Cytoprotection of ferrocene-containing curcumin analogue in human keratinocytes

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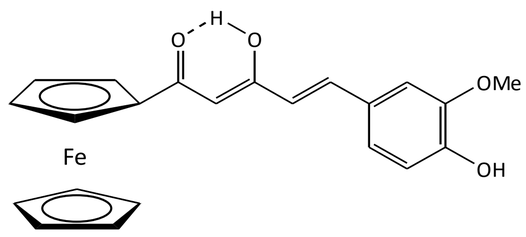
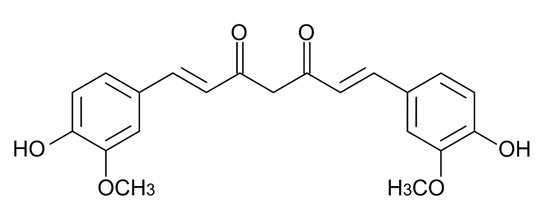
INTRODUCTION

Potential skin protective effects are attributed to polyphenols due to their antioxidant, anti-inflammatory and antiaging properties. Herein, we have selected curcumin (CRC) to investigate mechanisms involved in human keratinocytes protection. In addition, we tried to overcome the limitations of CRC application due to poor pharmacokinetics by introducing ferrocene into CRC structure. In novel (previously synthesized) ferrocene-containing curcumin analogue (FCA) one benzylidene group of CRC is replaced by ferrocene. CRC and FCA were tested in range 2.5 – 50 μM and cell viability was monitored by MTT assay, while intracellular effects were assessed by cytofluorimetric analysis.

Ferrocene-containing curcumin analogue (FCA)



Curcumin (CRC)



METHODOLOGY and RESULTS

CELL VIABILITY ASSAY

- initial cell concentration – 2.5x10^5 cells mL^{-1}
- V=100 μL of cell suspension per well (96-well plate)
- seeding cells in DMEM + 10 % FBS + 2.5 – 50 μM CRC or FCA
- control – DMSO (concentration did not exceed 1 % in final volume)
- determination of cell viability after 48 hours by MTT assay

ROS FORMATION

- initial cell concentration – 2.5x10^5 cells mL^{-1}
- V=100 μL of cell suspension per well (black 96-well plate)
- seeding cells in DMEM + 10 % FBS + 2.5 – 50 μM CRC or FCA
- control – DMSO (concentration did not exceed 1 % in final volume)
- determination of oxidative stress induced by 50 μM *tert*-butyl hydroperoxide (TBHP) after 24 h pre-incubation with CRC/FCA by fluorescent probes

INTRACELLULAR EFFECTS

- initial cell concentration – 2x10^4 cells mL^{-1}
- V=4 mL of cell suspension per well (6-well plate)
- seeding cells in DMEM + 10 % FBS
- after overnight incubation cells were treated with 20 μL of different CRC or FCA dilutions in DMSO to obtain the desired final concentration (5, 20 and 50 μM) in culture medium
- control – DMSO (concentration did not exceed 1% in final volume)
- determination of cell death after 48 h by cytofluorimetric analysis

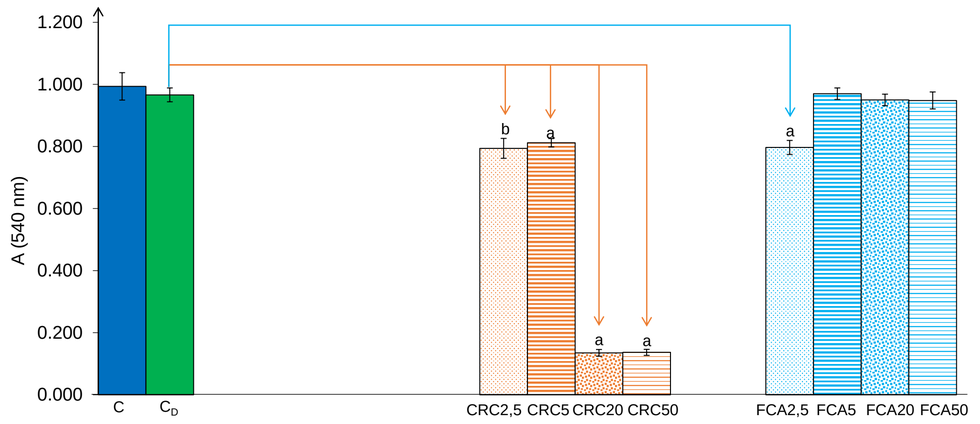


Fig. 1 Effects of 2.5 - 50 μM curcumin (CRC) and ferrocene-containing curcumin analogue (FCA) on HaCaT viability after 48 h of exposure obtained with MTT assay. C – nontreated cells. Data are presented as mean ± SEM. Statistically significant (Student's *t*-test) *vs.* control (cells treated with DMSO, C$_D$) $^a p<0.001$; $^b p<0.005$.

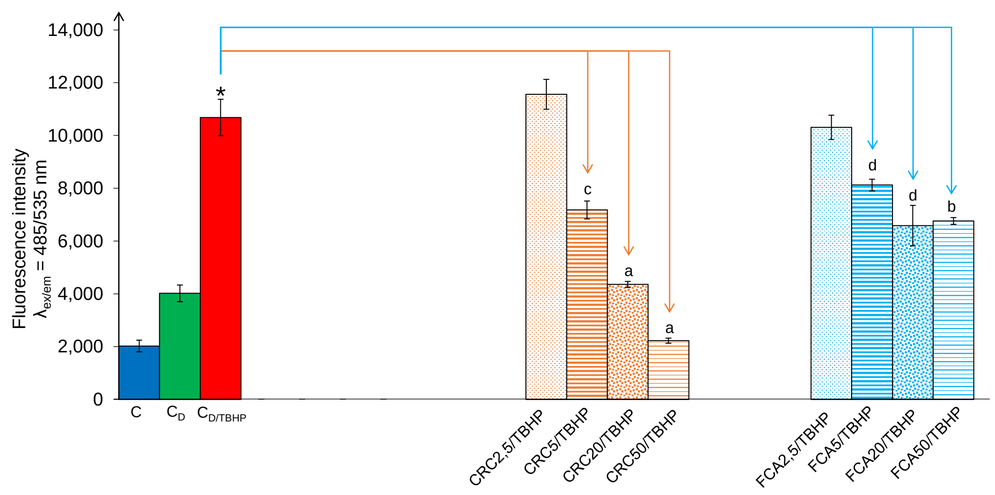


Fig. 2 Effect of pre-incubation with 2.5 – 50 μM curcumin (CRC) or ferrocene-containing curcumin analogue (FCA) on ROS formation in HaCaT cells treated with 50 μM *tert*-butyl hydroperoxide (TBHP). C – nontreated cells; C$_D$ – control (cells treated with 5 μL DMSO mL^{-1}); C$_{D/TBHP}$ – cells treated with 5 μL DMSO mL^{-1} and 50 μM TBHP. Data are presented as mean ± SEM. Statistically significant difference (Student's *t*-test): C$_{D/TBHP}$ *vs.* C$_D$ $^* p<0.001$; CRC/TBHP *vs.* C$_{D/TBHP}$ $^a p<0.001$, $^c p<0.01$; FCA/TBHP *vs.* C$_{D/TBHP}$ $^b p<0.005$, $^d p<0.025$.

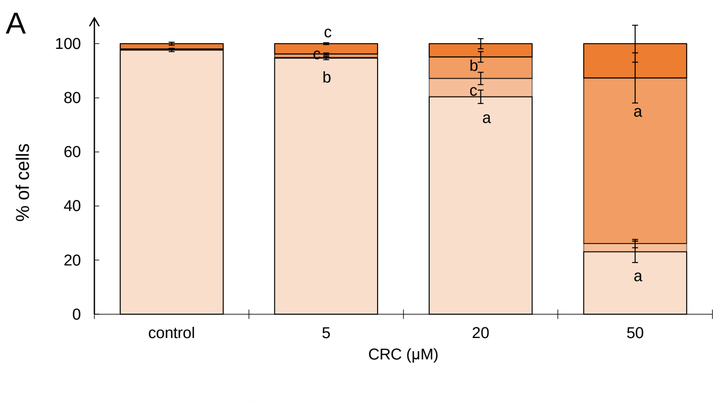


Fig. 3 Flow cytometric analysis of apoptosis/necrosis in HaCaT cells after exposure to 5 – 50 μM curcumin (CRC) for 48 h by Muse Cell Analyzer (Merck Millipore). (A) Percentage of live, early and late apoptotic, and dead cells in population (mean ± SEM) after treatment with CRC. Statistically significant difference (Student's *t*-test) *vs.* control: $^a p<0.001$, $^b p<0.01$, $^c p<0.025$. (a) Representative dot-plot profiles of untreated (control) and treated (5 and 50 μM CRC) HaCaT cells.

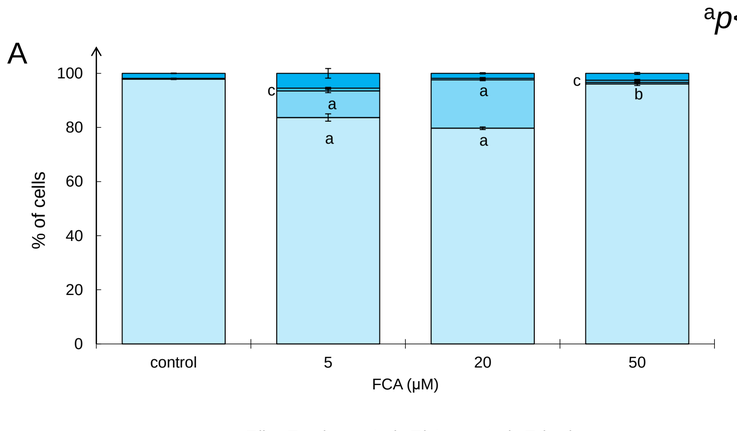


Fig. 4 Flow cytometric analysis of apoptosis/necrosis in HaCaT cells after exposure to 5 – 50 μM ferrocene-containing curcumin analogue (FCA) for 48 h by Muse Cell Analyzer (Merck Millipore). (A) Percentage of live, early and late apoptotic, and dead cells in population (mean ± SEM) after treatment with FCA. Statistically significant difference (Student's *t*-test) *vs.* control: $^a p<0.001$, $^b p<0.025$, $^c p<0.05$. (a) Representative dot-plot profiles of untreated (control) and treated (5 and 50 μM FCA) HaCaT cells.

CONCLUSION

- Strong growth inhibition in HaCaT cells after 48 h of CRC exposure was confirmed with MTT method with significant effects in concentrations ≥ 2.5 μM. Keratinocyte's survival at doses ≥ 20 μM was less than 15 %.
- Opposite to CRC-treated cells, cell culture treated with FCA showed an apparently higher viability and proliferation rate.
- Cytofluorimetric analysis confirmed notably elevated apoptotic cell fraction (late apoptotic/dead cells – 73.88 %) after treatment with 50 μM CRC, while FCA did not affect cell viability (level of apoptotic and necrotic cell death was low – 5.19 % at 100 μM FCA; data not shown).
- ROS induced by *tert*-butyl hydroperoxide were effectively suppressed by both, CRC and FCA. Our findings suggest that FCA has an important role in protection of skin cells against ROS-related pathological processes and associative cell death induction.

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