Photokilling of bacteria by curcumin in selected aqueous preparations.

*T. Haukvik [1,2], E. M. Bruzell [2], I. S. Dragland [2], S. Kristensen[1], H. H. Tønnesen [1]

[1] *University of Oslo, School of Pharmacy, Department of Pharmaceutics, Norway*
[2] *Nordic Institute of Dental Materials (NIOM), Norway*

e-mail: tone.haukvik@farmasi.uio.no

Antibacterial photodynamic therapy (PDT) offers a promising alternative to antibiotic treatment against localized infections in e.g. the mouth, skin and stomach. Curcumin (CU) has potential as a photosensitizer (PS) in PDT for localized superficial infections. CU is a natural, yellow compound with absorption maximum at 430 nm. CU is practically insoluble in water at acidic or neutral pH. At pH above neutral the water solubility increases, but the compound then undergoes rapid hydrolytic degradation. Therefore, it is a challenge to make an aqueous CU preparation with an acceptable solubility and stability at physiological pH by using excipients. Further, an optimal preparation of CU should act as a delivery vehicle which ensures a localized and adequate delivery of CU at the site of infection. In addition, the vehicle may interfere with the bacterial membrane causing a disruption of the outer barrier, thereby making the bacteria more susceptible to the treatment. The vehicle may also influence the effect of CU by preventing “stacking” and altering the photochemical and photophysical properties of CU. These changes may, in turn, lead to an increased formation of phototoxic species responsible for the killing.

The aim of this study was to investigate the phototoxic effects of CU in selected aqueous preparations against gram positive and gram negative bacteria. The gram positive *Enterococcus Faecalis* and the gram negative *Escherichia Coli* were used as models. The bacteria were exposed to 2.5 and 25 µM CU in DMSO, cyclodextrines, liposomes and surfactants known to interfere with membranes. After 30 min incubation the bacteria were irradiated with fluorescent tubes emitting blue light (em. max 430 nm). The irradiance was 17mW/cm^2 and the light dose 0.5 - 30 J/cm^2. The bacterial survival was calculated as a percentage compared to controls. Different post-irradiation incubation times were tested. CU possesses native fluorescence and the uptake of CU in bacteria was examined by fluorescence microscopy.

The gram positive bacteria *E. faecalis* was efficiently killed by low concentrations of CU (2.5 µM) in combination with a low light dose (0.5 J/cm^2) when CU was dissolved in DMSO or certain surfactant solutions (<0.5% survival). The gram negative bacteria *E. coli* was somewhat less susceptible to the treatment, but by increasing the CU concentration tenfold in combination with higher light dose (30 J/cm^2) a 100 % reduction of viable bacteria (n=12) was achieved with CU in a surfactant solution.

In conclusion, the phototoxic effect was dependent on CU concentration, light dose, post-irradiation incubation time and excipient used in the CU preparation. This work demonstrates the potential of CU as a PS in the killing of both gram positive and gram negative bacteria in combination with blue light, by selecting an appropriate pharmaceutical formulation. Ongoing work includes investigations of the mechanisms responsible for the observed phototoxicity, optimization of the experimental conditions, and evaluations of solubility, hydrolytic stability and photochemical and photophysical properties of CU in selected aqueous preparations.