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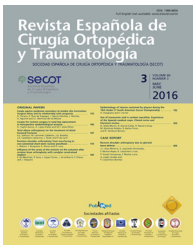

RESEARCH

In vivo bactericidal efficacy of farnesol on Ti6Al4V implants☆

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KEYWORDS
Ti6Al4V;
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Abstract
Objective: To evaluate the *in vivo* anti-staphylococcal bactericidal activity of farnesol on Ti6Al4V surfaces.
Material and methods: An experimental model of infection in biomaterials was developed by inoculation of *Staphylococcus aureus* ATCC 29213 into the canal of both femurs of 15 Wistar rats. A Ti6Al4V pin impregnated with 30 mM of farnesol was inserted into study femur, and a Ti6Al4V control was inserted into the control femur. To evaluate the bactericidal efficacy, a comparison was made between the median of the colony forming units recovered after inoculation in the study group and the control group for different times of euthanasia and inoculum size.
Results: The median expressed as Log_{10} CFU counts obtained with farnesol titanium pin was 4.26, and in control group, it was 4.86, which was statistically significant ($P = .001$) on applying the Student *t* test for related samples.
The median reduction obtained in farnesol pins relative to the control was 74%.
Conclusions: Treatment with farnesol 30 mM on Ti6Al4V pins appears to decrease the rate of colonisation by *S. aureus*.

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PALABRAS CLAVE
Ti6Al4V;
Farnesol;
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In vivo

Eficacia bactericida *in vivo* del farnesol sobre implantes de Ti6Al4V

Resumen
Objetivo: Evaluar *in vivo* la actividad bactericida antiestafilocócica del farnesol sobre superficies de Ti6Al4V.
Material y métodos: Se desarrolló un modelo experimental de infecciones en biomateriales inoculando *Staphylococcus aureus* ATCC 29213 en los fémures de 15 ratas wistar. Seguidamente se insertó una aguja de Ti6Al4V impregnada con farnesol 30 mM en el fémur estudio y una aguja control en el fémur control. Para valorar la eficacia bactericida se compararon las medianas de unidades formadoras de colonias recuperadas después de la inoculación en el grupo estudio y en el grupo control, para diferentes tiempos de eutanasia y tamaño de inóculos.
Resultados: La mediana expresada en Log_{10} de los recuentos de UFC obtenidos en agujas de titanio con farnesol fue de 4,26 y en agujas sin farnesol, controles, fue de 4,86. Esta diferencia, al aplicar la prueba de t de Student para muestras relacionadas, resultó ser estadísticamente significativa (p = 0,001). La reducción mediana obtenida en las agujas con farnesol respecto a las agujas control fue del 74%.
Conclusiones: El tratamiento con farnesol de agujas de Ti6Al4V, a una concentración de 30 mM, parece disminuir la tasa de colonización por *Staphylococcus aureus* en dichas agujas.

Introduction

The infections in biomaterials implanted in live beings are normally associated with the formation of a biofilm that is difficult to eradicate. In the majority of cases it is necessary to remove the infected implant. All of this gives rise to a considerable increase in morbidity, mortality and healthcare costs.

Pathogenic bacteria have developed many defence mechanisms against antibacterial agents, so that resistance against old and new pharmaceutical products is increasing.

Due to the gradual increase in the antibiotic resistance of these bacteria, researchers have studied different organic molecules with antibacterial capacity. In this context attention has focused strongly on natural products such as plant-derived compounds including the essential oils.

Farnesol ($C_{15}H_{26}O$) is a natural organic compound, an acyclic sesquiterpene alcohol that is found in many essential oils such as citronella oil.[1] It intervenes in the quorum sensing of *Candida*, blocking the formation of a biofilm as well as the production of other virulence factors by this fungus.[2] Farnesol affects the growth of a large number of bacteria and fungi, such as *Staphylococcus aureus*,[2,3] *Streptococcus mutans*[4] and *Fusarium graminearum*,[5] which underlines its potential use as an antimicrobial agent.[6]

Bhattacharyya et al. stated that farnesol penetrates the biofilm, accumulating in the cell membrane, where it increases the porosity of the same by its mechanism of action.[7] This increase in cell membrane permeability to different substances may increase the absorption of antibiotics if they are used together with farnesol. This would mean that a lower dose of antibiotic would be necessary, and this in turn would reduce the possible appearance of resistence. *i.e.*, farnesol would increase the susceptibility of bacteria to antibiotics and other antimicrobial compounds.[8]

In a recent paper Unnuntana et al. give an *in vitro* demonstration of the capacity of farnesol to inhibit the formation of methicillin-sensitive biofilms of *S. aureus* at concentrations of 30 mM on titanium discs.[9]

The aim of this work is to analyse whether treatment with farnesol of the surface of Ti6Al4V needles prior to their implantation in rat femurs reduces the rate of *S. aureus* colonisation on the said needles and in the femur containing them.

Material and methods

Osteosynthesis material: Ti6Al4V

The Ti6Al4V alloy was supplied by Kirschner Maschinenbau GmbH (Unterschneidheim, Germany) in the form of 1.2 mm × 150 mm wire, which was cut using a chisel into 1.2 mm × 20 mm pieces.

The needle-cleaning protocol prior to their implantation was as follows: Derquim DSF at 2%, sonication, immersion in distilled water at 60 °C during 15 min, 10 min in acetone at 70% and finally a Pasteur oven during 30 min at 40 °C.

The needles were divided into 2 groups:

a) Those which were to be used as control needles were not treated with any other process.
b) The needles which were used to study the bactericide effect of farnesol on Ti6Al4V alloy were subjected to the following process as well as those described above:
   1) Immersion in a piranha solution with 5 ml H_2SO_4 concentrate at 5 ml and 30% H_2O_2% during one hour.
   2) Washing with water and ethanol in an ultrasound bath during 10 min with each liquid.
   3) The needles were then immersed in a farnesol solution (30 mM) during 24 h.