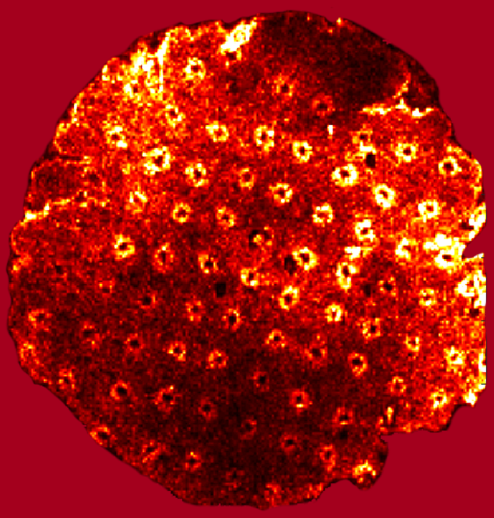
UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

Exploring bleomycin in dermato-oncology with energy-based topical drug delivery



PhD Thesis

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List of papers

I Hendel KK, Jemec GBE, Haedersdal M, Wiegell SR. Electrochemotherapy with bleomycin for basal-cell carcinomas: A systematic review. *J Eur Acad Dermatol Venereol 2021*

II Hendel KK, Bagger C, Olesen UH, Janfelt C, Hansen SH, Haedersdal M, Lerche CM. Fractional laser-assisted topical delivery of bleomycin quantified by LC-MS and visualized by MALDI mass spectrometry imaging. *Drug Deliv 2019;26:244–51.*

III Hendel KK, Hansen AC, Bik L, Bagger C, van Doorn M, Janfelt C, Olesen UH, Haedersdal M, Lerche CM. Bleomycin administered by laser-assisted drug delivery or intradermal needle-injection results in distinct biodistribution patterns in skin: in vivo investigations with mass spectrometry imaging. *Drug Deliv 28:1, 1141-1149*

The listed articles form the basis of the present thesis and will be referred to as study I, II, and III throughout the text. Paper I does not have Open Access status and is not available in this online version of the thesis. Please visit the resource online or find the print version at The Royal Library of Copenhagen.

I II III

Scan the QR codes with your phone to access the *doi* for each article directly.

Summary

Basal cell carcinoma (BCC) is a skin tumour and the most frequently occurring cancer in the world. Several treatment options are available including surgical approaches, topical options, energy-based treatments, and even systemic treatments. Incidence rates are rising, and effective, topical, and tissue-sparing treatments are in high demand. Bleomycin is an antitumour agent with great potential for treating BCCs, but it cannot cross neither skin barrier nor cell membrane unassisted. An emerging approach, electrochemotherapy, employs intratumoural injection to overcome the skin barrier, and subsequent localised electroporation to destabilise cell membranes, augmenting intracellular uptake. Several drug delivery systems are designed to overcome the skin barrier, but laser-assisted drug delivery presents an opportunity to apply bleomycin topically.

The literature basis for treating BCCs with bleomycin-based electrochemotherapy was systematically reviewed. A single randomised trial (RCT) showed good results similar to those seen with conventional excision surgery, reporting high efficacy and low recurrence rates. Despite a limited and heterogeneous basis of available literature, most studies reported results in keeping with those observed in the RCT.

Laser-assisted topical drug delivery (LADD) of bleomycin was established *in vitro* using an ablative fractional CO_2 laser to create a fractional pattern of microscopic ablation zones in pig skin. Uptake of bleomycin was enhanced with longer drug exposure times and increasing laser-channel dimensions i.e., drug penetration depth following topical application may be adjustable by modulating laser energy-levels. Assessed by state-of-the-art mass spectrometry techniques for imaging biodistribution and quantifying exact concentrations, bleomycin was visualised to concentrate in laser-channels and disperse into the surrounding tissue, with high concentrations available at all skin depths dependent on delivery parameters.

In a live pig trial, bleomycin was successfully delivered by way of both LADD and conventional needle injection (NI), resulting in distinct biodistribution patterns. As seen in the *in vitro* study, bleomycin delivery by LADD concentrated in laser-channels and dispersed into the surrounding tissue, creating a belt-shaped delivery zone, stretching from the superficial skin to the lower boundaries of the laser-channel dimensions. In contrast, NI delivery resulted in circular biodistrubtion with bleomycin dispersing in a radiating pattern from an initial intradermal deposit. Drug residence time was at least one hour for both delivery routes, with a small deposit still retained after four hours following NI. Subsequent electroporation did not modulate drug residence time or biodistribution. All delivery methods were tolerable to the skin, causing moderate and transient local skin reactions, and allowed almost full recovery within nine days.

LADD with bleomycin and electroporation can potentially become an effective, topical, and tissue-sparing treatment for BCCs. Conceivably a complement to needle injection, the resulting distinct belt-shaped biodistribution pattern can offer a topical approach and may be uniquely suited for superficial BCCs. With bedside imaging being introduced in the field of dermato-oncology, the perspectives include highly individualised and targeted treatment approaches using adjustable laser-treatments and electroporation.

Danish summary | Dansk resumé

Hudkræften basal celle karcinom (BCC) er den hyppigst diagnosticerede kræftype i verden. Der findes flere behandlingstilgange og tilbud, herunder kirurgiske, topikale, energi-baserede, og systemiske. Incidensen stiger ligeledes verden over, og der et tiltagende merbehov for effektive, topikale, og vævsbesparende behandlinger. Bleomycin er et kræftmiddel med stort potentiale i behandlingen af BCC. Imidlertid kan stoffet ikke trænge ind gennem huden eller ind i kræftceller uden for- eller efterbehandling. Med en ny model der vinder indpas, kan man injicere bleomycin ind i hudkræft, og efterfølgende tilføre strøm til huden (elektroporation) for at destabilisere kræftcellerne, hvorved det intracellulære optag øges. Kombinationen af kræftlægemiddel og elektroporation benævnes elektrokemoterapi (ECT). Der findes flere modeller udviklet til at facilitere lægemidler over hudbarrieren, men særligt forbehandling med ablativ fraktioneret laser udgør en mulighed for at benytte bleomycin topikalt.

I en systematisk litteraturgennenmgang undersøgtes evidensgrundlaget for behandling af BCC'er med ECT. Et enkelt randomiseret og kontrolleret forsøg viste man gode resultater der var sammenlignelige med en konventionel kirurgisk tilgang. På trods af et begrænset og heterogent litteraturgrundlag viste de fleste øvrige studier tilsvarende resultater.

I et *in vitro* forsøg blev grisehud forbehandlet med en ablativ fraktioneret CO_2 laser, hvilket faciliterede transport af topikalt appliceret bleomycin ned i huden. Laser-assisteret facilitering af lægemidler ned i huden er et resultat af mikroskopiske laser kanaler i det behandlede område, der er organiseret i et gittermønster. Lægemidler kan trænge ned gennem disse laser kanaler, og et højere energi-niveauer skaber dybere og bredere laser kanaler. Optag af bleomycin blev forøget ved tiltagende længere eksponeringstider, samt ved højere energi-niveauer. Med billeddannende og kvantificerende masse-spektrometri analyser blev det vist, at bleomycin opkoncentreres i laser kanalerne og derfra fordeler sig ud i det omkringliggende væv. Afhængigt af energi-niveauer og eksponeringstid kunne bleocmyin leveres til alle hudlag.

I et griseforsøg med levende dyr blev både laser-assisteret facilitering og konventionel nåle injektion benyttet som leveringsmetoder for bleomycin. Lægemidlet fordelte sig forskelligt afhængigt af leveringsmetode. Ved brug af laser-kanaler opkoncentreredes bleomycin heri, og spredte sig til det omkringliggende væv. Dette skabte en bæltelignende leveringszone der strakte sig fra hudoverfladen og ned langs laser-kanalerne i dybden. Ved nåle-injektion så man et cirkulært fordelingsmønster hvor bleomycin spredte sig ud fra et initielt intradermalt depot. Bleomycin var til stede mindst en time ved begge leveringsmetoder, og et lille depot ved det initielle injektionssted kunne ses efter fire timer når man benyttede nåle-injektion. Elektroporation påvirkede ikke fordelingsmønstre eller tilstedeværelsen af bleomycin i huden. Begge leveringsmetoder, med og uden efterfølgende elektroporation, var tolerable for huden. Der blev observeret forbigående moderate hudreaktioner, men for alle behandlingskombinationer var hudens integritet næsten genoprettet inden for ni dage.

Laser-assisteret facilitering af topikalt appliceret bleomycin kan blive en effektiv og vævsbesparende behandling for BCC'er. Metoden kan komplementere lokal nåleinjektion, hvor leveringsmetoderne resulterer i distinkte fordelingsmønstre i huden, der potentielt kan målrettes forskellige typer og lokalisationer af BCC'er. Udviklingspotentialet er stort. Hurtige og non-invasive scanninger bliver i tiltagende grad taget i brug i dermatoonkologien. Med scanninger af et BCC's udstrækning i dybden og bredden under huden, udvides perspektiverne for laser-assisteret facilitering. Med justérbare energi-niveauer kan forbehandlingen med laser og efterbehandlingen med elektroporation målrettes det enkelte BCC.