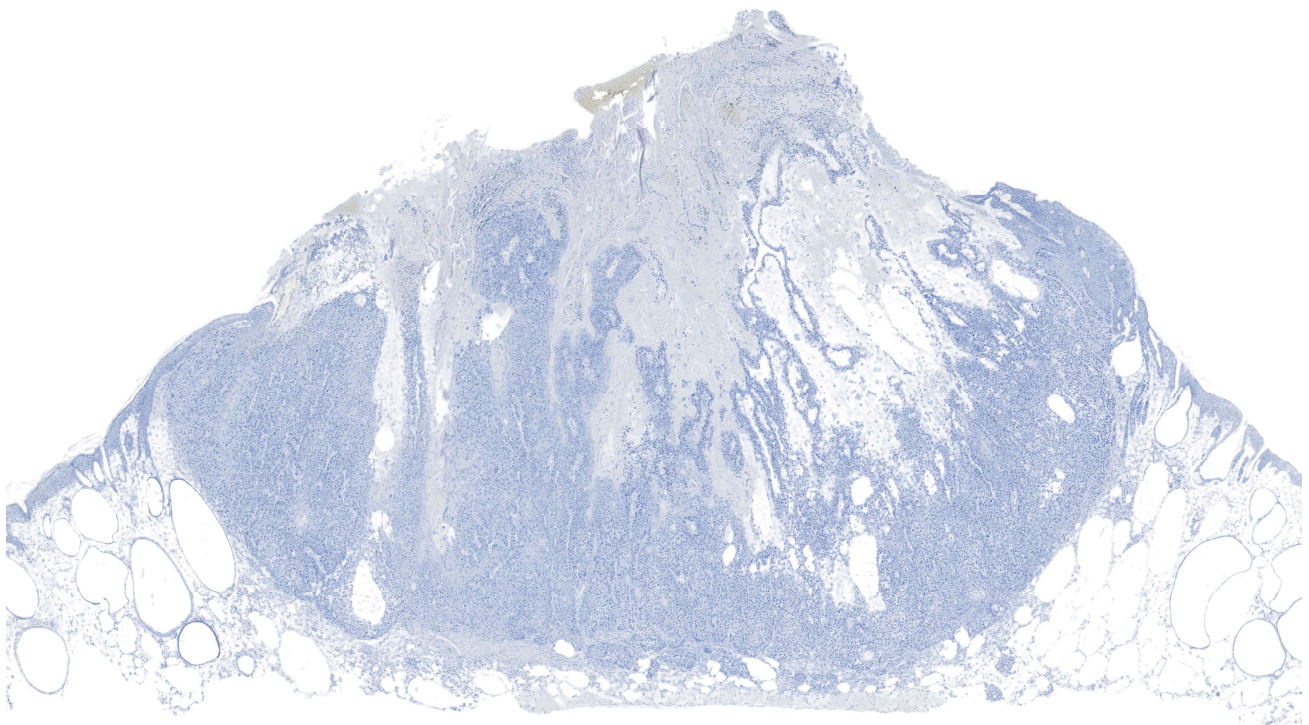


UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

DEPARTMENT OF DERMATOLOGY
COPENHAGEN UNIVERSITY HOSPITAL – BISPEBJERG HOSPITAL



PhD thesis

Celina Pihl

Pre-clinical investigations of oral photoprotection to prevent keratinocyte carcinoma

This thesis has been submitted to the Graduate School of Health and Medical Sciences,
University of Copenhagen on the 31st of January 2024.

List of papers

The PhD thesis is based on the scientific work conducted in the period between 2020-2024 as a part of the Danish Research Center for Skin Cancer and was performed at the Department of Dermatology, Copenhagen University Hospital – Bispebjerg and Frederiksberg Hospital. The papers and manuscripts presented below make up the basis of the thesis and will be referred to as *study I*, *study II*, *study III*, and *study IV*, respectively.

- I. Pihl C, Bendtsen KMS, Jensen HE, Andersen F, Bjerring P, Haedersdal M, Lerche CM. *Oral phytochemicals as photoprotectants in UVR exposed hairless mice: A study of hesperidin methyl chalcone, phloroglucinol, and syringic acid.* J Photochem Photobiol B. 2023 Sep;246:112760. doi: 10.1016/j.jphotobiol.2023.112760.
- II. Pihl C, Granborg JR, Pinto F, Bjerring P, Andersen F, Janfelt C, Haedersdal M, Lerche CM. *Oral administration of quercetin and fisetin potentiates photocarcinogenesis in UVR-exposed hairless mice.* Submitted 18th of November 2023.
- III. Pihl C, Bjerring P, Andersen F, Haedersdal M, Lerche CM. *Oral intake of bucillamine, carvedilol, metformin, or phenformin does not protect against UVR-induced squamous cell carcinomas in hairless mice.* Accepted for publication in Photochem. Photobiol. Sci. 2024.
- IV. Pihl C, Andersen F, Bjerring P, Haedersdal M, Lerche CM. *Efficacy of combinational treatment versus nicotinamide monotherapy in the prevention of ultraviolet radiation-induced skin cancer.* Submitted 24th of January 2024.

English Summary

Cutaneous squamous cell carcinoma is the second most common type of keratinocyte carcinoma with 2.4 million cases a year. Ultraviolet radiation (UVR) contributes to most of these cases, representing a major risk factor. Topical sunscreen is under-used with considerable adherence challenges. Oral photoprotection presents an emerging alternative. Through oral intake of photoprotective compounds, such as phytochemicals or pharmaceuticals, photoprotection may be boosted. Nicotinamide is reported to prevent the formation of new keratinocyte carcinomas in high-risk patients, but further optimisation of efficacy is still possible. The overall aim of this thesis was to explore the efficacy of orally delivered photoprotectants to protect against UVR-induced squamous cell carcinoma development, with an additional focus on exploring nicotinamide combinations.

The studies were performed in a hairless mouse model treated with the photoprotectants through either their drinking water or feed. The mice were exposed to UVR three times per week to induce tumour development. Photoprotection was primarily evaluated by the time until tumour onset.

Photoprotection was demonstrated by two phytochemical compounds, phloroglucinol and syringic acid, demonstrating a delay in tumour onset compared to UVR control mice (*Study I*). Phloroglucinol was shown to reduce tumour number but not size, indicating a protective role in the initial stages of tumour development. In contrast, the phytochemicals quercetin and fisetin accelerated tumour onset, with quercetin further increasing tumour growth (*Study II*). The opposite effects of the included phytochemicals highlight the bidirectional impact phytochemicals may have on carcinogenesis, indicating a need for caution. Four repurposed pharmaceuticals (*Study III*), including metformin thought to be photoprotective, and two phytochemicals (*Study I* and *II*) failed to impact tumour development, showcasing a lack of oral photoprotection potential for compounds otherwise considered to be photoprotective. Nicotinamide treatment provided effective protection in all three studies.

Given the photoprotection of nicotinamide, *study IV* explored combinational treatments with compounds of different mechanisms of action. Treatments included: nicotinamide monotherapy, nicotinamide combined with phloroglucinol, an antioxidant, and nicotinamide combined with metformin thought to act anti-proliferative. All treatments delayed tumour onset compared to UVR control mice. Nicotinamide combined with phloroglucinol was comparable to monotherapy, whereas the metformin combination was inferior. Nicotinamide combined with phloroglucinol reduced DNA damage compared to both the UVR control and monotherapy, indicating an improved photoprotection mechanism.

Overall, the studies provide important insight into potential compounds for oral photoprotection, indicating that nicotinamide remains an excellent candidate. Furthermore, the successful results of nicotinamide and phloroglucinol treatment demonstrate an avenue for enhancing photoprotection that should be further explored with additional compounds to improve skin cancer prevention.

Danish summary | Dansk resumé

Spinocellulære karcinomer er den anden mest hyppige form for keratinocyt karcinom hudkræft med 2,4 millioner nye tilfælde om året. Ultravioletstråling bidrager til størstedelen af disse tilfælde og repræsenterer derfor en stor risikofaktor for udviklingen af keratinocyt karcinomer. På grund af manglen på gentagen og tilstrækkelig brug af solcreme, må andre hudkræftforebyggelsestiltag betragtes. Oral solbeskyttelse er et koncept, hvori solbeskyttende stoffer såsom plantestoffer eller genanvendte lægemidler indtages for at øge solbeskyttelse. Nikotinamid har vist sig at forbygge fremkomsten af keratinocyt karcinomer i høj-risiko patienter, men optimering af nikotinamids effektivitet er stadig mulig. For at adressere disse udfordringer bør kombinationsbehandlinger med nikotinamid undersøges. Målet med denne afhandling var at undersøge effektiviteten af oralt leverede solbeskyttende stoffer i forebyggelsen mod hudkræft med særligt fokus på nikotinamidkombinationer.

Studierne blev udført i hårløse mus behandlet med solbeskyttende stoffer i enten deres drikkevand eller foder. Musene blev udsat for ultravioletstråling tre gange om ugen for at inducere udviklingen af tumorer. Solbeskyttelse blev primært evalueret ud fra tiden indtil tumorfremkomst.

To plantestoffer (Phloroglucinol og syrensyre) udviste god solbeskyttelse ved at forsinke tiden til tumorfremkomst sammenlignet med mus kun bestrålet med ultravioletstråling (*Studie I*). Phloroglucinol reducerede antallet af tumorer, men ikke det samlede tumorareal, hvilket kan indikere en beskyttende rolle i de indledende stadier af tumorudvikling. Behandling med quercetin og fisetin derimod accelererede udviklingen af tumorer (*Studie II*), hvilket understreger de modsatte effekter plantestoffer kan have på udviklingen af kræft. Fire genanvendte lægemidler (*Studie III*), inklusiv metformin tiltænkt at være solbeskyttende, og to plantestoffer (*Studie I og II*) viste sig at have ingen effekt på tumorudvikling, hvilket begrænser deres potentiale som oral solbeskyttelse. Behandling med nikotinamid var solbeskyttende i alle tre studier.

På grund af nikotinamids effektive solbeskyttelse fokuserede *studie IV* på at undersøge kombinationsbehandlinger med forskellige virkningsmekanismer. Behandlingerne bestod af nikotinamidmonoterapi, nikotinamid i kombination med antioxidanten phloroglucinol og nikotinamid kombineret med metformin, som menes at hæmme ukontrolleret cellevækst. Alle behandlinger udsatte tiden til tumorfremkomst sammenlignet med mus kun bestrålet med ultraviolette stråler. Nikotinamid kombineret med phloroglucinol var lige så effektiv som nikotinamidmonoterapi, mens kombinationen med metformin udviste værre solbeskyttelse. Nikotinamid kombineret med phloroglucinol reducerede også DNA-skader sammenlignet med både UV-kontrolgruppen og nikotinamidmonoterapi, hvilket indikerer en forbedret virkningsmekanisme for solbeskyttelse.

Sammen giver studierne vigtigt indsigt i mulige stoffer for oralt leveret solbeskyttelse og viser, at nikotinamid forbliver en god kandidat. Desuden indikerer de succesfulde resultater fra nikotinamid og phloroglucinol kombinationsbehandlingen nye alternative for at forbedre solbeskyttelse. Yderligere kombinationer bør undersøges med henblik på bedre hudkræftforebyggelse.