SHORT COMMUNICATION

Resveratrol role in *Staphylococcus aureus*-induced corneal inflammation

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Corneal inflammatory diseases are often associated to *Staphylococcus aureus* (limbitis, blepharo-conjunctivitis, superficial punctate keratopathy, staphylococcal marginal keratitis, and corneal abscesses). Except for corneal abscesses, *S. aureus* induced corneal inflammation seems to be related to host hypersensitivity rather than to a classical invasive infection. This new approach targeting the immune-modulation of the corneal epithelium seems to be an attractive alternative solution to conventional treatment consisting of corticosteroid drops.

Keywords
immunofluorescence; immunoperoxidase; innate immunity; phytoalexin.

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Under normal conditions, the cornea is highly resistant to microbial invasion. However, once the epithelial integrity is breached, pathogens may invade the cornea, leading to microbial keratitis (Kumar & Yu, 2006). *Staphylococcus aureus*, a commensal of the wet mucosa and skin, is a leading cause of invasive infection (Heimer et al., 2010). In the field of ophthalmology, ocular infections such as dacryocystitis, conjunctivitis and keratitis, which occur mainly in contact lens wearers and those with corneal injury, are often reported (Heimer et al., 2010; Sotozono et al., 2013). Infectious keratitis and endophthalmitis caused by methicillin-resistant *S. aureus* (MRSA) are increasing problems throughout the world (Major et al., 2010; Sotozono et al., 2013). Because of the incidence of reported antibiotic-resistant strains and failure of antimicrobial peptides treatment to manage keratitis, it has been suggested that a better understanding of the mechanisms by which the pathogen strains induce disease will be critical for the rational design of improved therapeutic strategies (Hazlett, 2004). The ability of corneal epithelial cells to recognize *S. aureus* is largely attributed to the Toll-like receptor 2 (TLR2) which, once activated, triggers pro-inflammatory cytokines expression in a TLR2/MyD88-dependent manner (Sun et al., 2006; Lambiase et al., 2011). Resveratrol (trans-3,5,4′-trihydroxystilbene) is a phytoalexin whose synthesis in plants can be induced by microbial infections (Alarcón de la Lastra & Villegas, 2005). Several studies within the last few years have shown that resveratrol...
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The primary antibody was excluded. The sections were analyzed and images acquired using a Zeiss LSM 5 DUO confocal laser scanning microscope.

For RNA extraction the superficial layers of the epithelium were removed using a 13-mm pre-autoclaved membrane filter (Redfern et al., 2011). Total RNA from the epithelium was extracted using an RNeasy Mini Kit (Qiagen) following the supplier’s instructions. PCR amplification was performed with an Applied Biosystem 7300 Real-Time PCR System, (Monza, Italy) coupled with the SYBR® green JumpStart™ Taq Ready Mix kit using specific primers for TLR2 (Kajikawa et al., 2005), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and interleukin (IL)-8 (Wang et al., 2007) at optimized concentrations and cycling conditions. GAPDH was used as housekeeping gene for normalization. The fold increase was compared with the cells of intact corneas not exposed to S. aureus and mRNA expression was determined using the 2 \(^{-\Delta\Delta C(T)}\) method (Livak & Schmittgen, 2008). Results were expressed as means ± SD from two experiments and statistically analyzed by a one-way ANOVA test, followed by Tukey’s HSD. Differences in groups and treatments were considered significant for \(P < 0.05\).

The external surface of the cornea is covered by a stratified epithelium, composed of an outer layer of squamous cells, an intermediate layer formed by both flattened wing cells, and deeper polygonal cells, as well as a basal layer of columnar cells resting on the Bowman’s layer (Fig. 1a). Immunohistochemical analyses were used to label TLR2.

The cells of intact corneas demonstrated an occasional mild immunostaining for TLR2 (Fig. 1a). The cells of abraded corneas presented a stronger staining located on a few cells (Fig. 1b). The cells of corneas activated by both strains revealed a higher staining diffused along the surface of all the epithelium exposed to strains (Fig. 1c and Fig 1d), whereas resveratrol-treated cells showed a pattern similar to those of cells of intact corneas. The images of vehicle-treated samples were not different from those of the activated corneas (data not shown).

These results were confirmed by quantitative RT-PCR. TLR2 mRNA levels were significantly higher in activated cornea cells (2.9 ± 0.3-fold and 3.2 ± 0.2-fold change for S. aureus ATCC 6538P and S. aureus ATCC 29213, respectively) than in resveratrol-treated cells (0.6 ± 0.1-fold and 1.1 ± 0.2-fold change, for S. aureus ATCC 6538P and S. aureus ATCC 29213, respectively) compared with abraded cornea cells (1.7 ± 0.2-fold change) or intact cornea cells (1.0 ± 0.2-fold change).

To study the TLR signalling efficiency and downstream effector responsiveness to S. aureus, the expression of IL-8 mRNA was measured. The results showed a significant upregulation of IL-8 mRNA levels in activated cornea cells (7.8 ± 1.9-fold and 3.6 ± 0.1-fold change for S. aureus ATCC 6538P and S. aureus ATCC 29213, respectively) against resveratrol-treated cells (0.5 ± 0.1-fold and 0.9 ± 0.2-fold change for S. aureus ATCC 6538P and S. aureus ATCC 29213, respectively) when compared with abraded cornea cells (1.9 ± 0.3-fold change) or intact cornea cells (1.0 ± 0.1-fold change). DMSO did not
influence the TLR2 or IL-8 expression in vehicle-treated samples (data not shown).

Human corneal epithelial cells express the TLR2, which recognize and respond to \textit{S. aureus} infection by expression and secretion of pro-inflammatory cytokines (IL-8) in a TLR2/MyD88-dependent manner (Lambiase \textit{et al.}, 2011). The anti-inflammatory properties of resveratrol against bacterial infection are due to inhibition of both transcriptional activity and translocation into nuclei of NF-\textit{kB} functioning downstream of TLR2 (Iyori \textit{et al.}, 2008).

Our results demonstrated that, in response to \textit{S. aureus}, epithelial cells increased expression of TLR2s and, at the same time, significantly upregulated expression of the IL-8 gene. Since the IL-8 gene has the consensus sequence in its promoter \textit{kB}, it is possible to hypothesize the involvement of the transcription factor NF-\textit{kB} by the TLR2/MyD88 pathway in the activation of the inflammatory response triggered by \textit{S. aureus} (Johnson \textit{et al.}, 2005). The resveratrol treatment significantly decreased cell surface TLR2 and downregulated expression of IL-8 gene in epithelial cells stimulated by \textit{S. aureus} with respect to untreated cells.

Our study is the first to demonstrate the anti-inflammatory effects of resveratrol against \textit{S. aureus}-induced corneal inflammation. The present results are very interesting and, although obtained on two strains of \textit{S. aureus}, suggest that resveratrol should be evaluated not only for its anti-inflammatory effects but also for its potential innate immune-suppressive properties. Further detailed studies will be needed to confirm the anti-inflammatory activity of resveratrol towards a greater number of strains. The experimental approach described here and based on \textit{ex vivo} corneal culture can be an alternative to \textit{in vivo} animal testing for achieving an understanding of the molecular events of bacterial–epithelial interactions and their inflammatory consequences. The corneal culture model tested can also be used to develop novel specific therapies.

References

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