INTRODUCTION

Mesenchymal stem cells (MSCs) play a key role in the regulation of tissue maintenance and repair. These cells have multipotential potential in vivo, which can be maintained when they are cultured in vitro. MSCs can be derived from various tissues including bone marrow, umbilical cord, Wharton’s jelly of the umbilical cord, adipose tissue, dental pulp, as well as from the lungs of lung transplant patients. In vitro, these cells are adherent to tissue culture flasks, display a fibroblast-like morphology, and maintain the ability to differentiate into osteoblasts, adipocytes, and chondroblasts. It has been demonstrated that MSCs are also capable of differentiating into cell types of different germ layers including neurons, skeletal epithelia, hepatocytes, and myocytes. Currently, only a few signaling pathways involved in the regulation of MSC proliferation and differentiation have been identified (e.g. Wnt signaling pathway). Disruption of these pathways drastically alters the ability of MSCs to proliferate and differentiate, therefore, altering the ability of MSCs to participate in tissue maintenance and repair. Service members have the potential to be exposed to many toxic chemicals. These chemicals could disrupt the normal functions of MSCs and interrupt the normal healing processes of a wound for injured service members preventing return to duty. Recently, it has been reported that bone marrow-derived MSCs express active acetylcholinesterase (AChE). The presence of AChE in these cells suggests that chemicals effects the ability of MSCs to differentiate into osteoblasts. The role AChE plays in MSC proliferation and differentiation is currently unknown. In this study, we examined the effects of organophosphates on AChE activity, MSC proliferation, as well as MSC differentiation.

MATERIALS AND METHODS

Experimental Chemicals: All chemicals of the organophosphate pesticides (OP) parathion and paraoxon were purchased from Sigma-Aldrich (St. Louis, MO). The pesticides were prepared in double distilled water (pH 7.4) at 1000 µM.

METHODS

Human MSCs: Primary human bone marrow-derived MSCs were obtained from cords (Walkersville, MD) and cultured as previously described [1].

Confirmation of MSC Identity: We isolated MSCs in the wells of 96-well tissue culture plates and allowed to attach for 1 h. MSCs were then exposed to increasing concentrations of parathion, paraoxon, or vehicle (EtOH) for 24 h. Then, MSCs were fixed and stained for Focal adhesion kinase (FAK) and cell viability (Diock, black). The cells were then incubated with anti-FAK antibodies, and incubated with anti-rabbit IgG antibodies conjugated to alexafluorophore. The Alexafluorophore-labeled antibodies were analyzed using the SpectraMax® 190 spectrophotometer to confirm the adherence of MSCs to the plate. These results confirmed the adherence of MSCs to the plate.

Differentiation of MSCs: MSCs were plated into 96-well tissue culture plates at a density of 5000 cells/well and allowed to grow to confluence. The media was replaced with Human MSC Adipogenic Differentiation Medium (ThermoScientific) or Human MSC Osteogenic Differentiation Medium (Pierce) and maintained in culture under standard conditions outlined above. The cells were then washed, fixed, and stained for FAFK and cell viability (Diock, black), and the resultant images were analyzed using the SpectraMax® 190 spectrophotometer to confirm the adherence of MSCs to the plate. These results confirmed the adherence of MSCs to the plate.

Differentiation of MSCs: MSCs were plated into 96-well tissue culture plates at a density of 4-6,000 cells/well. After attachment, but prior to confluence (10-14 days), the media was replaced with Ethylene-Glycol-based Differentiation Medium (b1; paraoxon, paraoxon, or vehicle) (100 µM). The media was replaced every 24 hours, and the cells were stained for FAFK and cell viability (Diock, black). The resultant images were analyzed using the SpectraMax® 190 spectrophotometer to confirm the adherence of MSCs to the plate. These results confirmed the adherence of MSCs to the plate.

Differentiation of MSCs: MSCs were plated into 96-well tissue culture plates at a density of 6-8,000 cells/well. After attachment, but prior to confluence (10-14 days), the media was replaced with Ethylene-Glycol-based Differentiation Medium (b1; paraoxon, paraoxon, or vehicle) (100 µM). The media was replaced every 24 hours, and the cells were stained for FAFK and cell viability (Diock, black). The resultant images were analyzed using the SpectraMax® 190 spectrophotometer to confirm the adherence of MSCs to the plate. These results confirmed the adherence of MSCs to the plate.

RESULTS

Figure 1: Effects of parathion and paraoxon on the viability of cultured human MSCs. MSCs were exposed to increasing concentrations of parathion (red), paraoxon (blue), or equivalent amounts of vehicle control (Diock, black) for 24 h at which they were assayed for viability. The results are reported as mean ± SEM of 3 conditions. *p < 0.05 versus vehicle control.

Figure 2: Effects of parathion and paraoxon on the proliferation of cultured human MSCs. MSCs were exposed to increasing concentrations of parathion (red), paraoxon (blue), or equivalent amounts of vehicle control (Diock, black) for 24 h at which they were assayed for proliferation. The results are reported as mean ± SEM of 3 conditions. *p < 0.05 versus vehicle control.

Figure 3: Effects of parathion and paraoxon on the activity and expression of AChE in human MSCs. A: MSCs were exposed to increasing concentrations of parathion (filled, parathion; cross-hatched, media alone (open) for 24 h. The cells were then assayed for AChE activity (vertical bars). B: Representative Immunoblot of AChE expression in MSCs (Diock, black) control conditions. n = 7 for each condition tested. *p < 0.05 versus media control. B: Human MSCs were exposed to parathion, paraoxon, or vehicle control (Diock, black) for 14 days and stained for AChE (magenta). C: Immunoblot of AChE expression in MSCs (Diock, black) control conditions (n = 7 for each condition tested. *p < 0.05 versus vehicle control).}

CONCLUSIONS

• Parathion/paraoxon reduce cellular viability of MSCs
  • Oxidative stress has a significant impact on MSC viability

• Parathion/paraoxon reduce the proliferative potential of MSCs

• Parathion/paraoxon reduce both AChE activity and protein levels

• Parathion/paraoxon reduce the ability of MSCs to differentiate into both adipoectyes and osteoblasts.

• AChE binds to the focal adhesion protein, vinculin, in MSCs. This interaction may be key to regulating MSC proliferation and differentiation.

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Role of Acetylcholinesterase in the Regulation of Mesenchymal Stem Cell Proliferation and Differentiation

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1027x374: Figure 1

1062x979: Figure 2

1105x1449: Figure 3