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Selective photodynamic inactivation of *Staphylococcus aureus* with Hypericin

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Introduction: *Staphylococcus aureus* is a gram positive bacteria responsible by localized and systemic infections. The bacterial resistance to antibiotics is a huge problem and a challenge for the development of new therapeutic modalities [1]. The photodynamic inactivation of microorganisms (PDI) is a promising technique to fight localized infections based on a association of a photosensitizer (PS), oxygen and visible light [2]. The main advantage of this technology is the existence of multiple targets, making improbable the upsurge of resistance [3]. The efficacy of PDI depends on type and concentration of PS as well as the action mode of the photosensitizer. The goal of this study was to investigate the selective photoinactivation of *S. aureus* using Hypericin (Hy) and a more soluble derivative (Hy-G, hypericin associated with N-methyl-glucamine, since this amine can act as a base, promoting the deprotonation of carboxylic groups, as well as a huge volume ion pair, avoiding the aggregation between the planar macrocicles). We have observed that the association with the glucamine enhances the molar extinction coefficient of Hy in DMSO, what support the general idea of this work. In order to have an extensive clinical application of PDI is necessary to have a selective photoinactivation of the microorganisms, i.e., causing negligible injure to the host tissue. The study involved the solubility, the phototoxicity as well as the photodynamic parameters of these compounds. **Methodology:** 100 µL of a suspension of *S. aureus* ($1x10^9$ cells mL^{-1}) was inoculated with 100µL of the PSs in several concentrations at 37 °C for 150 and 300 seconds. The samples were irradiated at the wavelength 590 nm and doses 3 and 6 J cm^{-2} with LEDs. After that the samples were centrifuged by 10 min at 1300 rpm. To the pellet were added 50 uL of MTT (2 mg mL^{-1}) incubated for 30 min at 37 °C, centrifuged for 5 min at 6000 rpm and followed by the addition of 150 uL of isopropanol incubated for 12h followed by the addition of 50 uL de PBS. The absorbance was obtained at 560 nm in order to calculate the survival index. Aiming the investigation of the effect of PDI parameters on human epithelial cells, HEp-2 cells were used as a model since this kind of cells are the constituents of some host tissues, such mucosas, skin and cavities. Therefore, a suspension of $1x10^5$ cells was submitted to the same conditions as the bacteria. In order to estimate the hydrophobicity of the photosensitizers, the partition coefficient between mutually saturated 1-octanol and phosphate buffered saline (PBS) pH=7.4 was determined by the well spread shake-flask method [4]. The quantum yield of singlet oxygen formation was determined following the oxidation of uric acid (UA) by spectrophotometric absorption measurement at 292 nm. The PS photodynamic activity was measured in sodium dodecylsulfate (SDS 2%). The solution containing PS and UA was irradiated with a LED 590nm. The samples were then collected to record the UV-vis spectra. The photodynamic activity was evaluated based on Fisher's method [5]. **Results:** Results confirmed that HY (log P=1.20) is more liposeluble than HY-G (P=1.06) which agrees with the photodynamic activities (Hy=25.8 ± 0.5 and Hy-G=21.3 ± 0.6 m^2 J^{-1}) as well as with the cytotoxicity. The median inhibitory concentration (IC_{50}) for HY in HEp-2 cells after 2h of incubation and irradiation with 6 J cm^{-2} of yellow light is 56 ± 6 while for HY-G this value is 142 ± 17 nM. Despite of the slightly better solubility of Hy-G, Hy is a better PS both in cells and in *S. aureus*. When bacteria and cells were treated with the same conditions (5 min incubation, D=6 J cm^{-2}), it is clear that *S. aureus* is much more sensitive than the cells (about 10 fold) what can be explained by the faster accumulation of these photosensitizers in bacteria (minutes) then in cells (~16 h). **Conclusion:** It is possible to adjust the PDI parameters to get a selective photoinactivation, i. e., were very low HY concentration (0.1–0.2 nM for Hy and 0.1–0.4 nM for Hy-G), low incubation time (5 min) and irradiation with LED 590 nm with 6 J cm^{-2}. The results suggest that Hy-mediated PDI could potentially be employed to treat localized infections of *S. aureus* with practically no harm for the host cells.

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Keywords: photodynamic therapy; photosensitizer; microorganism; selective photoinactivation; photodynamic activity; hypericin.

Small-molecule inhibitors of the type 1 pilus biogenesis in *Escherichia coli* and *Salmonella typhimurium*

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Uropathogenic *Escherichia coli* (UPEC) is the primary causative agent of urinary tract infections (UTIs). UTIs account for ~ 8 million physician-office visits and ~ 100, 000 hospital admissions per year in the US alone and represent 20 - 40% of nosocomial infections in the US and Europe. In UPEC, the presence of type 1 pili forms a critical factor in mediating adherence, invasion and establishment of biofilms in the bladder epithelium. This suggests that chemical inactivation of type 1 pili present promising strategies for treating and preventing UPEC-caused urinary tract infections. Type 1 pili are assembled by the chaperone-usher (CU) pathway, a conserved biosynthetic secretory system responsible for assembly of an array of pili in many bacterial pathogens. In this study, we have identified small compounds that inhibit type 1 pili assembly by specifically blocking a critical step during pilus biogenesis. Our study showed that these compounds not only inhibit the type 1 pili dependent-biofilm formation in UPEC but also in *Salmonella typhimurium*. Compound-treated UPEC exhibited reduced adherence to human bladder epithelial cells. We further demonstrated that the compounds are able to disrupt pre-assembled type 1 pili in UPEC. These compounds had no overall inhibitory effect on the growth rate of the bacterial species tested.