Tissue and Cell 47 (2015) 61–65

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

All trans retinoic acid modulates peripheral nerve fibroblasts viability and apoptosis

Nazila Niapour[a], Ali Niapour[a,*], Hamid Sheikhkanloui Milan[b], Mohammad Amani[b], Hossein Salehi[c], Nowrouz Najafzadeh[a], Mohammad Reza Gholami[d]

[a] Department of Anatomical Sciences, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran
[b] Department of Physiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran
[c] Department of Anatomical Sciences, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[d] Department of Anatomical Sciences, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

ARTICLE INFO

Article history:
Received 13 July 2014
Received in revised form 2 November 2014
Accepted 17 November 2014
Available online 21 November 2014

Keywords:
ATRA
Peripheral nerve fibroblasts
Viability
Apoptosis

ABSTRACT

Objective: Following peripheral nerve injury, residing fibroblasts start to proliferate and accumulate at the injury site and may participate in neuroma tissue evolution. Retinoic acid has been shown to regulate many cellular processes and to display anti-proliferative and anti-fibrotic properties. The aim of this study was to investigate the impact of all trans retinoic acid (ATRA) on rat peripheral nerve fibroblasts.
Materials and methods: Peripheral nerve fibroblasts and C166 cells were treated with increasing doses of ATRA (0.05 nM to 1 μM). The viability of cells was determined with 3-(4,5-dimethlthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In addition, the number of peripheral nerve fibroblasts was counted after two days of ATRA treatment and alternatively up to the end of next week. Acridine orange/ethidium bromide double staining was implemented to morphologically visualize the possible mechanism of cell death. For apoptosis, caspase 3/7 activity was measured using Caspase-Glo 3/7 assay kit.
Results: MTT assay revealed that 0.05–1 nM of ATRA reduces fibroblasts viabilities. Then, almost a plateau state was observed from 1 nM to 1 μM of ATRA exposure. Additionally, a deceleration in peripheral nerve fibroblasts growth was confirmed via cell counting. Quantification of acridine orange/ethidium bromide staining displayed highly increased number of early apoptotic cells following ATRA administration. Amplified activation of caspase 3/7 was in favor of apoptosis in ATRA treated peripheral nerve fibroblasts.
Conclusion: The data from the present study demonstrate that ATRA could interfere in peripheral nerve fibroblasts viabilities and induce apoptosis. Although more investigations are needed to be implemented, our in vitro results indicate that retinoic acid can probably help the regeneration of injured axon via reducing of fibroblasts growth.

1. Introduction

Axons of the peripheral nervous system (PNS) have the ability to grow beyond the lesion area (Yiu and He, 2006). Compelling evidence suggests the presence of permissive environment allowing axonal regeneration in PNS (Chen et al., 2007; Vargas and Barres, 2007). Indeed, a complex of cellular and molecular changes referred to Wallerian degeneration takes place within the distal stump of the damaged nerve as the natural mechanism for healing (Dubovy, 2011). Following loss of contact with axon, Schwann cells initiate proliferation and migration (Rotshenker, 2011) and participate actively with accumulated fibroblasts at the injury site, establishing guidance channel (i.e. Bands of Bungner) in a specialized cell sorting manner (Parrinello et al., 2010). The accomplishment or failure of peripheral nerve regeneration, to the most part, depends on the integrity of connective tissue which surrounds the nerve unit (Dreesmann et al., 2009). Disruption of nerve structure reduces the functional recovery since the regenerating axons gets stuck within a scar tissue (i.e. neuroma) made of fibroblasts originating from surrounding connective tissues and Schwann cells as well (Parrinello et al., 2010). Post-traumatic neuroma not only produces neurite growth repellent factors (Tannemaat et al., 2007),

* Corresponding author at: Department of Anatomical Sciences, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
Tel.: +98 451 5510052 287; fax: +98 451 5513424.
E-mail address: a.niapour@arums.ac.ir (A. Niapour).

http://dx.doi.org/10.1016/j.tice.2014.11.004
0040-8166/