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Research Article

Differential Effects of Methylisothiazolinone (MIT) on Dental pulp stem cells (DPSC)

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Abstract

Background: Methylisothiazolinone (MIT) is a strong preservative commonly found in household products such as cosmetics, soaps and baby wipes and may be linked with acute contact dermatitis (ACD). Evidence has now emerged that demonstrates the effects of MIT may not be limited to ACD and other skin allergies, but may also affect other tissue types, including stem cells. Relatively few studies have evaluated the effects of this compound on oral cells and tissues, therefore the primary objective of this study was to evaluate the effects of MIT on dental pulp stem cells (DPSC), which may be acutely sensitive to differentiation stimuli and environmental exposures.

Methods: Two dental pulp stem cell (DPSC) isolates (dpsc-11750, dpsc-11836) from an existing repository were selected for this study to test the effects of MIT. Cell viability was measured using the Trypan Blue exclusion assay. Cellular proliferation was measured using 96-well growth assays using different concentrations of MIT ranging between 25–75 uM.

Results: Differential effects of MIT were observed with DPSC viability. Comparison of baseline (control: 0 uM) viability of DPSC isolates revealed significantly increased viability among dpsc-11836 cells at all concentrations (25 uM, 50 uM, 75 uM, P=0.004) with significantly reduced viability among dpsc-11750 cells at all concentrations (P=0.03). In addition, differential effects of MIT were also observed with DPSC proliferation. Comparison of baseline (control: 0 uM) growth of DPSC isolates revealed significantly increased proliferation among dpsc-11836 cells at all concentrations (25 uM, 50 uM, 75 uM, P=0.05) with significantly reduced growth among dpsc-11750 cells at all concentrations (P=0.04).

Conclusions: Because MIT and other biocides are used in a wide variety of consumer products and industrial processes, a greater understanding of the potential effects of these compounds on specific cells and tissue types is warranted. As differential responses to MIT were observed among the DPSC isolates evaluated, a more research will be needed to elucidate the factors that regulate and mediate the cellular responses to MIT exposure.

Keywords: Methylisothiazolinone (MIT), dental pulp stem cells (DPSC).

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Introduction

Methylisothiazolinone (MIT) is a preservative commonly found in household products such as cosmetics, soaps and baby wipes[1,2]. Recent evidence has demonstrated that MIT and isothiazolinone (IT) are responsible for many

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cases of allergic contact dermatitis (ACD)[3,4]. With increased evidence of contact allergies caused by MIT and IT in non-cosmetic and other unusual sources, more research has recently focused on the adverse effects of these agents on cells and tissues other than the epidermis [5-7].

Evidence has now emerged that demonstrates ecotoxicological effects on multiple ecosystems, animals and even organ systems [8]. For example, genotoxic and histopathologic effects of MIT have been observed with low-level concentration exposure in rainbow trout [9]. In addition, MIT has been shown to exhibit cellular toxicity and inhibition of wound healing and regeneration in planaria [10]. Finally, wound healing and tail regeneration have also been linked with MIT exposure in Xenopus laevis [11].

This evidence may suggest that the effects of MIT are not limited to ACD and other skin allergies but may have effects in cells and tissue types, including dendritic cells and stem cells [12,13].In fact, neurological, neurodegenerative, and neurotoxic effects of MIT have been documented from developmental exposures, which may suggest long-term effects on stem cells or other tissue-specific progenitors[14]. Furthermore, MIT exposure has been demonstrated to harm promyeloblast cells through activation of apoptosis- and necrosis-associated pathways [15].

Despite the use of MIT in dental and orofacial products, relatively few studies have evaluated the effects of this compound on oral cells and tissues [5,16]. Based upon the paucity of available information regarding these effects, the overall objective of this study was to evaluate any effects of MIT on dental pulp stem cells (DPSC), which may be acutely sensitive to differentiation stimuli and environmental exposures [17]. The specific goals of this study were to determine any changes in DPSC-specific cellular growth or viability in response to MIT exposure.

Methods

Human subjects' approval

The original study involving the collection and long-term storage of DPSC was reviewed and subsequently approved by the University of Nevada, Las Vegas (UNLV) Office for the Protection of Research Subjects (OPRS) and Institutional Review Board (IRB) as OPRS#0907-3148 titled "Isolation of Non-Embryonic Stem Cells from Dental Pulp" on February 5, 2010 [18]. This study used the existing DPSC repository, which involved previously collected, non-identifiable samples and was therefore reviewed and approved as Exempt research by OPRS and IRB under protocol #763012 titled "Retrospective analysis of dental pulp stem cells (DPSC) from the UNLV School of Dental Medicine pediatric and clinical population" on August 3, 2015.

DPSC cell culture

DPSC isolates were initially cultured for a minimum of ten passages and subsequently cryopreserved in 10% dimethyl sulfoxide (DMSO)-containing media at -80oC. Two DPSC isolates with the highest viability (dpsc-11750, dpsc-11836) were selected and thawed for culture, as previously described [19]. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) High Glucose with the addition of Fetal Calf Serum (FCS) 10% and Penicillin-Streptomycin antibiotic 1% in a Biosafety Level (BSL)-2 humidified tissue culture chamber at 37oC with 5% CO2 [20,21].

Methylisothiazolinone (MIT)

Methylisothiazolinone or 2-Methyl-4-isothiazolin-3-one hydrochloride was obtained from Sigma-Aldrich (CAS #26172-54-3) with a molecular weight of 151.61 and empirical formula of C4H5NOS – HCl. MIT was resuspended in DMSO at 1 mg/mL for subsequent experimental use at the bioequivalent range of concentrations where biological effects were previously observed (25 – 75 uM) [13,15,17].

Viability assays

Cellular viability was measured using the Trypan Blue exclusion assay and a Bio-Rad TC20 automated cell counter. Trypan blue is a vital stain to selectively identify dead or dying cells, as live or vital cells selectively exclude compounds such as Trypan Blue from crossing the plasma membrane – which can also be described as a dye exclusion assay method. In brief, 100 uL of cells were mixed with 100 uL of Trypan Blue and loaded into the TC20 automated cell counter. This analysis provides information regarding the absolute (total) cell number and percentage of viable (live) cells during an experimental protocol [18,19].

Proliferation assays

Experimental growth assays were performed in 96-well tissue-culture treated, flat bottom Costar plates. In brief, cells were initially plated at a standardized concentration of 1.2 x 105 cells/mL for three days. Three replicates of each experiment were performed, which included three rows of n=8 wells of each DPSC isolate and each concentration (0 uM, 25 uM, 50 uM, 75 uM) for a total n=24. At the end of each time point (24 hours, 48 hours, 72 hours) plates were fixed with 10% buffered formalin and subsequently stained with Gentian Violet using a 1% aqueous solution. Absorbance was acquired using an Elx808 BioTek microplate reader at 595 nm to calculate cell number (and therefore cell proliferation) for comparison with controls, as previously described [20,21].

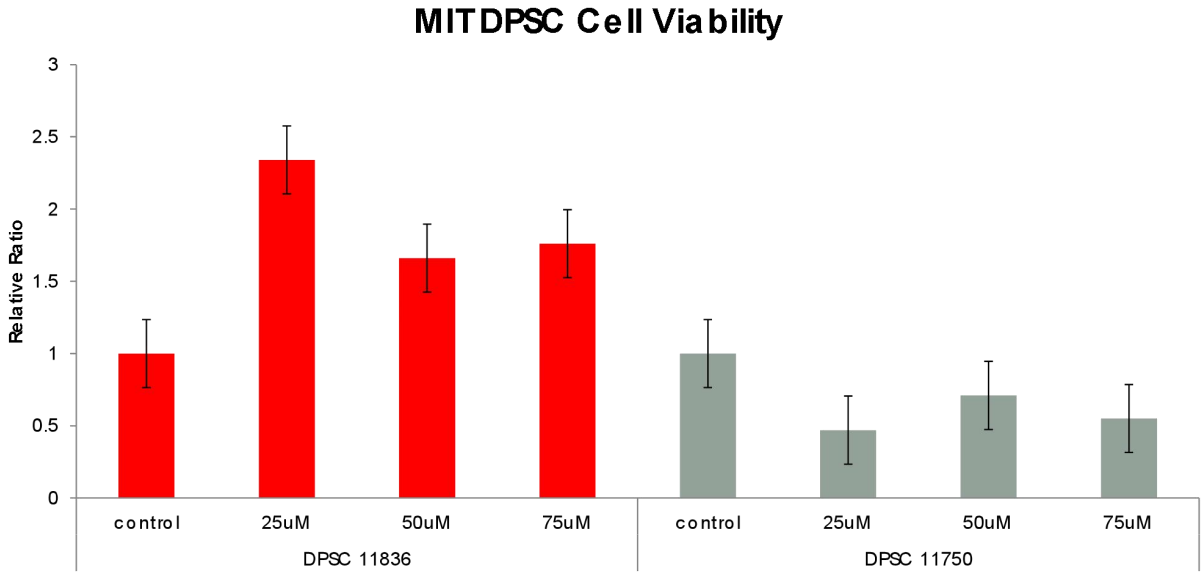
Statistical analysis

Measurements of cell viability and cellular proliferation are continuous data, which are most appropriately analyzed using parametric statistics. Data were exported to Microsoft Excel and analyzed using two-tailed Student's t-tests. Statistical significance was set using an alpha level = 0.05.

Results

Cellular viability assays were performed to evaluate any potential effects of MIT on DPSC (Figure 1). These data demonstrated that baseline viability for the DPSC isolates (dpsc-11836 28.3%, dpsc-11750 31.2%) was significantly but differentially altered by the administration of MIT. More specifically, the exposure of dpsc-11836 to MIT at all concentrations (25 uM, 50 uM, 75 uM) significantly increased viability over three days. Comparing the viability of the negative control (0 uM MIT) with each experimental condition revealed the greatest increase in viability at the lowest concentration of 25 uM (2.34-fold compared with control), P=0.002. In addition, the higher concentrations of 50 uM and 75 uM also significantly increased viability (1.66-fold and 1.76-fold) compared with baseline, P=0.004. However, the effects of MIT were not similar among both DPSC isolates. The exposure of dpsc-11750 to MIT also induced significant changes to viability over three days by reducing viability at all experimental concentrations

evaluated. More specifically, the comparison of viability with the negative control (0 uM MIT) at each experimental condition revealed the greatest reduction in viability at the lowest concentration of 25 uM (0.47-fold compared with control), P=0.02. In addition, the higher concentrations of 50 uM and 75 uM also significantly inhibited viability (0.71-fold, 0.55-fold, respectively) compared with baseline, P=0.03.

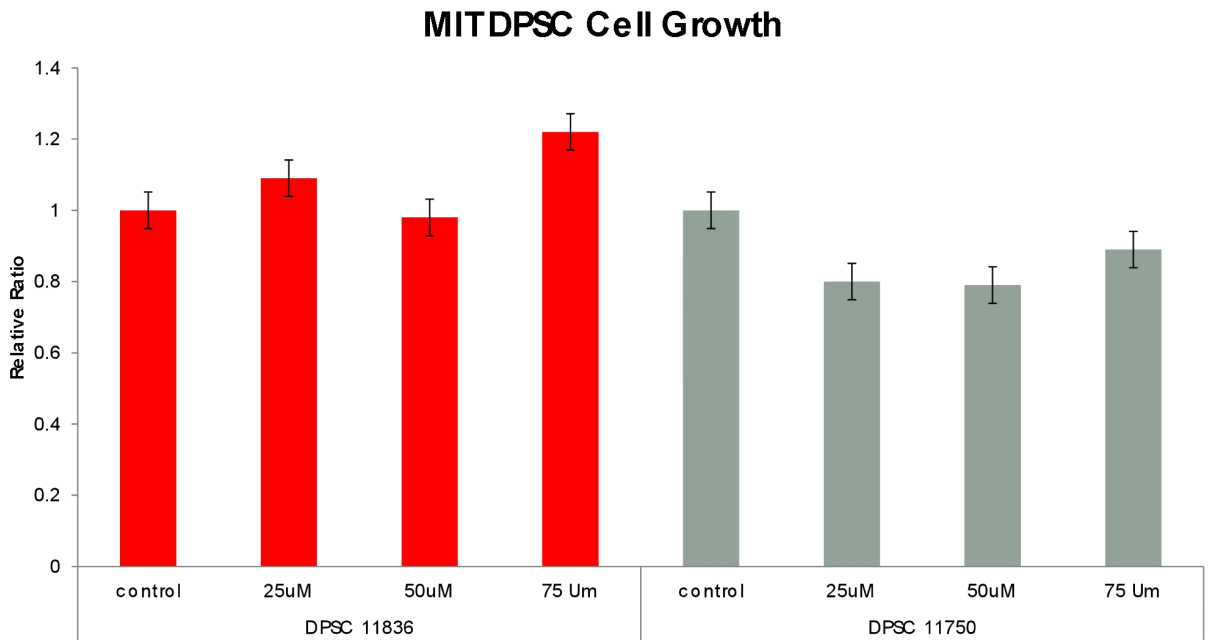


DPSC 11836				DPSC 11750			
Control	25 uM	50 uM	75 uM	Control	25 uM	50 uM	75 uM
1	2.34	1.66	1.76	1	0.47	0.71	0.55

Figure 1. Differential effects of MIT on DPSC viability. Comparison of baseline (control: 0 uM) viability of DPSC isolates revealed significantly increased viability among dpsc-11836 cells at all concentrations (25 uM, 50 uM, 75 uM, P=0.004) with significantly reduced viability among dpsc-11750 cells at all concentrations (P=0.03).

To evaluate whether the effects of MIT on cellular viability were associated with other changes to cellular phenotype, proliferation assays were also performed (Figure 2). These data also revealed differential effects of MIT on the DPSC isolates evaluated. More specifically, the growth of dpsc-11836 was increased at the lowest (25 uM, 1.09-fold) and highest concentrations (75 uM, 1.22-fold) over three days compared with baseline (0 uM), although the growth at the highest concentration was the only statistically significant change observed, P=0.05.

However, the evaluation of dpsc-11750 revealed significant inhibitions of proliferation over three days at all concentrations evaluated. For example, the reduction in growth at the lowest concentration (25 uM, 0.8-fold) was similar to the inhibition observed at 50 uM (0.79-fold), with slightly less robust effects observed at the highest concentration (75 uM, 0.89-fold), P=0.04.



DPSC 11836				DPSC 11750			
Control	25 uM	50 uM	75 uM	Control	25 uM	50 uM	75 uM
1	1.09	0.98	1.22	1	0.8	0.79	0.89

Figure 2. Differential effects of MIT on DPSC proliferation. Comparison of baseline (control: 0 uM) growth of DPSC isolates revealed significantly increased proliferation among dpsc-11836 cells at all concentrations (25 uM, 50 uM, 75 uM, P=0.05) with significantly reduced growth among dpsc-11750 cells at all concentrations (P=0.04).

Discussion

The primary objective of this study was to evaluate any effects of MIT on dental pulp stem cells (DPSC), which may be acutely sensitive to direct or indirect exposure to environmental biocides [12,13]. More specifically, changes in DPSC-specific cellular growth or viability in response to MIT exposure were evaluated, which demonstrated differential responses among the two DPSC isolates evaluated. These observations may support previous research that indicated MIT may induce different effects on different cell types, which may be related to their differentiation status and tissue-specific gene expression [22,23].

Although much remains to be discovered regarding the potential mechanisms through which MIT may induce these phenotypic changes, some studies have begun to evaluate the potential pathways associated with MIT-induced cytotoxicity. For example, some studies have demonstrated that focal adhesion kinase (FAK) tyrosine phosphorylation may be indirectly down-regulated in part by direct inhibition of SRC tyrosine kinases by MIT [24,25]. Other studies have proposed mitochondrial transmembrane hyperpolarization as one mechanism of cell death induced by MIT exposure in HL60 cells [15]. However, there is no consensus regarding the common responses and pathways directly or indirectly affected by MIT exposure.

The limited nature of this study did not allow for characterization of the mechanisms that might be responsible for the differential responses observed among

the DPSC isolates evaluated in this study. However, future studies might include an evaluation of the growth characteristics and differentiation potential of these DPSC isolates, which may help explain the potential factors responsible for differential responsiveness to MIT administration [26,27].

Conclusions

Because MIT and other biocides are used in a wide variety of consumer products and industrial processes, a greater understanding of the potential effects of these compounds on specific cells and tissue types is warranted. As differential responses to MIT were observed among the DPSC isolates evaluated, a more research will be needed to elucidate the factors that regulate and mediate the cellular responses to MIT exposure.

Conflicts of Interest

None

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