Porphyrins and heme in microorganisms

Porphyrin content and its relation to phototherapy and antimicrobial treatments *in vivo* and *in vitro*

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Abstract

One of the greatest threats to human health is increasing antimicrobial resistance among pathogens, and finding alternatives for treatment of bacterial infections is of highest importance together with a more controlled use of antibiotics. Porphyrins and heme have both been shown to be a promising class of compounds for inactivation of bacteria; porphyrins by their excellent properties to act as a photosensitizer, and heme by its importance as an iron source during a bacterial infection in vertebrates.

This thesis describes the development of analytical methods for the identification and determination of porphyrins and heme using liquid chromatography coupled to tandem mass spectrometry. Subsequently, these developed methods were applied to bacterial samples to investigate different culture conditions and additives effect to the intracellular porphyrin and heme composition. Singlet oxygen production of three naturally occurring porphyrins have been determined together with the photosensitivity for blue light and the porphyrin content in *E. coli*. Toothbrushes equipped with a LED, emitting light with a wavelength of 450 nm, were used in an eight week randomized clinical trial to investigate any positive periodontal effect of blue light.

Porphyrin and heme content in *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* were highly affected by the different cultivation conditions. The culture age of *A. actinomycetemcomitans* affected the porphyrin profile, while only small changes were observed for *P. gingivalis* during growth. A large change of the porphyrin profile could be observed when the bacteria were passaged onto a new growth medium. Additional porphyrins were detected and the total porphyrin content increased up to 28 times. These findings highlight the need for more standardized cultivation procedures when performing *in vitro* experiments.

Heme content in *Escherichia coli* was affected when different additives related to biosynthesis of heme were added to the growth medium. The uptake of heme could be reduced with 52% when a compound that chemically looks similar to heme was added to the growth medium. Since heme acquisition is important for many pathogens, this could be a promising target for antimicrobial drugs.

E. coli showed no sensitivity for 405 nm light using light doses up to 172.8 J/cm^2 and only low concentrations of porphyrins could be quantified. By adding a porphyrin precursor to *E. coli* the intracellular concentration of porphyrins increased remarkably and a light dose of 57.6 J/cm^2 reduced the bacterial number with > 5 $\log_{10}$ steps. This shows that *E. coli* can be killed due to their endogenous porphyrins.

In the clinical study we could see a weak trend that the 450 nm LED toothbrush possessed a phototherapeutic effect for three clinical indices. All indices were decreased in the intervention group, but there were no statistically significant difference compared to the control group. However, four inflammation markers were significantly decreased in the intervention group while only one decreased significantly in the control group.

In conclusion, this thesis has shown that porphyrins and heme are produced endogenously in microorganisms and that the porphyrin profiles vary depending on culture conditions and different additives. Furthermore, porphyrins may be used as endogenous photosensitizers to inactivate bacteria, but more research is necessary to determine if there is a specific porphyrin that contributes more to the photosensitivity.

Keywords: *Porphyrins, heme, phototherapy, antimicrobial resistance, singlet oxygen, photosensitizer, bacteria, HPLC-MS/MS, oral bacteria, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Escherichia coli.*

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