4	10^6	0	$4,6x10^7$	$4,8x10^7$
5	10^6	0	$5,5x10^7$	$4,5x10^4$
6	10^6	0	$2,8x10^7$	$5,0x10^5$
7	10^6	0	$3,3x10^7$	$2,6x10^6$
8	10^6	0	$5,8x10^7$	$5,1x10^5$

Table 3: Bacterial proliferation on the implant.

No	Inoculated colony count	Group 1	Group 2	Group 3
1	10^6	0	$5,6x10^7$	$2,8x10^5$
2	10^6	0	$2,5x10^6$	$1,6x10^6$
3	10^6	0	$3,2x10^7$	$3,7x10^6$
4	10^6	0	$4,4x10^7$	$3,2x10^7$
5	10^6	0	$2,7x10^7$	$3,2x10^4$
6	10^6	0	$1,6x10^6$	$4,1x10^5$
7	10^6	0	$1,1x10^7$	$2,6x10^6$
8	10^6	0	$4,3x10^7$	$1,5x10^5$

Table 4: In terms of the number of bacteria reproducing difference on the vertebral colon was not statistically significant observed between group 2 and group 3 ($p > 0.05$).

Group	Colony count				Total
	(10^4)	(10^5)	(10^6)	(10^7)	
2	0 (0%)	0 (0%)	1 (12.5%)	7 (87.5%)	8 (100%)
3	1 (12.5%)	2 (25.0%)	3 (37.5%)	2 (25.0%)	8 (100%)
Total	1 (6.3%)	2 (12.5%)	4 (25.0%)	9 (56.3%)	16 (100%)
p					0.079

Table 5: Subjects in terms of the number of bacteria reproducing on the implant a statistically significant observed between group 2 and group 3 ($p = 0.05$).

Group	Colony count				Total
	(10^4)	(10^5)	(10^6)	(10^7)	
2	0 (0%)	0 (0%)	2 (25%)	6 (75%)	8 (100%)
3	1 (12.5%)	3 (37.5%)	3 (37.5%)	1 (12.5%)	8 (100%)
Total	1 (6.3%)	3 (18.8%)	5 (31.3%)	7 (43.8%)	16 (100%)
p					0.05

DISCUSSION

Using implants in spinal surgery increase the risk of infection by three times[8,21]. These infections increase with the increasing duration of hospital stay, require long-term medication, adversely affect surgical results and lead to socio-economic losses. Infections that do not respond to medical treatment generally require revision surgery[11]. Two models have been designed so far on the "implant-related infections in animal spine". First one is the model designed by Poelstra & Ark in rabbit spine[18]. The other one was designed by Ofluoğlu in rat spine and it was the one and the first implant related infection model designed in rat spine[16]. This model, which has also been used in our study, depends on the basis that at least 10^6 colonies of MRSA implant infection can be created by planting. S. aureus is the most frequently isolated bacterial strain in implant-related infections. In the literature, various methods have been used in order to prevent implant related infections. Most of them depend on the usage of biomaterials which were imbrued with antibiotics[15].

Since the antiquity, honey has been used for infected wounds and burns[22]. Several studies have been carried out that show manuka honey naturally speeds up wound healing[4,9,14,17,19]. Recently, manuka honey has been used in various clinical and experimental studies for its antibacterial and wound healing characteristics; and its positive effects have been reported[4,9]. MH shows its antibacterial effect both with its osmotic feature, low pH value, enzymatic factors such as hydrogen peroxide, inhibine and glucose oxidase acting with various different mechanisms[3]. Studies demonstrate that MH is also effective against MRSA and it prevents bacterial growth[4,9]. Moreover, several studies indicate that MH is effective against MRSA both in vitro and in vivo conditions[2,5,6,12,13].

Within this study, the effectiveness of MH in preventing IRIs in rat spine was investigated by using MRSA, the most frequently isolated bacteria in IRIs, and standard strains in standard numbers. Absence of bacterial growth in the rats of Group 1 suggests that no exogenous inoculation of bacteria had occurred and that the experiment was conducted in a sterile environment. The rats in the Group 2 were successfully infected and the planted MRSA strain was isolated in all members of this group. This result

indicates that MRSA planted rats, which were not exposed to MH, were entirely planted with an appropriate number of bacteria and that the grown bacteria match the planted bacterial strain; hence, no bacterial infection had occurred from outside. This research, which was conducted by using MRSA, the most frequently isolated bacteria in IRIs, and standard strains in standard number also assessed the effectiveness of MH statistically in preventing IRIs in rat spine. Macroscopically, abscess formation was observed in subcutaneous tissues, fascia, paravertebral muscles and in the spine of the rats in the Group 2 and 3. However, no macroscopic infectious findings were observed in the Group 1. MRSA growth was comparably lower in Group 3 than Group 2. Growth rate on the implants in the Group 3 was significantly lower as compared to Group 2. As for the vertebral column, the growth in Group 3 was lower than the Group 2; however the difference was not found to be significant. Having a general look on the data, MH decreased the bacteria growth both on the tissues and the implant; whereas it could not entirely eradicate the infection.

Our study suggests that while MH has a mitigating impact against MRSA in IRIs in rat spine, its impact on preventing infections could not been demonstrated. MH's antibacterial impact against MRSA, which is noted in the literature, should be experimented in other strains of Staph. aureus as well. MH's inability to prevent the infection against MRSA does not necessarily imply that it is not effective against MRSA. In the vast majority of the studies which demonstrate the effectiveness of MH against MRSA, MH was applied on superficial scars and with regular intervals. In our model, MH could be applied only once after MRSA was planted.

In conclusion, in this model of "manuka honey" could not eradicate MRSA infection completely; however, it has prominently decreased intensity of the infection. With the constitution of a different model, if manuka honey could be used in infections regularly, more objective and promising results would be revealed. We consider that experimenting "manuka honey" on different bacteria and other Staph. aureus strains would be appropriate through constituting different models.

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