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Vitamin E supplementation in old mice induces antimicrobial activity and improves the efficacy of daptomycin in an animal model of wounds infected with methicillin-resistant *Staphylococcus aureus*

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Sir,

Antibiotic-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), are a recognized problem in healthcare settings, leading to refractory infections and potentially life-threatening illnesses.[1] MRSA is often isolated from patient wounds in both the hospital and the community setting. A variety of factors determine whether a wound remains harmlessly colonized or succumbs to infection. Some of the most important factors include individual vulnerability to infection and the size and location of the wound, balanced against the number of microorganisms present and their virulence factors. Vulnerability factors include age, with the elderly being more prone to MRSA infection as they have a compromised immune response (immunosenescence), and underlying diseases, such as diabetes or cardiovascular disease, and malnutrition, all of which affect the wound-healing process.[2,3]

Daptomycin is a branched cyclic lipopeptide antibiotic of non-ribosomal origin and the prototype of the acidic lipopeptide family. It was approved in 2003 for the non-topical treatment of skin structure infections caused by Gram-positive pathogens, including MRSA, and in 2006 for the treatment of bacteraemia.[4]

Vitamin E is a family of essential micronutrients composed of lipid-soluble tocopherols and tocotrienols with strong antioxidant and immunomodulating activity. Vitamin E supplementation is associated with increased resistance to several pathogens, especially in the elderly, who are at greater risk of inadequate dietary intake of vitamin E.[5,6]

We studied whether supplementation of old mice with vitamin E was effective in inducing antimicrobial activity and in increasing the effect of daptomycin in a mouse model of wound infection due to MRSA.

Sixteen-month-old male BALB/c mice (average weight 30 g) were assigned to four groups ($n=8$ per group): a group pretreated with vitamin E alone (60 mg/kg by oral gavage 30 days prior to challenge); a group pretreated with vitamin E plus intraperitoneal daptomycin (7 mg/kg) after challenge; a group given only daptomycin (7 mg/kg) after challenge; and a control group that did not receive any treatment. Antibiotic was administered daily for 7 days. The experiments were repeated twice. Mice were anaesthetized by an intramuscular injection of ketamine and xylazine and hair on the back was shaved and the skin cleansed with 10% povidone–iodine solution. Using a 1.0×2.0 cm template, one full-thickness wound was established through the panniculus carnosus of the subcutaneous tissue on the back of each animal. A small gauze was placed over each wound and the gauze then inoculated with 5×10^7 cfu of *S. aureus* ATCC 43300. The pocket was closed by means of skin clips. This procedure resulted in a local abscess at 24 h. The procedure and facilities complied with ethical standards and followed the requirements of Commission Directive 86/609/EEC concerning the protection of animals used for experimental and other scientific purposes. Italian legislation is defined in D.L. No. 116 of 27 January 1992.

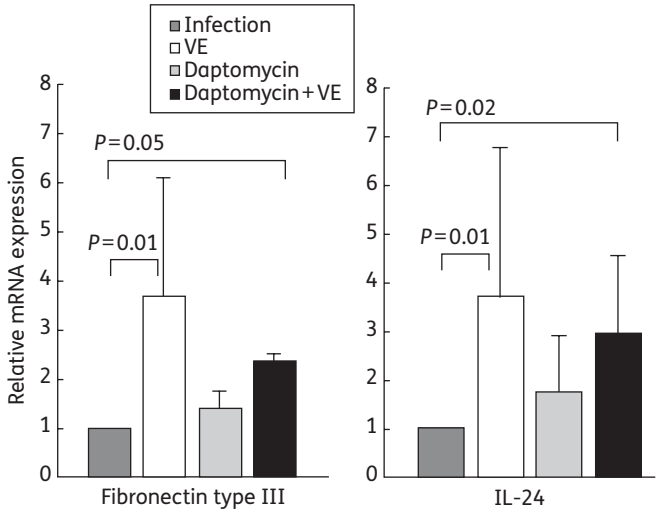

Figure 1. Effect of *in vivo* treatment with vitamin E (VE) and/or daptomycin on markers of tissue repair. Old BALB/c mice were treated with VE and/or daptomycin and analysed for fibronectin and IL-24 mRNA levels by quantitative real-time PCR. Data are reported as means ± SD and are cumulative of two different experiments. Differences in fibronectin and IL-24 mRNA expression were evaluated by ANOVA followed by the Student–Newman–Keuls *post hoc* test when appropriate.

Quantitative cultures of viable bacteria were performed in excised tissues. When mice were challenged with *S. aureus* and immediately afterwards treated with saline (control group), mean bacterial numbers were significantly higher than those in cultures from all other groups ($1.1\times10^8\pm0.1\times10^8$ cfu/mL, ANOVA, $P<0.001$). Groups treated either with vitamin E or daptomycin alone showed a 2 or 3 log reduction in counts ($3.2\times10^6\pm1.1\times10^6$ or $8.5\times10^5\pm2.3\times10^5$ cfu/mL, respectively). The most significant reduction in quantitative cultures of excised tissues was found in mice receiving vitamin E plus daptomycin, in which there was a 4 log reduction in count ($2.0\times10^4\pm0.3\times10^4$ cfu/mL, ANOVA, $P<0.001$). In order to evaluate whether the antimicrobial effect of vitamin E and/or daptomycin was associated with better tissue repair, mRNA for either fibronectin type III or interleukin-24 (IL-24) was evaluated by quantitative PCR on day 8 after infection. Vitamin E treatment induced significant up-regulation of both fibronectin and IL-24 mRNA of >2-fold (3.6- and 3.7-fold, respectively) compared with untreated-infected mice ($P=0.01$; Figure 1). The vitamin E+daptomycin combination significantly increased both fibronectin and IL-24 expression compared with control infected animals: increases of 2.3-fold ($P=0.05$) and 2.9-fold ($P=0.02$), respectively. However, daptomycin alone did not modify marker expression compared with infected animals.

The antimicrobial effect of vitamin E alone or with daptomycin was associated with immune modulation. In mice treated with vitamin E the percentage of both Gr-1+ cells ($P=0.03$) and CD49b+ cells ($P=0.04$) was increased in comparison with control infected animals. Daptomycin alone did not change any of the leucocyte populations in comparison with control infected mice. The vitamin E+daptomycin combination significantly increased CD49b+ cells compared with mice treated with vitamin E alone ($P=0.03$) or control infected animals ($P=0.002$).

In conclusion, this study demonstrates that vitamin E displays potential antimicrobial benefit with respect to MRSA both by itself and when used in combination with daptomycin. The significant bacterial inhibition occurring in animals treated with either vitamin E alone or vitamin E+daptomycin was associated with increased markers of tissue repair and immunological changes. Future studies will be required to evaluate the potential use of vitamin E as an enhancer of antibiotic therapy in humans, especially for the management of infected wounds, particularly in those populations who are at greater risk of inadequate dietary intake of vitamin E,[2] such as elderly subjects.

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Transparency declarations

None to declare.

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Long-term carriage of NDM-1-producing *Escherichia coli*

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