Suppression of oxidative-stress induced melanocyte death: Role of poly(ADP-ribose) polymerase in vitiligo pathogenesis

Sir,

Vitiligo is an acquired depigmentation disorder linked with genetic and non-genetic etiologic factors. Oxidative stress has been implicated as the initial triggering factor for melanocyte destruction in vitiligo.[1] The poly adenosine diphosphate-ribose polymerases (PARPs) are a group of nuclear enzymes that repair DNA damage by polyADP-ribosylation using NAD^+ as a substrate and implicated in vital cellular processes. Oxidative stress triggers DNA damage and hyperactivation of PARP1s, leading to cell dysfunction and death.[2] The present study aimed to assess the impact of inhibition of PARPs using 1,5-dihydroxyisoquinoline on hydrogen peroxide ($H_2O_{2)}$ induced oxidative stress in *in vitro* cultured normal human melanocytes.

The study plan was approved by the university's Institutional Ethics Committee for Human Research (FS/IECHR/BC/RB/1). The skin samples were procured by punch biopsy and/or from the left-over skin after surgery to be used for melanocyte culturing. The rescue from oxidative stress in H_2O_2 treated normal human melanocytes was studied by inhibition of PARPs using 1,5-dihydroxyisoquinoline. Cell viability was monitored by trypan blue exclusion assay; cleavage and activity of PARPs were monitored by Western blotting and the transcript levels were estimated by real-time polymerase chain reaction. For *in vitr*o studies, triplicate experiments' data was analysed by Student's *t*-test and one-way analysis of variance (Prism 6, GraphPad Software, Inc; San Diego, CA) as mean±standard error of the mean.

Our study observed a dose-dependent decrease in cell viability on H_2O_2 treatment, that is, 100 μM (P = 0.0248), 250 μM (P = 0.0014) and 500 μM (P < 0.0001) [Figure 1b]. 100 μM H_2O_2 (Lethal concentration 50% dose) was selected for downstream experiments. 1,5-dihydroxyisoquinoline (50 μM, 100 μM and 200 μM) could not significantly affect the morphology and viability of normal human melanocytes [Figure 1c]. Further, we monitored rescue of normal human melanocytes death with and without oxidant treatment by 1,5-dihydroxyisoquinoline. Interestingly, 100 μM 1,5-dihydroxyisoquinoline pre-treated cells showed a significant rescue from the cytotoxic effects of H_2O_2 on cell morphology [Figure 1a] and viability [Figure 1d]. A significant rescue was observed at 250 μM (P = 0.0022) and 500 μM (P = 0.0002) H_2O_2. However, no significant rescue was seen at 100 μM H_2O_2 (P = 0.0471) [Figure 1d].

Earlier reports suggested the activation of caspases 3, 8 and 9 and elevated cleavage of PARPs in the depigmented epidermis compared with the normally pigmented one. Tulic group[3] also observed increased activation of p38 and poly (ADP-ribose) polymerases cleavage in vitiligo patients. We further studied PARylation and PARP-1 activation on 1,5-dihydroxyisoquinoline mediated rescue from H_2O_2 induced apoptosis. 1,5-dihydroxyisoquinoline exhibited a significant restoration of a few normal human melanocytes proteins which were affected by H_2O_2. Hyperactivation of PARP-1 led to PARP-1 cleavage resulting in release of 89 kDa fragment followed by apoptosis. On the contrary, 1,5-dihydroxyisoquinoline suppressed the PARP-1 cleavage and apoptosis [Figure 2b]. Furthermore, normal human melanocytes exhibited a significant restoration in polyADP-ribosylation pattern and PARP-1 activation in 1,5-dihydroxyisoquinoline+H_2O_2 treated group compared to only H_2O_2 treated group [Figures 2a and 2b]. In line with our observations, the previous studies showed 1,5-dihydroxyisoquinoline rescued human cardiac myoblasts and rat cardiomyocytes exposed to H_2O_2. It has also been shown that 1,5-dihydroxyisoquinoline inhibit PARP activity and protect endothelial cells from oxidative stress.[4] The effect of oxidative stress on microphthalmia-associated transcription factor-M (MITF-M), tyrosinase and intercellular adhesion molecule-1 (ICAM-1) expression was also studied in 50 μM and 100 μM of H_2O_2 treated normal human melanocytes. We observed, significantly decreased MITF-M transcript (P = 0.0083 and P = 0.0383) and protein (P = 0.0024 and P < 0.0001) levels at both H_2O_2 doses,

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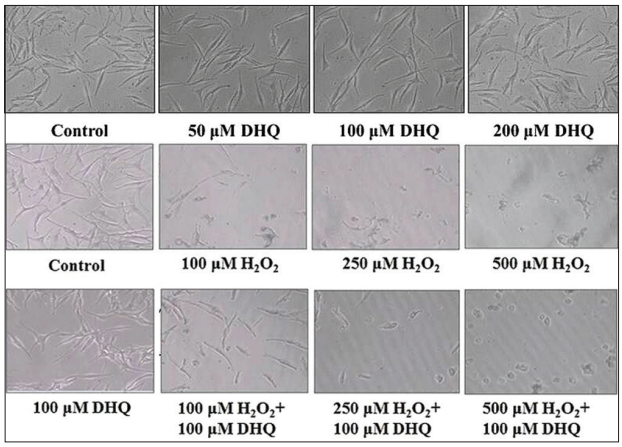

Figure 1a: Morphological effect of 1,5-dihydroxyisoquinoline on normal human melanocytes: No significant morphological effect was observed on 1,5-dihydroxyisoquinoline exposure on normal human melanocytes morphology. A significant rescue was observed from a dose-dependent H_2O_2 mediated cell death on 1,5-dihydroxyisoquinoline pre-treatment. Magnification ×10

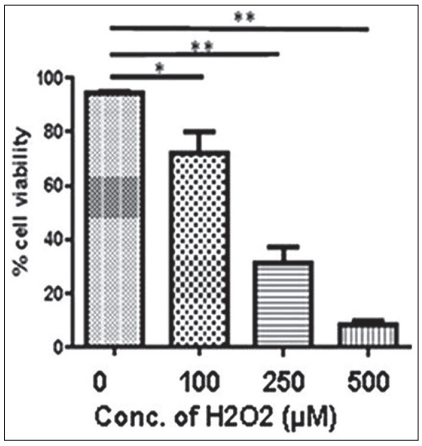

Figure 1b: A significant cell death was seen at 100 µM, 250 µM and 500 µM H_2O_2, respectively, (P = 0.0248, P = 0.0027 and P = 0.0021) compared to control

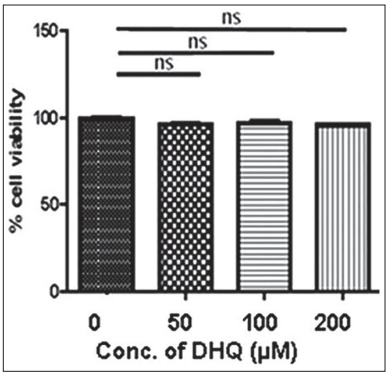

Figure 1c: No significant effect on normal human melanocytes viability was observed on 1,5-dihydroxyisoquinoline exposure

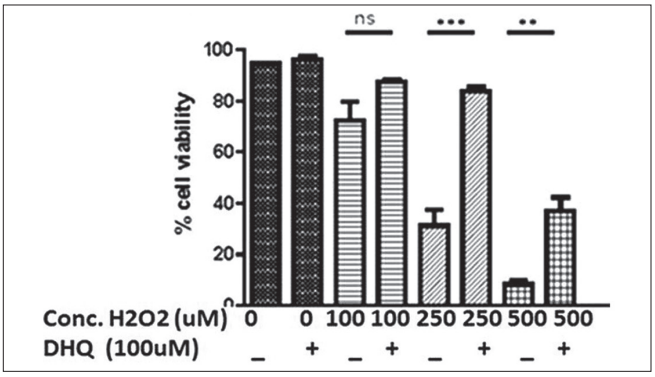

Figure 1d: A dose-dependent effect of H_2O_2 on normal human melanocytes viability with and without pre-treatment (4 hours) of 100 µM 1,5-dihydroxyisoquinoline. Significant rescue in cell death was observed at higher doses of H_2O_2 (P = 0.0471; P = 0.0002 and P = 0.0022); the values represent mean ± standard deviation of three independent experiments

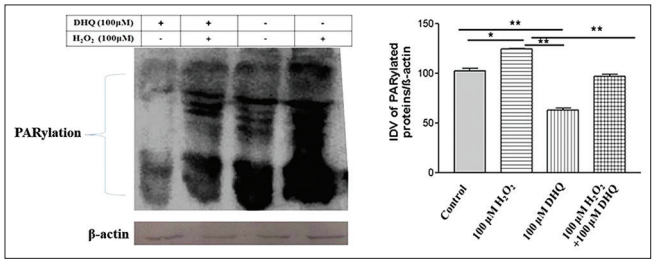

Figure 2a: Analysis of PARylation and poly (ADP-ribose) polymerase 1 activation on 1,5-dihydroxyisoquinoline mediated rescue from H_2O_2 induced cell death: Densitometric analysis for 1,5-dihydroxyisoquinoline and 1,5-dihydroxyisoquinoline + H_2O_2 groups revealed a significant difference (P = 0.0076) in PARylation suppression

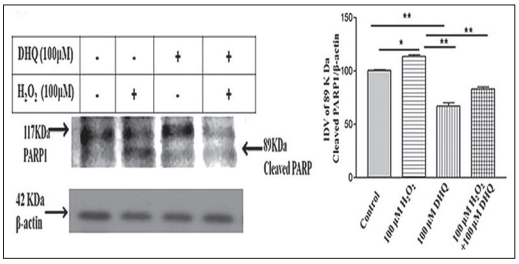

Figure 2b: Analysis of PARylation and PARP-1 activation on 1,5-dihydroxyisoquinoline mediated rescue from H_2O_2 induced cell death: PARP-1 hyperactivation was observed in (H_2O_2) group (89 kDa cleaved fragment). Densitometric analysis for 1,5-dihydroxyisoquinoline alone group and 1,5-dihydroxyisoquinoline + H_2O_2 group also revealed a significant difference (P = 0.0062) in PARP-1 activation. Beta-actin: A protein loading control. Results are mean ± standard deviation of three independent experiments

respectively, [Figures 3a, 3d and 3e]. MITF regulates the expression of melanocyte-specific proteins required for melanin synthesis. Tyrosinase, the target gene for MITF-M was decreased in 50 µM (P = 0.0109) and 100 µM (P = 0.0439) H_2O_2 treated cells [Figure 3b]. However, there was no significant difference found in ICAM-1 transcript post-treatment with 50 µM (P = 0.0772) and 100 µM H_2O_2 (P = 0.1325) [Figure 3c]. In a recent review,[5] parthanatos, which is PARP-1 dependent cell death, has been suggested as instrumental in oxidative stress-related diseases such as vitiligo and hence, inhibiting parthanatos to make melanocyte step back from the brink of parthanatotic cell death might

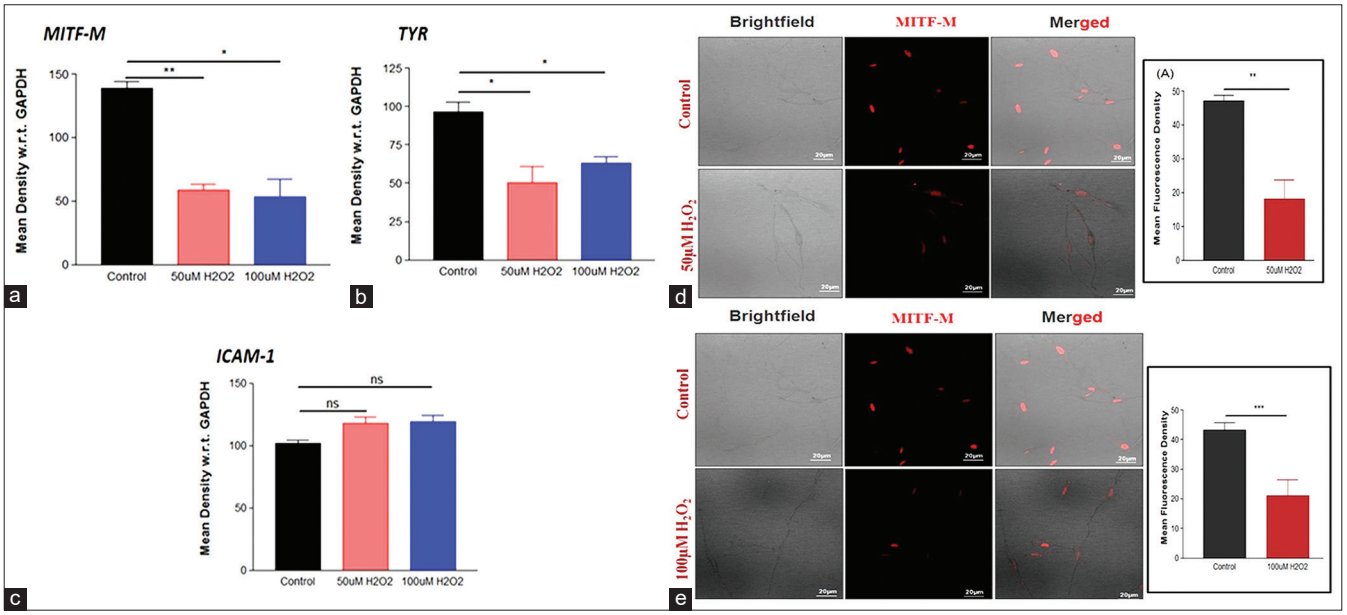

Figure 3: Effect of oxidative stress on microphthalmia-associated transcription factor-M, Tyrosinase and Intercellular Adhesion Molecule 1 and A dose-dependent effect of H_2O_2 on microphthalmia-associated transcription factor-M: (a) Microphthalmia-associated transcription factor-M transcript was significantly reduced in H_2O_2 treated cells compared to control ($P = 0.0083$ and $P = 0.0383$). (b) Tyrosinase transcript was also significantly reduced in H_2O_2 treated cells as compared to control ($P = 0.0439$ and $P = 0.0109$). (c) There was no significant difference in Intercellular Adhesion Molecule 1 transcript on H_2O_2 treatment compared to control ($P = 0.0772$ and $P = 0.1325$). Immunofluorescence analysis revealed a significant decrease in microphthalmia-associated transcription factor-M in normal human melanocytes treated with (d) 50 μM and (e) 100 μM of H_2O_2 for 24 h ($P = 0.0024$ and $P = 0.0001$). Results are mean ± standard deviation of three independent experiments (magnification ×63)

be well pursuing. However, the exact role of parthanatos in vitiligo pathogenesis through PARP-1 activation need to be be explored *in vivo* and *ex vivo* using animal and human skin models.

Collectively, this novel preliminary study supports that inhibition of poly (ADP-ribose) polymerases 1 by 1,5-dihydroxyisoquinoline attenuates H_2O_2 induced melanocyte death, signifying the role of PARP-1s in oxidative-stress mediated melanocyte death and in developing a potential therapeutic target for vitiligo.

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Declaration of patient consent

Institutional Review Board (IRB) permission obtained for the study.

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Conflicts of interest

There are no conflicts of interest.

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