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Research Article

Vitamin E Pretreatment of Mesenchymal Stem Cells: The Interplay of Oxidative Stress and Inflammation

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Abstract

Oxidative stress occurs as a result of breaking down the balance between oxidants (e.g., reactive oxygen species (ROS)) and antioxidants in cells. Several studies have shown that there is a close relationship between oxidative stress and inflammation at the sites of injury. Mesenchymal stem cells (MSCs) are exposed to endogenous and exogenous oxidants generated during their *ex vivo* expansion or following *in vivo* transplantation. *α*-tocopherol (vitamin E) is a fat-soluble compound known for its anti-oxidant and anti-inflammatory properties. In many studies, the immunomodulatory effects of vitamin E have been observed *in vivo*. This study aimed to determine whether pretreatment of MSCs with antioxidants like vitamin E, will enhance the anti-inflammatory and immunomodulatory properties of these cells. For this purpose, adipose-derived MSCs (ASCs) were treated with vitamin E (600 µM) for 48 h. Quantitative PCR (qPCR) experiments were performed to evaluate the expression of genes related to inflammation (*IL-1β, IL-6, IL-17, IL-10*) or immunomodulation (*TSG-6, COX-2, TDO2, TGF-β1*). Results indicated that vitamin E significantly increased the expression of *COX-2*, *TSG-6*, and *IL-1β* genes at the mRNA level compared with the control group, while it significantly decreased *IL-6* and *TGF-β* expressions. No effect was observed for *IL-17*, *IL-10*, and *TDO2* genes. These results suggest that *in vitro* preconditioning of ASCs with vitamin E may allow the cells to improve their anti-inflammatory and immunoregulatory capacities. Vitamin E pretreatment could lead to the improvement of their therapeutic abilities in conditions that are influenced by oxidative stress.

***Keywords*:** Mesenchymal Stem Cells, Vitamin E, Immunomodulation, Oxidative stress, Preconditioning

Introduction

Reactive oxygen species (ROS), which are generated during cellular metabolisms (Schieber and Chandel, 2014), are neutralized by antioxidants to gain a balance between oxidants and anti-oxidizing agents. Oxidative stress occurs as a result of excessive levels of ROS or low levels of antioxidants (Barrows et al., 2019). Oxidative stress as a pathophysiological condition is closely related to inflammation. ROS can initiate intracellular signal transductions and mediates the activation of various transcription factors (e.g., Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)) (Yuan et al., 2019). These transcription factors, in turn, raise the expression of pro-inflammatory genes and induce chronic inflammatory status (Biswas, 2016). Concurrently, inflammatory cells promote oxidative stress by releasing numerous reactive species at the sites of inflammation (Droge, 2002).

Antioxidant therapy seems to be a beneficial strategy to prevent or improve inflammatory diseases caused by oxidative stress. Nevertheless, some clinical studies were not promising (Kelly et al., 2008; Mahmood et al., 2018; Mishra et al., 2003). *α*-tocopherol (vitamin E) is the most effective lipid-soluble antioxidant that protects polyunsaturated fatty acids (PUFAs) of biological membranes (Azzi, 2007) and, is critical in the regulation of the immune response (Lee and Han, 2018).

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Numerous studies have illustrated that vitamin E has a modulatory effect on the immune system. Xue *et al*. showed that this vitamin improved experimental autoimmune neuritis (EAN) in a rat model by suppressing the production of pro-inflammatory cytokines and inhibiting progressive oxidative damages (Kihara et al., 2019). It was also suggested that vitamin E modulates the phase conversion between naïve T cells and T helper1 (Th1) or T helper (Th2) cells, as a response to the stimulation of dendritic cells (Xue et al., 2016). The anti-inflammatory effects of vitamin E have also been reported *in vivo,* which seems to be independent of its antioxidant properties. Tahan *et al*., in 2011, found that vitamin E suppresses inflammatory cytokines and inhibits the acetic acid-induced chronic inflammation in a rat model (Tahan et al., 2011). Xue *et al*. in 2016, revealed that vitamin E decreases the number of inflammatory cells in lymph nodes and spleens of the animals *in vivo* and inhibits the proliferation of stimulated splenocytes *in vitro* (Xue et al., 2016).

Stem cell-based therapy is a proper strategy for controlling the symptoms of inflammatory and immune-mediated diseases. Mesenchymal stem/stromal cells (MSCs) have been widely used for allogeneic cell therapy to treat autoimmune diseases (Rad et al., 2019), inflammation-mediated disorders (Francis et al., 2019; Zhao et al., 2019), and cardiovascular diseases (Yun and Lee, 2019). The successful isolation of MSCs from a variety of adult tissues, e.g., bone marrow and adipose tissues, has provided a powerful tool for applied biological research (Wei et al., 2013).

Many studies revealed that some environmental and pharmacological stimuli (e.g., small molecules) or preconditioning strategies could influence the functional properties of MSCs in the context of immunotherapy (Linares et al., 2016; Pittenger and Martin, 2004; Schaefer et al., 2016). Furthermore, endogenous and exogenous oxidants that MSCs may expose to them during *ex vivo* expansion or *in vivo* transplantation procedures are considered as significant bottlenecks in cell therapy experiments (Yang et al., 2015). High levels of ROS are harmful to preserve self-renewal, reparative, and immunoregulatory functions of MSCs (Denu and Hematti, 2016; Yang et al., 2015). ROS, as a metabolic side product, increases adipogenic differentiation, enhances senescence, diminishes osteogenic differentiation, and hinders the immunomodulatory properties of MSCs (Denu and Hematti, 2016). Moreover, inflammatory responses, in addition to the production of ROS at the ischemic target sites, lead to the loss of transplanted MSCs. Hence, it is vital to reduce ROS either by manipulating the cells or their target sites (Devine et al., 2001; Pittenger and Martin, 2004; Yang et al., 2015).

Accordingly, understanding the effects of ROS on MSCs biology could shed light on the immunomodulatory behaviors of the cells under inflammatory conditions.

To the best of our knowledge, this is the first study that evaluates the effects of vitamin E on immunomodulatory and anti-inflammatory properties of human ASCs. Considering the vitamin E's evident antioxidant and anti-inflammatory properties, we anticipated that priming of ASCs with vitamin E could boost the beneficial effects of these therapeutically valuable cells.

Materials and Methods

Isolation and culture of human ASCs

Adipose tissues were obtained from three healthy donors undergoing elective liposuction at a private cosmetic day clinic in Mashhad, Iran. All three patients signed the informed consent form. The Academic Center for Education, Culture, and Research (ACECR) Biomedical Research Ethics Committee authorized all downstream protocols (IR.ACECR.JDM.REC.1398.009).

200 ml of adipose tissues were washed three times with phosphate-buffered saline (PBS) containing 0.1% penicillin-streptomycin (pen-strep) (Biowest, Canada) and incubated for one hour in constant–temperature bath at 37°C in the presence of 0.1% collagenase type I (Invitrogen, USA). Fetal bovine serum (FBS, Gibco, USA, 10%) was applied for collagenase I inactivation. Then, the mixture was centrifuged at 800 *g* for 10 min to remove adipose cell debris. In the following, pellets were suspended in Dulbecco's Modified Eagle Medium (DMEM, Biowest, Canada) contained 10% FBS and 0.1% pen-strep. Then, the cells were transferred into cell culture vessels and kept in a 5% CO_2 incubator at 37°C (Naderi-Meshkin et al., 2016). We changed the culture medium every three days. All the following experiments were conducted with the cells at passage number 3.

Characterization of human ASCs

Flowcytometric approach was applied for the identification of mesenchymal lineage-specific surface markers. A suspension of 10^6 single cells was transferred into the staining buffer contained PBS and 5% FBS. Then, anti-human monoclonal

antibodies (*all from* Cytognos, Spain) against clusters of differentiation 44 (CD44), CD90, CD73, CD13, CD14, CD34, and human leukocyte antigen– DR (HLA-DR) antigens were mixed with the cells and incubated for 45 min at 4 °C. FACS Calibur cytometer equipped with 488 nm argon laser (Bioscience, US) was used for data acquirements. Data analysis was performed using FlowJo (7.6.1) software.

The capacity of the cells for differentiation toward osteogenic and adipogenic lineages was qualitatively determined based on the previously described alizarin red and oil red O staining methods, respectively (Naderi-Meshkin et al., 2016). Briefly, adipogenesis was induced through the culture of ASCs in the presence of DMEM supplemented by 10% FBS, 200 mM indomethacin, 10 mM β-glycerophosphate, and 1 mM dexamethasone. After 14 days, the cells were rinsed with PBS and fixed in 10% formalin solution. Then, they were stained with 0.5% Oil Red O (Sigma, Germany) for 15 min.

The osteogenic inductive medium was composed of DMEM, 10% FBS, 0.5 mM acid ascorbic, 10 mM β-glycerophosphate, and 1 mM dexamethasone. The cells were incubated in this medium for 21 days. Then, they were fixed and stained with alizarin red (Sigma, Germany) for 30 min to detect the mineralized matrix of the bone, secreted by differentiated cells.

Preconditioning of human ASCs with vitamin E

Human ASCs were cultured in DMEM supplemented with 10% FBS and 1% pen-strep. Upon reaching 80% confluency, the proper concentration of vitamin E (Sigma, Germany) was added to the cultures. Untreated ASCs or cells that had been cultured with ethanol-containing media were applied as controls.

MTT assay

MTT (2, 3-bis (2-methoxy-4-nitro-5-5 sulfoxyphenyl)-2H-tetrazolium assay was carried out to evaluate the possible toxic effects of various concentrations of vitamin E against human ASCs. 10^4 cells were seeded in 96-well plates, and after reaching 80% confluency, they were treated with 200, 400, 600, 800, and 1000 µM of vitamin E for 24 to 72 hours. Cells cultured in the presence of DMEM or DMEM supplemented by an equal volume of ethanol were used as blank and control groups, respectively. Cell viabilities were determined following the addition of MTT dye (5 mg/ml) to the wells, incubating the vessels at 37°C for 4 hours, and recording optical densities (ODs) at 540nm by NanoDrop spectrophotometer (Nanodrop, BIO-TEK, Winooski, VT).

RNA extraction and quantitative PCR (qPCR)

Total RNAs were extracted from ASCs after 48 hours of treatment with 600 µM of vitamin E and control cells using TriPure according to the protocol provided by the manufacturer (Roche, Germany). The integrity of RNA samples was indicated using 1% agarose gel, and their concentrations were assessed via a NanoDrop spectrophotometer (Nanodrop, BIO-TEK, Winooski, VT).

One µg of DNase *I*-treated total RNA was used for cDNA synthesis in each case (Thermo Scientific, USA). cDNA synthesis steps were performed according to the kit instructions (Takara, Japan).

qPCR was accomplished by SYBR Green PCR Master Mix (amplicon, USA) according to the kit protocol with The CFX Connect™ Real-Time PCR Detection System (Bio-Rad, Germany). Ribosomal protein lateral stalk subunit P (*RPLP0*) gene was used as an internal control (reference gene) to normalize the transcript level of tested genes. Primers were designed by AlleleID 6 software and are shown in table 1.

Table 1. Primer sequences used for qPCR.

Genes	Primer sequences (5'→ 3')	product length (bp)
RPLP0	**F**: TGGTCATCCAGCAGGTGTTCGA **R**: ACAGACACTGGCAACATTGCGG	119
TGF-β1	**F**: GTTCAAGCAGAGTACACACAGC **R**: GTATTTCTGGTACAGCTCCACG	153
TSG-6	**F**: GCTGCTGGATGGATGGCTAAG **R**: CTCCTTTGCGTGTGGGTTGTAG	156
COX-2	**F**:CCAGAGCAGGCAGATGAAATACC **R**: ACCAGAAGGGCAGGATACAGC	168
IL-1β	**F**: CCTCTCTCACCTCTCCTACTCAC **R**: CTGCTACTTCTTGCCCCCTTTG	186
IL-17	**F:**CGGCAGGCACAAACTCATCC **R:**TTGTCCTCAGAATTTGGGCATCC	163
IL-10	**F:**GAGATGCCTTCAGCAGAGTGAAGA **R:**AGGCTTGGCAACCCAGGTAAC	114
TDO2	**F:** ACCTCCGTGCTTCTCAGACAG **R:** GACCTCCTTTGCTGGCTCTATTC	151
IL-6	**F**: ACTCACCTCTTCAGAACGAATT **R**: GCAAGTCTCCTCATTGAATCCAG	196

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6. Data were expressed as mean of independent experiments±SEM. *One way* ANOVA and two samples T-test were used for statistical analysis. Events with *p* values less than 0.05 were considered significant.

Results

Characterization of human ASCs

Cultured ASCs were characterized through investigation of surface markers expression levels and their potential for multi-lineage differentiation. Flow cytometry analysis results indicated that > 98% of these cells expressed ASC specific markers including CD13, CD44, CD90 and CD73 and < 3% showed the expression of hematopoietic cell-specific markers including CD14, CD34, CD45, and HLA-DR (Figure 1).

The differentiation potential toward adipocytes and osteocytes was investigated using adipogenic and osteogenic differentiation media and staining with Oil Red O and Alizarin Red, respectively (figure 2 D, E & F).

Both lipid depositions and mineralization of the extracellular matrixes were visualized following the staining procedures, which confirmed the adipogenesis and osteogenesis of ASCs.

Investigating the toxic effects of vitamin E on ASCs

The effects of various concentrations of vitamin E (200-1000 µM) on ASCs' survival rate was explored by MTT assay. The results showed that after 24, 48, and 72 h, no significant difference (P<0.05) was observed between cell viabilities of control and sample groups among all concentrations and investigated time points (Figure 3).

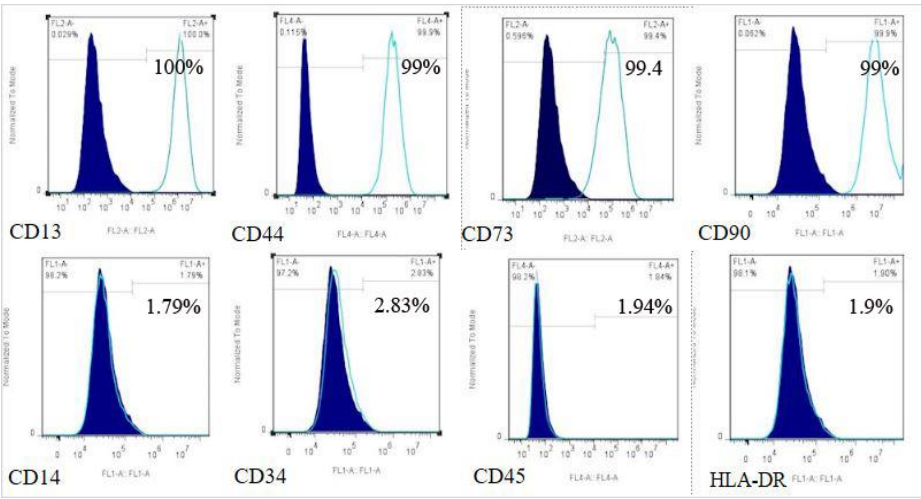

Figure 2. Immunophenotype characterization of sub-cultured MSCs at passage 3. Diagrams show flow cytometry data for MSC specific surface markers (CD13, CD44, CD73 & CD90) and hematopoietic markers (CD14, CD34, CD45 & HLA-DR). Data presents the percentages of the cells which were positive for each marker.

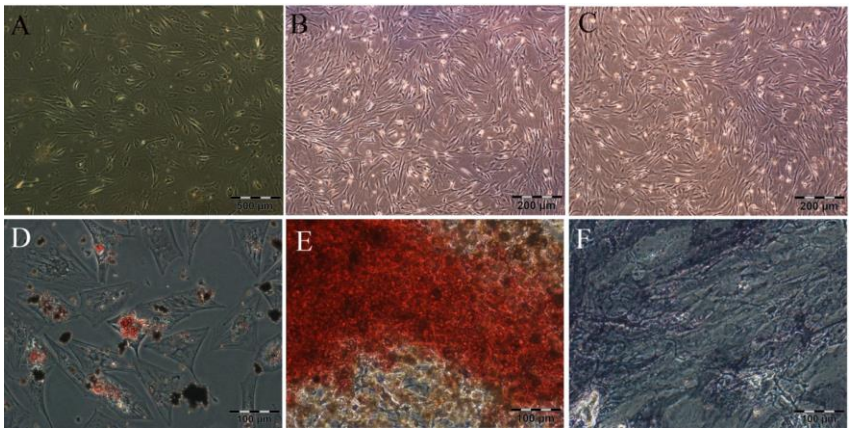

Figure 1. Characterization of human ASCs. Photomicrographs show the morphology and differentiation capacity of ASCs. A) Spindle-like morphology of human ASCs 8 days after harvesting from adipose tissues. B) Morphology of the control group after 48h. C) Morphology of ASCs preconditioned with 600 µM of vitamin E after 48 h. D) Oil Red O staining to detect adipogenic differentiation. E) Alizarin Red staining to measure osteogenic differentiation. F) Alkaline phosphatase assay to confirm osteogenic differentiation.

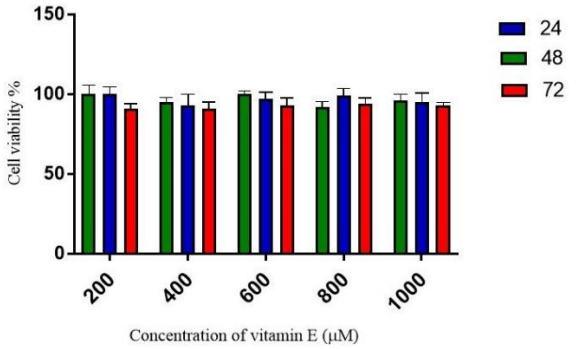

Figure 3. Mean of cell viabilities (%) calculated for pre-conditioned ASCs as obtained by MTT assay. As demonstrated, differences were not significant (p <0.05) in comparison to control groups at different concentrations and time points.

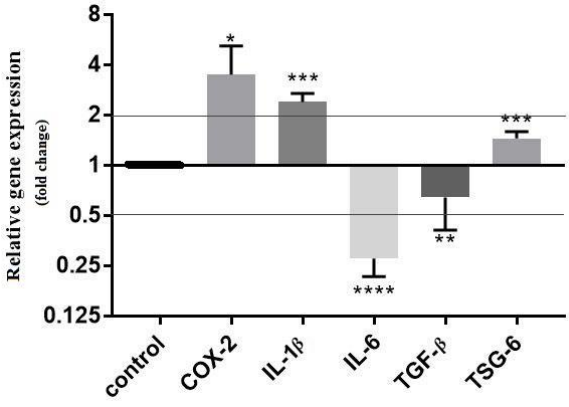

Figure 4. Vitamin E preconditioning of ASCs changed the expression of *IL-1β* and *IL-6* (inflammatory markers), in addition to *TSG-6*, *COX-2*, and *TGF-β* (immunomodulatory markers). Vitamin E treatment suppressed the expression of *IL-6* and *TGF- β* and enhanced the expression of *IL-1β*, *COX-2*, and *TSG-6* when compared to the control group. Results were expressed as mean±standard deviation (SD), and (*) represents $p < 0.05$, (**) represents $p < 0.01$, (***) represent $p < 0.001$ and (****) represent $p < 0.0001$. The expression levels of all investigated genes were considered equal to 1, conventionally.

Gene expression profiling of vitamin E stimulated ASCs

The expression levels of two categories of genes were investigated in this study: inflammatory-related genes including interleukin 1-betta (*IL-1β*), *IL-6*, *IL-17*, *IL-10* and immunomodulatory genes such as TNF-stimulated gene 6 (*TSG-6*), cyclooxygenase-2 (*COX-2*), tryptophan 2,3-dioxygenase (*TDO2*) and transforming growth factor- beta (*TGF-β*). The qPCR results showed that pretreatment with vitamin E markedly enhanced the gene expression of *TSG-6*, *IL-1β*, and *COX-2* at mRNA level and significantly (p<0.05) reduced the expressions of *IL-6* and *TGF-β* compared with the control group (Figure 4). In contrast, ASCs pretreatment did not affect *IL-10*, *IL-17*, and *TDO2* gene expressions compared to the control group.

Discussion

As a recommended supplement, vitamin E inhibits the production of ROS molecules and pro-inflammatory cytokines and depicts immunosuppressive properties (Lee and Han, 2018b). The findings of this study demonstrated that Vitamin E when applied as a small molecule for preconditioning of ASCs, altered the expression of some genes which are involved in immunomodulation and inflammation. Here, we argued that the pretreatment of stem cells with Vitamin E before cellular therapy could have beneficial effects on their immunoregulatory capacities.

ASCs are multipotent cells with a high capability for interacting with a variety of immune cells. These cells release various factors with immunomodulatory potential such as cytokines and chemokines, which make them a decent choice to treat numerous immune-mediated diseases accompanied by chronic inflammation (Baer et al., 2018). Priming MSCs with appropriate agents can promote the efficacy of some specific immunotherapeutic applications (Hu and Li, 2018; Silva et al., 2018; Tang et al., 2014; Wisel et al., 2009).

Vitamin E is recognized not only for its antioxidant properties but also for its regulatory effects on signaling pathways through the induction of gene expression modifications (Azzi, 2018; Sangiorgi et al., 2016; Zingg, 2015). We studied the consequences of vitamin E treatment at a high concentration (600 μM) on the cell proliferation rate and cytokine production status of the cells *in vitro*. Our findings showed that the preconditioning of MSCs by 600 μM of vitamin E significantly attenuated the expression of *IL-6* at least by two folds and altered the expression of *TGF-β* slightly. We also observed a significant increase in the expression of *COX-2*, *TSG6*, and *IL-1β*.

Wang et al. found that rat bone marrow-derived MSCs could ameliorate peritoneal injury by repairing mesothelial cells. They also showed that MSCs lacking *TSG-6* (TSG-6-siRNA MSCs) had no apparent effects on the peritoneal fibrosis. Thus, it was confirmed that the secretion of TSG-6 by MSCs made a significant contribution to their clinical outcomes (Wang et al., 2012). In line with their findings, Roddy et al. reported that

intravenous administration of human MSCs primed to express TSG-6 suppressed the inflammatory damages of the cornea following the induction of chemical injury in rats. Additionally, Roddy et al. demonstrated that the siRNA knockdown of TSG-6 impeded the anti-inflammatory effects of these cells on damaged corneal epithelial cells (Roddy et al., 2011). Given these observations, we suggest that preconditioning of MSCs with vitamin E could improve their immunomodulatory properties by enhancing the expression of TSG-6.

IL-6 is a pleiotropic and multifunctional cytokine involved in many physiological events, such as inflammation through NF-κB and signal transducers and activators of transcription (STAT) signaling pathways. It was shown that blockade of *IL-6* prevents the progression of autoimmune-based diseases and tumor formation (Barnes et al., 2011; Schaper and Rose-John, 2015; Tanaka et al., 2014). The blockade of *TGF-β1* also causes anti-tumor immunity and tumor regression (Mariathasan et al., 2018; Shangguan et al., 2012), which increases safety concerns in tumorigenesis. COX-2 is a crucial enzyme in prostaglandin E2 synthesis, which promotes the anti-inflammatory features of macrophages (M2) (Lu et al., 2017; Németh et al., 2009). The pro-inflammatory cytokine, IL-1*β*, is regulated through NF-κB and c-jun signaling pathways (Libby, 2017; Palomo et al., 2015; Rodriguez et al., 2019). In contrast, MSC pre-treatment did not affect *IL-10*, *IL-17*, and *TDO2* gene expressions in the current study. Their weak expression by naïve MSCs could explain it (Ben-Zwi et al., 2019).

There was a strong correlation between the changes in redox potential and the production of pro-inflammatory cytokines with the inflammatory pathways, e.g., NF-κB. NF-κB is a transcription factor thought to be modulated by oxidative stress (Behl et al., 1994; Lingappan, 2018). Antioxidants like vitamin E are believed to prevent the activation of NF-κB and other inflammatory pathways through the inhibition of lipid peroxidation (Saxena et al., 2019).

Together, these findings support the notion that vitamin E improves the anti-inflammatory characteristics of ASCs. This effect could be due to inhibition of the activation of some inflammatory signaling pathways, such as NF-Ķb, in human MSCs that inhibits the production of pro-inflammatory cytokines.

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Conflict of interest

The authors declared no competing interest.

References

Azzi A. (2007) Molecular mechanism of α-tocopherol action. Free Radical Biology and Medicine 43:16-21.

Azzi A. (2018) Many tocopherols, one vitamin E. Molecular Aspects of Medicine 61:92-103.

Baer P. C., Overath J. M., Urbschat A., Schubert R., Koch B., Bohn A. A. and Geiger H. (2018) Effect of different preconditioning regimens on the expression profile of murine adipose-derived stromal/stem cells. International Journal of Molecular Sciences 19:1719.

Barnes T. C., Anderson M. E. and Moots R. J. (2011) The many faces of interleukin-6: the role of IL-6 in inflammation, vasculopathy, and fibrosis in systemic sclerosis. International Journal of Rheumatology.

Barrows I. R., Ramezani A. and Raj D. S. (2019) Inflammation, Immunity, and Oxidative Stress in Hypertension—Partners in Crime? Advances in Chronic Kidney Disease 26:122-130.

Behl C., Davis J., Lesley R., and Schubert D. (1994) Hydrogen peroxide mediates amyloid β protein toxicity. Cell 77:817-827.

Ben-Zwi M., Petrou P., Halimi M., Karussis D. and Kassis I. (2019) Neuralized mesenchymal stem cells (NMSC) exhibit phenotypical, and biological evidence of neuronal transdifferentiation and suppress EAE more effectively than unmodified MSC. Immunology Letters 212:6-13.

Biswas S. K. (2016) Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? Oxidative Medicine and Cellular Longevity.

Denu R. A. and Hematti P. (2016) Effects of oxidative stress on mesenchymal stem cell biology. Oxidative Medicine and Cellular Longevity.

Devine S. M., Bartholomew A. M., Mahmud N., Nelson M., Patil S., Hardy W., Sturgeon C., Hewett T., Chung T. and Stock W. (2001) Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. Experimental hematology 29:244-255.

Droge W. (2002) Free radicals in the physiological control of cell function. Physiological Reviews 82:47-95.

Francis E., Kearney L. and Clover J. (2019) The effects of stem cells on burn wounds: a review. International Journal of Burns and Trauma 9:1.

Hu C. and Li L. (2018) Preconditioning influences mesenchymal stem cell properties in vitro and in vivo. Journal of Cellular and Molecular Medicine 22:1428-1442.

Kelly R. P., Poo Yeo K., Isaac H. B., Lee C.-Y. J., Huang S. H., Teng L., Halliwell B. and Wise S. D. (2008) Lack of effect of acute oral ingestion of vitamin C on oxidative stress, arterial stiffness or blood pressure in healthy subjects. Free Radical Research 42:514-522.

Kihara H., Konno S. and Fujioka T. (2019) Alpha-Tocopherol Ameliorates Experimental Autoimmune Neuritis (P1. 2-089). AAN Enterprises.

Lee G. Y. and Han S. N. (2018) The role of vitamin E in immunity. Nutrients 10:1614.

Libby P. (2017) Interleukin-1 beta as a target for atherosclerosis therapy: biological basis of CANTOS and beyond. Journal of the American College of Cardiology 70:2278-2289.

Linares G. R., Chiu C.-T., Scheuing L., Leng Y., Liao H.-M., Maric D. and Chuang D.-M. (2016) Preconditioning mesenchymal stem cells with the mood stabilizers lithium and valproic acid enhances therapeutic efficacy in a mouse model of Huntington's disease. Experimental Neurology 281:81-92.

Lingappan K. (2018) NF-κB in oxidative stress. Current Opinion in Toxicology 7:81-86.

Lu L. Y., Loi F., Nathan K., Lin T. h., Pajarinen J., Gibon E., Nabeshima A., Cordova L., Jämsen E. and Yao Z. (2017) Pro-inflammatory M1 macrophages promote Osteogenesis by mesenchymal stem cells via the COX-2prostaglandin E2 pathway. Journal of Orthopaedic Research 35:2378-2385.

Mahmood L. A., Al Saadi R. and Matthews L. (2018) Dietary and antioxidant therapy for autistic children: Does it really work? Archives of Medicine and Health Sciences 6:73.

Mariathasan S., Turley S. J., Nickles D., Castiglioni A., Yuen K., Wang Y., Kadel III E. E., Koeppen H., Astarita J. L. and Cubas R. (2018) TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature 554:544.

Mishra G., Malik N., Paul A., Wadsworth M. and Bolton-Smith C. (2003) Childhood and adult dietary vitamin E intake and cardiovascular risk factors in mid-life in the 1946 British Birth Cohort. European Journal of Clinical Nutrition 57:1418-1425.

Naderi Meshkin H., Matin M. M., Heirani,Tabasi A., Mirahmadi M., Irfan-Maqsood M., Edalatmanesh M. A., Shahriyari M., Ahmadiankia N., Moussavi N. S. and Bidkhori H. R. (2016) Injectable hydrogel delivery plus preconditioning of mesenchymal stem cells: exploitation of SDF-1/CXCR4 axis toward enhancing the efficacy of stem cells' homing. Cell Biology International 40:730-741.

Németh K., Leelahavanichkul A., Yuen P. S., Mayer B., Parmelee A., Doi K., Robey P. G., Leelahavanichkul K., Koller B. H. and Brown J. M. (2009) Bone marrow stromal cells attenuate sepsis via prostaglandin E 2–dependent reprogramming of host macrophages to increase their interleukin-10 production. Nature Medicine 15:42.

Palomo J., Dietrich D., Martin P., Palmer G. and Gabay C. (2015) The interleukin (IL)-1 cytokine family–Balance between agonists and antagonists in inflammatory diseases. Cytokine 76:25-37.

Pittenger M. F. and Martin B. J. (2004)

Mesenchymal stem cells and their potential as cardiac therapeutics. Circulation Research 95:9-20.

Rad F., Ghorbani M., Roushandeh A. M. and Roudkenar M. H. (2019) Mesenchymal stem cell-based therapy for autoimmune diseases: emerging roles of extracellular vesicles. Molecular Biology Reports 46:1533-1549.

Roddy G. W., Oh J. Y., Lee R. H., Bartosh T. J., Ylostalo J., Coble K., Rosa R. H., Jr. and Prockop D. J. (2011) Action at a distance: systemically administered adult stem/progenitor cells (MSCs) reduce inflammatory damage to the cornea without engraftment and primarily by secretion of TNF-alpha stimulated gene/protein 6. Stem Cells 29:1572-1579.

Rodriguez L. A., Mohammadipoor A., Alvarado L., Kamucheka R. M., Asher A. M., Cancio L. C. and Antebi B. (2019) Preconditioning in an Inflammatory Milieu Augments the Immunotherapeutic Function of Mesenchymal Stromal Cells. Cells 8:462.

Sangiorgi B., De Freitas H. T., Schiavinato J. L. D. S., Leão V., Haddad R., Orellana M. D., Faça V. M., Ferreira G. A., Covas D. T. and Zago M. A. (2016) DSP30 enhances the immunosuppressive properties of mesenchymal stromal cells and protects their suppressive potential from lipopolysaccharide effects: a potential role of adenosine. Cytotherapy 18:846-859.

Saxena A., Sonowal H. and Ramana K. V. (2019) Transcriptional Factor Modulation by Lipid Peroxidation-Derived Aldehydes. In The Molecular Nutrition of Fats. Elsevier. 419-431.

Schaefer R., Spohn G. and Baer P. C. (2016) Mesenchymal stem/stromal cells in regenerative medicine: can preconditioning strategies improve therapeutic efficacy. Transfusion Medicine and Hemotherapy 43:256-267.

Schaper F. and Rose-John S. (2015) Interleukin-6: biology, signaling and strategies of blockade. Cytokine & growth factor reviews 26:475-487.

Schieber M. and Chandel N. S. (2014) ROS function in redox signaling and oxidative stress. Current biology 24:R453-R462.

Shangguan L., Ti X., Krause U., Hai B., Zhao Y., Yang Z. and Liu F. (2012) Inhibition of TGF-β/Smad signaling by BAMBI blocks differentiation of human mesenchymal stem cells to carcinoma-associated fibroblasts and abolishes their protumor effects. Stem cells 30:2810-2819.

Silva L. H., Antunes M. A., Dos Santos C. C., Weiss D. J., Cruz F. F. and Rocco P. R. (2018) Strategies to improve the therapeutic effects of mesenchymal stromal cells in respiratory diseases. Stem Cell Research & Therapy 9:45.

Tahan G., Aytac E., Aytekin H., Gunduz F., Dogusoy G., Aydin S., Tahan V. and Uzun H. (2011) Vitamin E has a dual effect of anti-inflammatory and antioxidant activities in acetic acid-induced ulcerative colitis in rats. Canadian Journal of Surgery 54:333.

Tanaka T., Narazaki M. and Kishimoto T. (2014) IL-6 in inflammation, immunity, and disease. Cold Spring Harbor Perspectives in Biology 6:a016295.

Tang J., Xiong J., Wu T., Tang Z., Ding G., Zhang C., Wang S. and Liu Y. (2014) Aspirin treatment improved mesenchymal stem cell immunomodulatory properties via the 15d-PGJ2/PPARγ/TGF-β1 pathway. Stem cells and Development 23:2093-2103.

Wang N., Li Q., Zhang L., Lin H., Hu J., Li D., Shi S., Cui S., Zhou J., Ji J., Wan J., Cai G. and Chen X. (2012) Mesenchymal stem cells attenuate peritoneal injury through secretion of TSG-6. PLoS One 7:e43768.

Wei X., Yang X., Han Z.-p., Qu F.-f., Shao L. and Shi Y.-f. (2013) Mesenchymal stem cells: a new trend for cell therapy. Acta Pharmacologica Sinica 34:747-754.

Wisel S., Khan M., Kuppusamy M. L., Mohan I. K., Chacko S. M., Rivera B. K., Sun B. C., Hideg K. and Kuppusamy P. (2009) Pharmacological preconditioning of mesenchymal stem cells with trimetazidine (1-[2, 3, 4-trimethoxybenzyl] piperazine) protects hypoxic cells against oxidative stress and enhances recovery of myocardial function in infarcted heart through Bcl-2 expression. Journal of Pharmacology and Experimental Therapeutics 329:543-550.

Xue H., Ren H., Zhang L., Sun X., Wang W., Zhang S., Zhao J. and Ming L. (2016) Alpha-tocopherol ameliorates experimental autoimmune encephalomyelitis through the regulation of Th1 cells. Iranian Journal of Basic Medical Sciences 19:561.

Yang S.-R., Park J.-R. and Kang K.-S. (2015) Reactive oxygen species in mesenchymal stem cell aging: implication to lung diseases. Oxidative Medicine and Cellular Longevity 2015.

Yuan T., Yang T., Chen H., Fu D., Hu Y., Wang J., Yuan Q., Yu H., Xu W. and Xie X. (2019) New insights into oxidative stress and inflammation during diabetes mellitus-accelerated atherosclerosis. Redox Biology 20:247-260.

Yun C. W. and Lee S. H. (2019) Enhancement of functionality and therapeutic efficacy of cell-based therapy using mesenchymal stem cells for cardiovascular disease. International Journal of Molecular Sciences 20:982.

Zhao L., Han F., Wang J. and Chen J. (2019) Current understanding of the administration of mesenchymal stem cells in acute kidney injury to chronic kidney disease transition: a review with a focus on preclinical models. Stem Cell Research & Therapy 10:385.

Zingg J.-M. (2015) Vitamin E: a role in signal transduction. Annual Review of Nutrition 35:135-173.