Undecanoic acid toxicity toward *Trichophyton rubrum* relies on an oxidative stress status

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INTRODUCTION

The fungitoxicity of fatty acids to dermatophytes has long been known. The presence of these compounds in skin and sweat is associated to protection against superficial infections. In this respect, the antidermatophytic activity of undecanoic acid (UDA), a medium-chain saturated fatty acid, is highlighted in the series of C_{7:0}-C_{18:0} compounds, and it has been used to treat cutaneous mycoses. On the other hand, its mode of action is not entirely understood and seems to be related to non-specific interactions with fungal cell proteins and enzymes as well as to fatty acid metabolism. A high throughput assay was previously performed by our research group to unveil key physiological aspects in *T. rubrum* response to UDA exposure (Mendes et al, 2018). Among the main pathways involved in UDA detoxification, the response to oxidative stress drew our attention. For this reason, this work aimed to assess the effect of UDA on *T. rubrum* cellular antioxidant system.

METHODS

A serial drop dilution assay was carried out to analyze the effect of UDA, menadione, and the association of UDA with menadione on *T. rubrum* mycelial growth. Different concentrations of a conidia suspension were inoculated on 24 well plates (in a range of 10⁶ to 10² cell/mL), followed by incubation for 96h at 28°C.

The gene expression analysis of two encoding enzymes, Glutathione transferase, and Cytochrome c peroxidase, involved in oxidative stress response were evaluated using *rpb2* as the normalizer, and 2^{-ΔΔCt} method. The relative expression was assessed using *T. rubrum* grown on Sabouraud (without drugs) as the reference, and the test conditions were menadione (5μM, 10μM, 20μM) or UDA (17.5 μg/mL) for 3 and 12h of exposure.

REFERENCES

Mendes, N.S., Bitencourt, T.A., Sanches, P.R., Silva-Rocha, R., Martinez-Rossi, N.M., and Rossi, A. (2018). Transcriptome-wide survey of gene expression changes and alternative splicing in *Trichophyton rubrum* in response to undecanoic acid. *Scientific Reports* 8.

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RESULTS

The sensitive phenotype assay showed a marked growth inhibition by UDA in concentration assayed (12.5 μg/mL), and no growth inhibition in the lower concentration tested (3.125μg/mL), whereas menadione showed a pronounced colony growth reduction in the highest concentrations assayed (40μM and 20μM), followed by a slight reduction in the lower tested concentrations of 10 μM, and 5μM. In both cases, the growth inhibition was in a dose-dependent manner. The association of menadione (5μM) with UDA (3.125μg/mL) showed a possible synergism effect, with a reduction in colony growth similar to menadione concentration of 20μM. (Fig. 1)

Fig.1 Effect of the oxidizing agent menadione and the fatty acid UDA on *T. rubrum* growth. Plates were inoculated, from left to right in each panel, with 10⁶, 10⁵, 10⁴, 10³, and 10² cells/mL of conidial suspension.

Besides, the gene modulation of the oxidizing enzymes showed their up-regulation after 3h of UDA exposure, as well as for menadione exposure. (Fig. 2)

Fig. 2 qRT-PCR for *T. rubrum* genes encoding the oxidizing enzymes Glutathione transferase, and Cytochrome c peroxidase. Significantly different values are indicated by asterisks (P<0.05)

CONCLUSION

These results may be related to the first line defence employed by *T. rubrum* to overcome the oxidative stress status, and also hinted the enhance UDA-based antifungal therapy by oxidant agent menadione. Therefore, these data strengthen the cellular antioxidant system as a possible target of UDA.