Short research article

Cryptococcus neoformans – New science for discovering melanin modifiers

Abstract

Aim- The present study was taken up to establish the effect of niacinamide on phenoloxidase lead melanogenesis and to prove the reliability of *C. neoformans* based screening methodology.

Methods

The organism was grown in the Minimal media in presence and absence of L- DOPA and Niacinamide and checked for its pigment producing ability at different time intervals.

Results- Niacinamide did not affect the pigmentation in *Cryptococcus neoformans* in the absence or presence of L-Dopa.

Conclusion - *Cryptococcus neoformans* as a biological tool for studying the mechanism of action of various melanin promoters/ inhibitors. The present study highlights the importance and usefulness of Cryptococcus neoformans based screening invention as it is cost effective rapid and 'living cell model'.

Keywords

Vitamin B3, Tyrosinase, Hyperpigmentation, L-DOPA

Introduction

Niacinamide, is otherwise called as Vitamin B3 or Nicotinamide or 3-pyridinecarboxamid. This is a biologically effective form of niacin that is found in root vegetables of many plants and also in certain yeast fungi. Niacinamide functions as a precursor for the co-factors such as Nicotinamide adenine dinucleotide (NAD) and Nicotinamide adenine dinucleotide phosphate (NADP). Along with their reduced forms NADH and NADPH, and that would act as antioxidant [1].

Niacinamide has several medicinal applications for skin care including anti-inflammation, prevention of photo-immunosuppression and increased intercellular lipid synthesis. Topical Niacinamide is known to offer anti-aging benefits to the skin, improved barrier function and significant improvement in the appearance of photoaged facial skin such as texture, hyperpigmentation, redness, fine lines and wrinkles. [2, 3, 4 &5]

Additionally, Niacinamide is believed to influence the cutaneous pigmentation by down-regulating the transfer of melanosomes from melanocytes to keratinocytes. Studies were done by Hakozaki *et al.* suggest that Niacinamide has no effect on tyrosinase activity, melanin synthesis or melanocyte number in a monolayer culture system. The authors also found that Niacinamide had down-regulated the number of melanosomes transferred from melanocytes to keratinocytes from 35 to 68% in a co-culture model system. The actual process by which Niacinamide down-regulates melanosome transfer yet to be established [6, 7, and 8].

Cryptococcus neoformans (*C. neoformans*) is yeast like fungus belongs to the class basidimycota and is known to produce melanin like pigment. The pigment production is associated with virulence and drug resistance [9,10]. Cryptococcal disease typically manifests when latent infection is reactivated after a person becomes immunosuppressed (e.g., receives long-term steroids or immunosuppressive medications for an organ transplant or has advanced HIV infection) [11]. The mechanism of melanogenesis in *C. neoformans* is through an enzyme analogue of tyrosinase- Phenoloxidase. It is well known that Niacinamide doesn't affect tyrosinase or melanin synthesis, however, would abrogate melanin transfer to keratinocytes.

We have already established the usefulness of *C. neoformans* in rapid screening of actives that may have the pigment modifying the property. However, the absolute reliability of the *C. neoformans* based screening approach requires testing with a known tyrosinase non-inhibitors. The present study was taken up to establish the effect of niacinamide on phenoloxidase lead melanogenesis and to prove the reliability of *C. neoformans* based screening methodology. Findings are presented in the paper.

Materials and methods

C. neoformans culture was obtained from Y.R. Gaitonde Centre for AIDS Research and Education (YRG CARE), Chennai. *C. neoformans* was grown in a defined minimal media (15 mM glucose, 10 mM $MgSO_4$, 29.4 mM KH_2PO_4, and 13 mM glycine, 3 mM thiamine,

with and without 1.0 mM L-dopa. The organism was grown in the above media was incubated for 14 days at room temperature. The intensity of the pigment produced was observed at different time intervals.

Evaluation of Niacinamide in the melanisation of *C. neoformans*

To the above-defined media containing L-dopa, 1% Niacinamide was incorporated. The chemical formula of Niacinamide was given in Fig. 1. Media without L-dopa was used as negative control. All the media plates in triplicate were inoculated with *C. neoformans* and were incubated for 14 days at room temperature. The intensity of pigment produced by the organism in media plate containing L- DOPA and Niacinamide was observed and the similarity in the observation was compared with control plate which was devoid of L-dopa.

Fig-1. Chemical structure of Niacinamide

Result

C. neoformans required 14 days to produce melanoid pigmentaion. The *C. neoformans* grown in media containing L- Dopa (10mM) on day 2, mild pigmentation was observed and which further deepened from day 4 to day 14. Table- 1

When *C. neoformans* was grown in media containing Niacinamide and L- DOPA, the intensity and extent of pigmentation was similar to that in L- DOPA alone treated media. Niacinamide did not seem to either positively or negatively influence the pigment formation in *C. neoformans* where phenoloxidase is involved in melanoid pigmentogenesis. Table- 1

Experiments	**Presence of pigment vs days**			
	2	4	7	14
C.neoformans	-	-	-	+++

C.neoformans+ Dopa	+	++	+++	+++
C.neoformans+ Niacinamide	-	-	-	+++
C.neoformans+ Niacinamide+ Dopa	+	++	+++	+++
Dopa alone	-	-	-	-

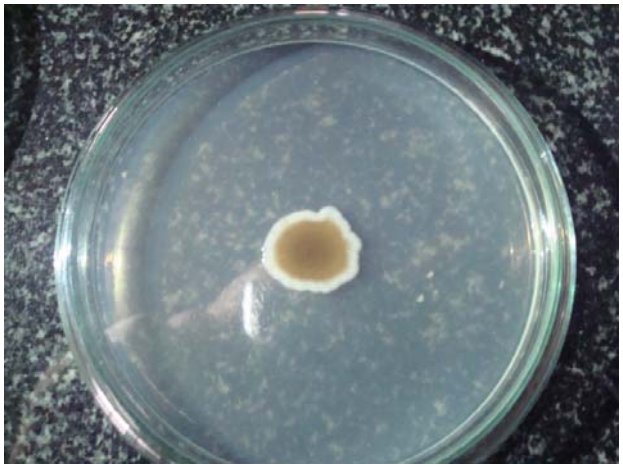
- = No black pigmentation

\+ = Mild pigmentation

++ = Moderate pigmentation

+++ = Deep pigmenttaion

Fig -2. 4 day old C.neoformans (Control) **Fig- 2 4 day old *C. neoformans* treated with Niacinamide**



Discussion

The present study has undoubtedly established the usefulness of *Cryptococcus neoformans* as a biological tool for studying the mechanism of action of various melanin promoters/

inhibitors. Further, the above tool also has established the mechanism of action of Niacinamide.

Addition of Niacinamide did not alter the pigment-producing ability of *C. neoformans* when DOPA was supplemented in the media which suggests Niacinamide does not inhibit the enzymatic pathway in melanogenesis.

It's already established that Niacinamide does not affect the process of melanogenesis through tyrosinase enzyme pathway. *Cryptococcus neoformans* produce melanin through an alternate mechanism by using tyrosinase analogue-phenoloxidase. However, the effect of Niacinamide on phenoloxidase is not clearly known. The present study has also revealed that Niacinamide does not affect phenol oxidase lead melanogenesis like that of tyrosinase linked melanogenesis. This proves that *C. neoformans* are quite a reliable tool for screening ingredients that may have melanin promotion/inhibition property. Tyrosinase based assays, as well as the cell culture-based assays, are followed for the above purpose. However, the *in vitro* studies may provide only indicative results whereas *C. neoformans* model is a perfect living cell biological model and can predict the results more accurately than the *in vitro* studies.

In the present, we have used two known positive indicators to predict the usefulness of *C. neoformans* based screening method. The first indicator is Niacinamide which does not affect the tyrosinase activity. The second indicator being *Cryptococcus neoformans* which produce melanoid pigmentation in selective media supplemented with L- DOPA. However, the pigmentation in *C. neoformans* is due to phenol oxidase enzyme which is an analogue of tyrosinase enzyme seen largely among vertebrates.

It is already known that Niacinamide does not affect the enzymatic pathway in melanogenesis, however, block the melanin transfer from melanocytes to keratinocytes. Since the Niacinamide has not affected the melanoid pigmentation in *C. neoformans* which proves phenoloxidase based screening shall go in concordance with the findings obtained through tyrosinase assay. This validates the scientific credence and sanctity of *C. neoformans* based screening method for melanin promotors/inhibitors. This method is reliable, rapid, cost-effective as well as 'living cell model' than *in vitro* cell culture based assay.

Conclusion

Cryptococcus neoformans as a biological tool for studying the mechanism of action of various melanin promoters/ inhibitors. The present study highlights the importance and usefulness of Cryptococcus neoformans based screening invention as it is cost effective rapid and 'living cell model'.

References

1. Jody P. Ebanks, R. Randall Wickett, and Raymond E. Boissy. Mechanisms Regulating Skin Pigmentation: The Rise and Fall of Complexion Coloration. Int J Mol Sci. 2009 Sep; 10(9): 4066–4087.
2. Bissett D, Miyamoto K, Sun P, Li J, Berge C. Topical niacinamide reduces yellowing, wrinkling, red blotchiness, and hyperpigmented spots in aging facial skin. Int. J. Cosmet. Sci. 2004;26:231–238.
3. Bissett D, Oblong J, Berge C. Niacinamide: A B vitamin that improves aging facial skin appearance. Dermatol. Surg. 2005;31:860–865.
4. Bissett D. Topical niacinamide and barrier enhancement. Cutis. 2002;70S:8–12. [PubMed]
5. Bissett D, Oblong J, Saud A, Berge C, Trejo A, Biedermann K. Topical niacinamide provides skin aging appearance benefits while enhancing barrier function. J. Clin. Dermatol. 2003;32S:9–18
6. Hakozaki T, Takiwaki H, Miyamoto K, Sato Y, Arase S. Ultrasound enhanced skin-lightening effect of vitamin C and niacinamide. Skin Res. Technol. 2006;12:105–113
7. Greatens A, Hakozaki T, Koshoffer A, Epstein H, Schwemberger S, Babcock G, Bissett D, Takiwaki H, Arase S, Wickett R, Boissy R. Effective inhibition of melanosome transfer to keratinocytes by lectins and niacinamide is reversible. Exp. Dermatol. 2005;14:498–508
8. Boissy R. Melanosome transfer to and translocation in the keratinocyte. Exp. Dermatol. 2003;12:5–12.[PubMed]
9. D. C. McFADDEN & A. CASADEVAL. Capsule and melanin synthesis in *Cryptococcus neoformans*. Medical Mycology 2001, 39, Supplement 1, 19-30

10. Polacheck I. The discovery of melanin production in Crypto- coccus neoformans and its impact on diagnosis and the study of virulence. Zentralbl Bakteriol 1991; 276: 120–123.
11. Saha DC, Goldman DL, Shao X, Casadevall A, Husain S, Limaye AP, et al. Serologic evidence for reactivation of cryptococcosis in solid-organ transplant recipients. Clin Vaccine Immunol. 2007;14:1550–4. 10.1128/CVI.00242-07