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Antibacterial Effect of Royal Jelly for Preservation of Implant-Related Spinal Infection in Rat

Sıçan Omurgasında İmplant İlişkili Spinal Enfeksiyonu Önlemede Arı Sütünün Antibakteriyel Etkisi

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ABSTRACT

AIM: Implant-related infections are still a significant problem in spinal surgical procedures. Many drugs and methods have been tried to prevent implant-related infections. Our objective in this study was to evaluate whether royal jelly, which was found to hinder the growth of MRSA, has any preventive role in the prognosis of an infection in rats in an implant-related infection model.

MATERIAL and METHODS: Rats were divided into 3 groups of eight rats. Group-1 consisted of rats that underwent only a spinal implant, group-2 included those rats that were inoculated bacteria together with a spinal implant and group-3 was administered royal jelly in addition to a spinal implant and infection.

RESULTS: The amount of bacteria that grew in vertebral columns and implants was more in Group-2 than in Group-3, which meant that the number of bacteria colonies that grew was more quantitatively. This difference was found to be statistically significant in vertebral columns, but not in implants.

CONCLUSION: Royal jelly could not fully prevent the MRSA infection in this model, but decreased the severity of infection noticeably. More objective and promising results may be obtained if royal jelly can be used at regular intervals in a different model to be designed with respect to implant-related infections.

KEYWORDS: Implant, Infection, Royal jelly, Spine, Staphylococcus aureus, Rat

ÖZ

AMAÇ: Spinal cerrahi prosedürlerde implant ilişkili enfeksiyonlar, hala önemli yer tutmaktadır. İmplant ilişkili enfeksiyonları önlemeye yönelik birçok ilaç ve yöntem uygulanmıştır. Arı sütünün, in-vitro ve in-vivo şartlarda metisilin rezistan stafilokokus aureusun üremesini engellediği tespit edilmiştir. Bu çalışmada, implant ilişkili spinal enfeksiyon modelinde arı sütünün etkisini değerlendirdik.

YÖNTEM ve GEREÇLER: Sıçanlar sekizerli 3 gruba ayrıldı. Grup-1; sadece spinal implant uygulanan grup, Grup-2; spinal implantla beraber bakteri ekilen grup, Grup-3; spinal implant ve enfeksiyona ilave arı sütü 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.

BULGULAR: 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, vertebral kolonda istatistiksel olarak anlamlı bulunurken, implant üzerinde anlamlı bulunmadı.

SONUÇ: Arı sütü bu modelde, MRSA enfeksiyonunu tam olarak önleyememiş ancak, enfeksiyonun şiddetini belirgin olarak azaltmıştır. Arı sütün implant ilişkili enfeksiyonlarda farklı bir model oluşturularak, düzenli aralıklarla kullanılabilmesi durumunda daha objektif ve umut verici sonuçlar ortaya konabilir. Ayrıca arı sütün, farklı modeller oluşturularak, farklı bakterilerde ve diğer Staph. aureus suşlarında da denenmesinin uygun olacağı kanaatindeyiz.

ANAHTAR SÖZCÜKLER: İmplant, Enfeksiyon, Arı sütü, Omurga, Stafilokokus aureus, Sıçan

INTRODUCTION

Implant-related infections (IRIs) are still an important issue in spinal surgical procedures. Although infections are seen at a rate of 1% in frequently used spinal procedures such as disk surgery and laminectomy, this rate increases up to 2.1%-8% in patients who undergo an implant surgery (1,6,8). The dead space in the surgical area, foreign bodies, necrotic tissue, and long-lasting surgical procedures are considered to be the factors increasing the risk of IRI (15). Clinicians have used many local and systemic agents to prevent IRIs (10). Royal jelly

(RJ), a product secreted by worker bees to feed the queen bee, has been shown to have an antimicrobial action against many microorganisms including Staphylococcus aureus (2). This action of royal jelly that was shown to be effective under in vitro and in vivo conditions has never been tested in spinal infections until now. In this study, we explored whether locally used RJ had any infection-preventing effect on rats in an implant-related spinal infection model.

MATERIAL and METHOD

The study was conducted at the Experimental Animals Research Laboratory of the Cukurova University Medical School under the permission of Cukurova University Animal Experiments Local Ethics Committee (January 25, 2013; Decision No: 7). A RJ of New Zealand origin that is produced by worker honeybees from Leptospermum scoparium was used in the study. The model designed by Ofluoglu et al. (11) was used as the implant-related infection model.

Preparation of Bacteria

Standard strains of methicillin-resistant Staphylococcus aureus (MRSA ATCC 33591) were used in the study to create infections. Bacterial suspensions were prepared in phosphate buffered saline (PBS) in accordance with the McFarland turbidity standard 6 from the testing strain colonies of MRSA that were grown after a night's incubation at the Department of Medical Microbiology of the Cukurova University Medical School.

Surgical Procedure

Twenty four Wistar Albino rats that were 10-12 weeks old and weighed 200-250 g were used in the study. Since the study was an infection study, prophylactic antibiotics were not used to prevent them from affecting the results of the study. General anesthesia was performed using a 50mg/kg dose of Ketamine hydrochloride (Ketalar, Parke-Davis, Eczacıbaşı, Istanbul) + a 10 mg/kg dose of Xylazine hydrochloride (Rompun) intraperitoneally. After the rat was fixated on the operation table, the operation area was brushed for 10 min. with povidone iodine scrub (MEDICA brush; 4% chlorhexidine soap, MEDICA BV, Holland) and stained with povidone iodine (POVIOD; 10% polyvinylpyrrolidone-iodine complex, Saba, Turkey) for disinfection. The operation area was covered with sterile sheets. The L1 level was determined. Then, a skin incision of approximately 3 cm was made from midline on spinal processes. The paraspinous muscles of this distance were detached through blunt dissection. Then, a laminectomy was performed. The rats were divided into three groups: Group 1 (Control): Only an implant was placed in the rats in this group. Group 2, the MRSA Group (n=8): The implants used in the rats in this group were dipped into, and held for 5 minutes in, the MRSA suspension that was prepared in accordance with the McFarland turbidity standard 6 and then were put in their places. Group 3, the treatment group (n=8): The rats in this group were received implants that were left in the MRSA solution as in the case of the implants administered to Group 2 but these also contacted RJ in a beaker for 5 minutes after removal from the solution (Table I). The rats were left to live for 2 weeks and then 5 ml of blood was first taken from each rat and added to the liquid media containing 20 ml of tryptic soy broth (TSB) The rats were then euthanized by administering a high dose (75-100 mg/kg) of Thiopental Sodium (Pentothal Sodium, Abbott, Italy) intraperitoneally. Relevant vertebral columns were removed en bloc. The rats that had a dura mater rupture or an injury of nerve root, and those that were found to have a postoperative neurological deficit were excluded from the study. They were replaced by new rats. The histopathological examination was performed in a blind fashion. Whether there was a growth of bacteria in the groups and the rates of such growth were evaluated both within the groups and by comparing them with the control group, and the results were compared statistically.

Microbiological Examination

The bone, muscle, and fascia samples taken from each animal under aseptic conditions were placed in tared falcon tubes containing 10 ml of sterile normal saline and were rushed to the laboratory within at most 2 hours at 4±8°C. The samples were reweighed at the laboratory to determine the sample weight in 10 ml. The tissue samples were mechanically cut into pieces using sterile glass rods with sharp tips and were homogenized through vortexing. Following the homogenization, sequential dilutions were made from the samples and trypticase soy agar was spread on 5 separate plates from each dilution for counting colonies. The plate media that were inoculated were left for incubation for 24 hours at 37°C. At the end of this period, the mean number of colonies that was taken from countable plates (10-100 colonies have grown) and from the plates where growth was detected in amounts within the standard deviation (where colony counts were within ±10 of the mean) and the number of bacteria grown in the tissue were detected quantitatively (CFU/gm). The quantification of the growth on titanium screws was determined by removing the implanted screws and placing them in 1 ml of TSB and vortexing them. Then, the implants were taken away from the tubes. Dilutions were prepared from the TSB media in the tubes using PBS according to $\log_{10}$ basis and inoculations were done to TSA media from each dilution as was the case in tissue samples. It was observed that only Staphylococcus aureus grew in the inoculated media. It was also observed

Table I: Colony Count 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

that there was no growth on the 3rd, 7th and 15th days in the passages made to the solid media from the blood cultures that were kept waiting after inoculating to TSB from the blood samples taken from the animals before they were euthanized.

RESULTS

There was no bacterial growth in the tissue, implant and blood cultures of any of the Group 1 rats. The standard MRSA strain that was inoculated grew in all of the rats in Group 2 and 3. The amount of bacterial colonies in Group 2 and 3 was recorded separately for vertebral columns and implants (Table II, III).

Statistical Evaluation

The data was evaluated using Fisher's exact test. The groups were compared with each other in terms of infection intensity. It was found that the number of bacteria grown on vertebral columns and implants in Group 2 was more intense than in Group 3 and the number of growing bacterial colonies was higher. This difference was found to be statistically significant on vertebral columns, but not on implants (Table IV, V).

DISCUSSION

The use of implants in spinal surgery raises the risk of infection by approximately 3 times (7,15). Such infections increase hospitalization times, require use of medication for a long time, affect surgical results negatively, and cause socioeconomic losses. Infections that do not respond to medical treatment usually necessitate a revision surgery (9).

Until now, only two models have been designed for implant-related infection in animal spine. The first was the model designed for rabbit spine by Poelstra et al. (12). The other was the model designed for rat spine by Ofluoglu et al. (11) and it was the first and only implant-related infection model designed for rat spine (10). This second model which was used in our study is based on the assumption that an implant infection can be created by inoculation of at least 10^6 colonies of MRSA.

S. aureus is one of the bacteria strains that are isolated most frequently in implant-related infections. Looking at the literature, one sees many methods that have been used to

Table II: Bacterial Proliferation on the Vertebral Column

No	Colony count	Group 2	Group 3
1	10^6	$2.6x10^7$	$4.8x10^6$
2	10^6	$1.8x10^7$	$3.5x10^6$
3	10^6	$3.5x10^6$	$1.9x10^6$
4	10^6	$2.7x10^7$	$4.4x10^7$
5	10^6	$6.6x10^7$	$6.1x10^5$
6	10^6	$4.1x10^7$	$6.0x10^4$
7	10^6	$4.1x10^7$	$4.3x10^5$
8	10^6	$4.9x10^7$	$1.8x10^5$

Table III: Bacterial Proliferation on the Implant

No	Colony count	Group 2	Group 3
1	10^6	$4.2x10^7$	$4.7x10^6$
2	10^6	$5.2x10^6$	$1.6x10^7$
3	10^6	$5.5x10^7$	$6.3x10^6$
4	10^6	$4.0x10^7$	$5.1x10^5$
5	10^6	$3.1x10^7$	$5.5x10^4$
6	10^6	$2.8x10^6$	$1.9x10^6$
7	10^6	$6.4x10^7$	$4.6x10^5$
8	10^6	$5.2x10^7$	$2.9x10^5$

Table IV: Subjects in Terms of the Number of Bacteria Reproducing the Observed Difference Between Group 2 and Group 3 was Statistically Significant ($p<0.05$)

Group	Colony count on the vertebral column								Total	
	10^4	n (%)	10^5	n (%)	10^6	n (%)	10^7	n (%)		
2	0	(0)	0	(0)	1	(12.5)	7	(87.5)	8	(100)
3	1	(12.5)	1	(12.5)	5	(62.5)	1	(12.5)	8	(100)
Total	1	(6.3)	1	(6.3)	6	(37.5)	8	(50)	16	(100)
p									0.027	

Table V: Subjects in Terms of the Number of Bacteria Reproducing Over Implant the Observed Difference Between Group 2 and Group 3 was not Statistically Significant ($p<0.05$)

Group	Colony count on the spinal implant								Total	
	10^4	n (%)	10^5	n (%)	10^6	n (%)	10^7	n (%)		
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									0.051	

prevent implant-related infections. A large number of these are based on the use of biomaterials soaked in antibiotics (10).

RJ is essentially a creamy secretional product generated in the hipopharyngeal glands by young worker bees for the queen bee. RJ is the source of nutrition for the queen (16). RJ is a nourishing substance with its rich protein, carbohydrate, and mineral content (13). Many studies have been conducted on the medical activities of RJ. Some of these were related to its antibacterial effects (4). Many antimicrobial peptides have been discovered in the composition of RJ including royalactin, apisimin, jeleines I, II, III, IV, 10-HDA, and apalbumin α (2). In vitro studies have demonstrated that royalactin that is obtained from RJ was effective against gram positive bacteria including MRSA and some gram negative bacteria (3,5,14).

We explored in our study the efficacy of RJ in preventing IRIs in rat spine by using a standard number of standard strains of MRSA, the most frequently isolated bacteria in IRIs. Abscess formation was observed macroscopically in subcutaneous tissues, fascia, paravertebral muscles and spine in both Group 2 and Group 3. No macroscopic finding was observed relating to an infection in Group 1. The fact that there was no growth of bacteria in any of the Group 1 rats shows that no bacterial inoculation was made to the study from outside and it was performed in a sterile environment. The entire Group 2 rats were infected and the inoculated MRSA strains were isolated from all of them. This result showed that appropriate amount of MRSA bacteria were inoculated to the entire rats that were not administered honey, the bacterial strain that grew was the same strain that was inoculated and no bacteria were introduced from the outer environment. Although there was less growth in Group 3 rats than in Group 2 rats, the standard MRSA strains that were inoculated still grew. The amount of growth in vertebral columns in Group 3 was statistically significantly less than in Group 2. The amount of growth on implants was not found statistically significant although growth was somewhat less in Group 3. According to these results, RJ reduced bacterial growth on both tissues and implants, but it could not prevent it.

Although honey had a reducing effect on the severity of implant-related MRSA infection in rat spine, we could not demonstrate its infection preventing effect in our study. The antibacterial effects of RJ against MRSA as mentioned in the literature should be tried on other bacteria and other strains of stp aureus. The failure of RJ in fully preventing MRSA infection in this model does not mean that it is of no effect against MRSA. RJ has been used on surface wounds and in regular intervals in most of the experimental researches that showed RJ was effective against MRSA. In the model we used, RJ could be administered only once after inoculation of MRSA.

In conclusion, RJ could not fully prevent MRSA infection in this model, but reduced the severity of infection noticeably. More objective and promising results can be obtained if a different model is designed for the use of RJ in IRIs in regular intervals. We believe that it will be appropriate to design different models and to try RJ on different bacteria and other strains of Staphylococcus aureus.

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