Research article

Glutathione-related inflammatory signature in hepatocytes differentiated from the progenitor mesenchymal stem cells☆

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ABSTRACT

N-acetylcysteine (NAC) as a glutathione inducer is known for its anti-inflammatory effects in inflammatory conditions. The aim of the present study was to know if supplementation of the culture medium with NAC can improve anti-inflammatory activities of hepatocytes during their differentiation from mesenchymal stem cells (MSCs). For this, in vitro hepatic differentiation of MSCs was performed in culture medium supplemented with NAC and selected pro- and anti-inflammatory factors were monitored for two weeks. Treatment of the MSCs undergoing hepatic differentiation with NAC (0.1 and 1.0 mM) caused a significant (~5-fold) increase in pro-liferation rate of MSCs, whereas the rate of hepatic differentiation was declined in NAC-treated cells as compared to those untreated with NAC. Under these circumstances, NAC caused a significant increase in total glutathione in cell lysate during 2 weeks of differentiation as compared to untreated group. NAC-related increase in glutathione was associated with significant alterations in tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-8 and IL-10 levels secreted in the culture medium. A substantial decrease in the IL-6, IL-8 and TNF-α levels in the culture medium supplemented with NAC was obvious in hepatocytes recovered 14 days after differentiation. In contrast, the secretary IL-10 was significantly increased as a result of NAC treatments. These data suggest that NAC sup-plementation can improve anti-inflammatory activities of the hepatocytes derived from MSCs. NAC function mediated by glutathione synthesis can also help in modulation of proliferation of the stem cells and their dif-ferentiation into hepatocyte-like cells.

1. Introduction

Mesenchymal stem cells (MSCs) with wide differentiation potential and self-renewal capacity have been considered as a promising candidate for tissue engineering and transplantation for both regenerative and transplant medicine. In this line, in vitro differentiation of MSCs into the biologically active hepatocytes is implicated in stem cell-based therapy of chronic liver diseases. The therapeutic effects of MSCs are justified by their differential potential as well as their immunomodulatory properties (Gebler et al., 2012; Puglisi et al., 2011).

The safety of the liver cells prepared for pre-clinical or clinical ap-plications, particularly those differentiated from stem cells has always been a concern (Allameh and Kazemnejad, 2012). Besides, the

hepatocytes differentiated from stem cells under conventional culture conditions are vulnerable to oxidative damages involving protein, lipids and DNA molecules (Khajeniazi et al., 2013).

We previously showed that under the conventional condition, during hepatogenic differentiation of MSCs, the newly formed hepatocytes are more susceptible to lipid and protein oxidation rather than the DNA damage (Esmaeli et al., 2017; Khajeniazi et al., 2013).

Hepatic differentiation of MSCs is a process associated with devel-opmental changes in the biochemical, metabolic as well as immunolog-ical activities. MSCs as the progenitor stem cells are well known for their immunomodulatory functions (Nasef et al., 2008). Likewise, the hepa-tocytes perform a number of important immunological roles in addition to their essential metabolic functions (Robinson et al., 2016).

The immunological and inflammatory reactions and overall liver homeostasis are mainly regulated by cytokines as the cell signaling factors. Therefore, it is assumed that during the hepatic differentiation of MSCs a significant change in the profile of cytokines and their signaling patterns can occur. During the *in vitro* differentiation process of MSCs into functional hepatocytes, growth factors and cytokines produced are involved in suppression of inflammatory responses, reduction of apoptosis and enhancing metabolic and biochemical activities of the hepatocytes (Puglisi et al., 2011). According to Irudayam et al. the progenitor endoderm cells express lower levels of pro-inflammatory interleukins, interleukin (IL)-receptors and chemokines compared to the stem cell derived hepatic cells (Irudayam et al., 2015). Experiments carried out on knockout mice showed that tumor necrosis factor-α (TNF-α) and IL- 6 secreted by bone marrow derived cells play important role in liver regeneration (Sudo et al., 2008).

The role of N-acetylcysteine (NAC), as a glutathione precursor, in detoxification process and protection against oxidative damage in liver is well established. However very little is known about the role and the mechanism of action of NAC on inflammatory reactions, particularly during the differentiation of hepatocyte-like cells from their progenitor stem cells.

Earlier we reported that cellular glutathione plays an important role in balancing the antioxidant and prooxidant factors during the hepatogenic differentiation of MSCs. The antioxidant properties of NAC in hepatocytes was elucidated by showing the improvement in metabolic and biochemical functions of stem cell derived hepatocytes (Allameh et al., 2014). The present study is aimed to know the impact of glutathione biosynthesis on expression of cytokines and homeostasis of the hepatic differentiation of MSC derived hepatocytes.

The role of MSCs as guardians of inflammation has been reviewed (Prockop and Oh, 2012). In the present study, *in vitro* experiments were designed to know if the pro- and anti-inflammatory properties of the MSCs are transferred to the hepatocytes differentiated from these progenitor cells. Moreover, the impact of the NAC-dependent increase in glutathione on the inflammatory status in hepatocyte-like cells was evaluated during the hepatic differentiation.

2. Materials and methods

2.1. Chemicals and reagents

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, glutamine, antibiotics and trypsin-ethylene diamine-tetra acetic acid (EDTA) solution were purchased from Gibco BioCult, Paisley, UK. NAC, dexamethasone (DEX), hepatocyte growth factor (HGF), Oncostatin M (OSM), mouse anti-human antibodies for albumin and α-fetoprotein (AFP), rabbit anti mouse fluorescein isothiocyanate-conjugated immunoglobulin G and oil red O-staining kit were from the Sigma Chemical Co. (St. Louis, MO, USA). Antibodies used for flow cytometry analysis were from Dako (Glostrup, Denmark) and Oxford Biomedical Research (Oxford, UK).

2.2. Preparation of human bone marrow MSCs and culture

In this study, 3 surplus bone marrow biopsies from young healthy donors were used for isolation of MSCs. The samples were collected from the Shariati Bone Marrow Transplantation Center, Tehran, Iran after informed consent was taken according to the guidelines of the Medical Ethics Committee. MSCs were isolated routinely in our laboratory as described previously (Jazayeri et al., 2008; Kazemnejad et al., 2009). Briefly, the aspirates were diluted with phosphate buffer saline (PBS) and the cell suspension was gently overlaid over the Ficoll-Hypaque (1.077 g/ml) gradient to eliminate unwanted cell types in the marrow aspirate. Mononuclear cells were recovered from the gradient interface and washed with PBS after centrifugation at 400g for 30 min. The isolated mononuclear cells were seeded into polystyrene

culture plates (75 cm^2) containing DMEM-low glucose supplemented with 15% FBS, 2 mM glutamine, 100 mg/ml streptomycin and 100U/ml penicillin and was maintained at 37 °C in a humidified 5% CO2 incubator for 3–4 days. The non-adherent cells were washed away and adherent cell population that were growing as fibroblastic cells in clusters were maintained to obtain 70–90% confluency. The cells were harvested with trypsin-EDTA solution and plated in 25-cm^2 culture flasks at a density of 10^4 cells/cm2.

The human bone marrow derived MSCs were characterized for their morphology, immunophenotyping profile and differentiation potential as described in our previous publications (Allameh et al., 2014; Kazemnejad et al., 2009). The differentiation potential of the MSCs was approved by performing trans-differentiation assay into osteoblasts and adipocytes. In the process of osteogenic differentiation was accomplished for two weeks in α-MEM supplemented with 10% FBS, 0.1 mM DEX, 10 mM β-glycerophosphate and 50 mM ascorbate-phosphate. During this period, the culture media were changed twice weekly. Then the cells were fixed with methanol for 10 min at room temperature and identified by staining procedures for calcium using alizarin red staining kit and examined under a phase contrast microscope (Nikon, Tokyo, Japan). In case of adipogenic differentiation, the MSCs were subjected to differentiation in DMEM supplemented with 10% FBS, 1 mM DEX, 200 mM indomethacin, 1.7 mM insulin, 500 mM isobutyl-methylxanthine, 0.05 U/ml penicillin and 0.05 mg/ml of streptomycin for two weeks, followed by adipocyte-specific staining using oil red O-staining.

2.3. Differentiation of MSCs into hepatocyte-like cells

The cultured MSCs at their third passage (2×10^4 cells/cm^2) were seeded on 24-well plastic cell culture plates. Hepatic differentiation was initiated using a 2-step protocol as described in our previous publication (Kazemnejad et al., 2008). Briefly, in the first step which lasted for seven days, the cells were seeded in DMEM-low glucose medium containing 15% FBS, HGF (20 ng/ml), and DEX (10^{-7} mol/L). The second phase of differentiation was started by adding OSM (10 ng/mL) to the culture to obtain hepatocytes-like cells. During this period, the culture media were changed twice weekly.

2.4. Characterization of hepatocytes-like cells

Morphological changes in the cells during the differentiation process were used to identify the hepatocyte-like cells. Also the hepatocyte were characterized by using different liver-specific markers. The liver cells were routinely checked for their biochemical markers such as albumin, AFP, cytokeratins (CK18, CK19), and cytochrome P450 type 3A4 (CYP3A4) as described in our previous publications (Allameh et al., 2018; Ghaderi et al., 2011; Kazemnejad et al., 2009). Briefly, CK18, CK19 and CYP3A4 expression in the differentiated hepatocytes (day14 of differentiation) and their progenitor MSCs were assayed using reverse transcription polymerase chain reaction (RT-PCR). Expression of albumin and AFP in the cells was detected by RT-PCR and ICC techniques.

2.5. Supplementation of culture medium with NAC

MSCs in passage 3 were transferred to 24-well culture plates, the medium was removed and cells were washed with PBS prior to NAC treatment. The cells were then treated with NAC (0.1 or 1.0mM) after dissolving in DMEM.

During the differentiation process, the culture medium was changed every 3 days, and the cells obtained after 2, 7 and 14 days of differentiation along with the untreated cells (no NAC), were collected and processed for the assays. All the assays were carried out in triplicate using samples obtained from 3 wells in culture.

2.6. Cell viability and cell proliferation assays

Cell viability was checked using the trypan blue exclusion assay. The proliferation rate was assessed following the cell incubation with 10 μM 5-bromo-2′-deoxyuridine (BrdU) for 2 h at room temperature. The cells were then fixed and made permeable with FixDenat solution for 30 min before incubation with anti-BrdU peroxidase-conjugated antibody for 90 min. The peroxidase activity was determined by using tetramethylbenzidine as chromogenic substrate. Finally, the reaction was stopped by adding sulfuric acid followed by reading the absorbance at a wavelength of 450 nm using a spectrophotometer.

2.7. Total glutathione assay

The cells were collected at time points indicated and the cell lysate was prepared. Total glutathione in undifferentiated MSCs as well as the hepatocyte-like cells (NAC-treated and untreated) was determined using a commercially available kit (Alexis Biochemicals, San Diego, CA, USA).

2.8. Measurement of cytokines

In this study, selected cytokines were determined during the hepatic differentiation of MSCs exposed for 2, 7 and 14 days to NAC. The data were compared with the cells differentiated under similar condition without NAC treatment. Quantitative assay of TNF-α, IL- 6, IL- 8 and IL-10 was performed by enzyme-linked immunosorbent assay (ELISA) using commercially available kits on supernatants collected after 2, 7 and 14

days of culture. TNF-α kit was from the e-Bioscience, San Diego,California,USA, IL-6 and IL-10 kits were the products of Diaclon, Besancon Cedex, France. IL-8 ELISA kit was from the Assay Design, USA.

2.9. Statistical analysis

Statistical analysis of the data was performed using the SPSS software. The significant difference between control and treated groups was analyzed by Student's t-test. A P- value of less than 0.05 is considered statistically significant. Biochemical data and BrdU results are expressed as mean $\pm$ SD.

3. Results

3.1. Characterization of human bone marrow MSCs

Isolated MSCs were characterized routinely by their spindle-shape morphology as well as immunophenotyping assays using flow cytometry. The cells were positive for CD44 (H-CAM), CD105 (Endoglin) and CD166 (ALCAM) (Figure 1A), but they were negative for CD34 and CD45 (Figure 1B).

Furthermore, the differentiation potential of the MSCs was confirmed by showing the capacity of the cells to differentiate into adipocytes (Figure 2B) and osteoblasts (Figure 2D) following the staining of cells with oil red O and alizarin red, respectively. The unstained non-differentiated MSCs used as control were negative for both staining (Figure 2C and Figure 2E).
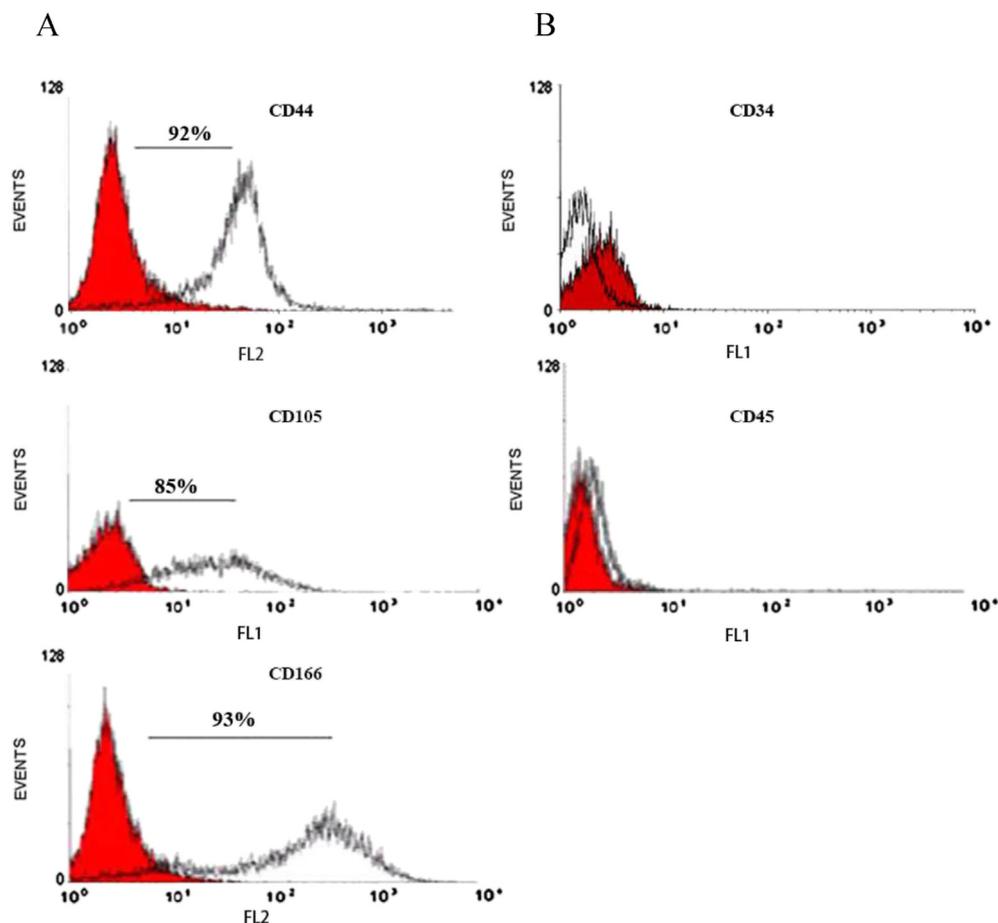
Figure 1. Characterization of human bone marrow MSCs using flow cytometry. (A) Histograms illustrate the immunophenotypes of MSCs isolated from human bone marrow. The MSCs were positive for CD44, CD105, and CD166 markers. The shaded area shows the profile of negative control. (B) The cells were negative for CD34, and CD45 markers. The shaded area shows the profile of the negative control.
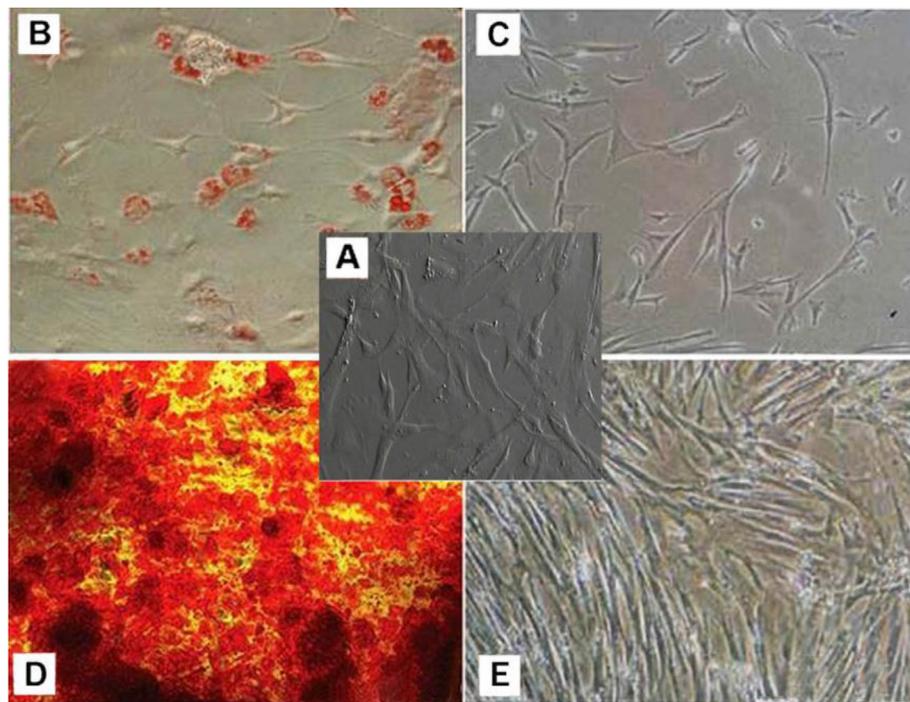
Figure 2. Multi-differentiation capacity of MSCs isolated from human bone marrow. (A) Homogeneous population of spindle-shaped MSCs. (B) Adipogenic cells differentiation identified by oil-red O staining. (C) Negative control for adipogenic cells (D) Osteogenic cells differentiated identified by alizarin red staining. (E) Negative control for osteoblasts.

3.2. Differentiation of MSCs into hepatocyte-like cells

Morphological characteristics as well as immunocytochemistry (ICC) staining for albumin and AFP were used to confirm hepatogenic differentiation of the stem cells as described in our previous publication (Kazemnejad et al., 2009).

The positive staining for albumin and AFP determined by ICC showed that these proteins appeared on day 7 with a steady increase in the expression levels of both proteins until day 14 following differentiation induction. Undifferentiated MSCs were negative for both the markers. These findings were further corroborated by RT- PCR analysis. Furthermore, the RT-PCR data showed the expression of CK18, CK19 and CYP3A4 in the cells at day 7 after hepatogenic induction that increased with progression in differentiation (day 14 after hepatogenic induction) as shown in Figure 3. The CK18, CK19 and CYP3A4 expressions was negative for the undifferentiated cells.

3.3. Effects of NAC supplementation on cell proliferation

The growth curve presented in Figure 4A, shows that supplementation of the culture media with NAC stimulates cell proliferation rate beginning from undifferentiated MSCs (BD) into hepatocyte-like cells. The cell proliferation rate monitored at different time intervals (2, 7 and 14 days) during hepatic differentiation was coordinated with the increased number of cells (2–5 folds) when compared to untreated MSCs.

3.4. Effect of NAC on total glutathione during hepatic differentiation of MSCs

As shown in Figure 4B, there is a surge in cellular total glutathione in hepatocyte-like cells differentiated from MSCs in presence of NAC (0.1 and 1 mM). Total glutathione was gradually increased during differentiation and reached to approximately 3 folds in the cells recovered two days after differentiation onset. Total glutathione was further elevated to about 4 and 5 folds in hepatocytes collected after 7 and 14 days of differentiation in presence of NAC.
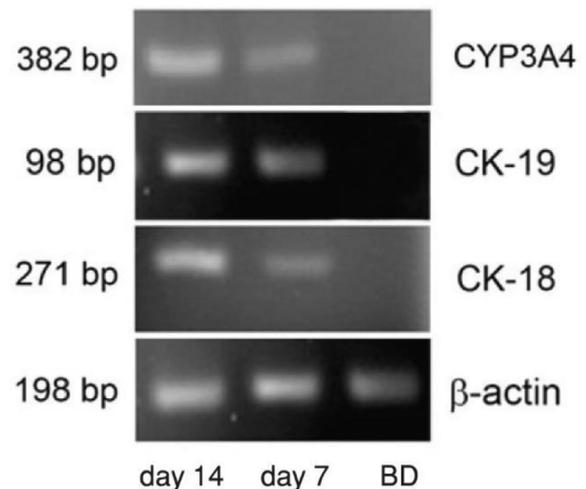
Figure 3. Liver-specific genes expressed by hepatocyte-like cells during differentiated from the MSCs. The expression of liver markers was estimated with reverse transcription PCR (RT-PCR). The cytokeratin-18 (CK-18), cytokeratin-19 (CK-19) and cytochrome P450 3A4 (CYP3A4) expression at mRNA levels were determined in cells on days 7 and 14 of differentiation and compared with non-differentiated cells (BD). Expression of β-actin gene was simultaneously determined as internal control. Please see supplementary material for un-cropped images.

3.5. Changes in pro-inflammatory and anti-inflammatory factors in presence of NAC during hepatic differentiation

The data on anti-inflammatory parameter measured during hepatogenic differentiation are as shown in Figure 5 (A,B and C). Section A, shows that there was a significant increase in TNF-α concentration in hepatocytes during the differentiation process. This increase was approximately 2 folds ($P < 0.05$) in the cells recovered after 14 days of
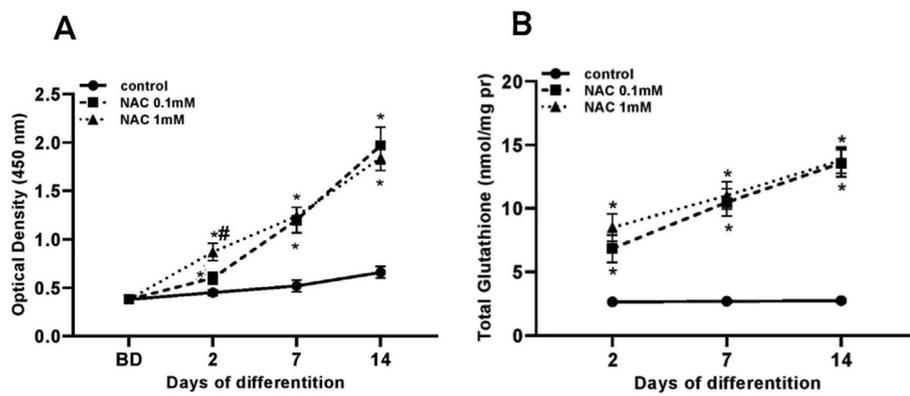
Figure 4. Effects of N-acetylcystein (NAC) on cell proliferation rate and total glutathione levels in MSCs undergoing hepatic differentiation. The MSCs culture was supplemented with NAC (0.1 or 1.0 mM), and cells were examined on day 2, 7 and 14. The cell proliferation rate was estimated by BrdU incorporation assay (section A). Section B shows the changes in total glutathione levels in cells which was measured by ELISA kit. The results are mean ± SD of 3 independent experiments, *, $P < 0.05$ compared with the group treated with lower concentration of NAC (0.1 mM) at the same day. #, $P < 0.01$ compared with the control group at the same day. BD: before differentiation, BrdU: 5-bromo-2′-deoxyuridine, MSCs: mesenchymal stem cells, NAC: N-acetyl cysteine.
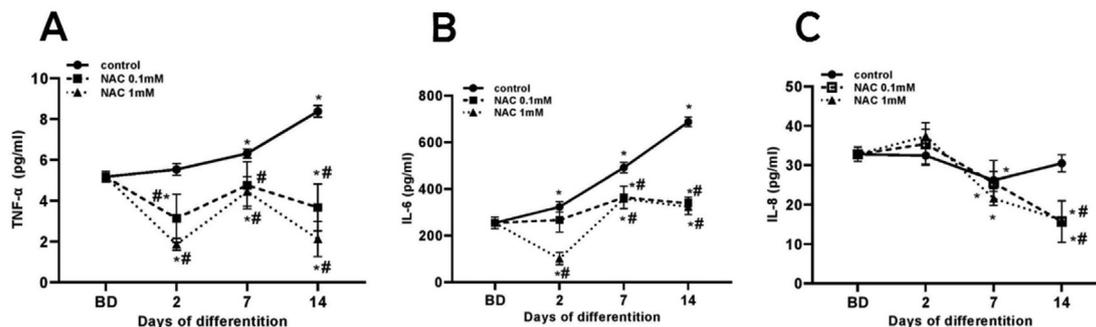
Figure 5. Effect of N-acetyl cysteine treatments on selected pro-inflammatory factors in mesenchymal stem cells (MSCs) undergoing hepatic in treatment with NAC. The MSCs were cultured and incubated with or without NAC (0.1 or 1.0 mM) for 2, 7 and 14 days. Section A, shows the time-dependent effects of NAC on TNF-α levels. Section B, shows the effects of NAC treatments of IL-6 and the results of IL-8 levels in NAC-treated cells are presented in section C. ELISA was used to determine these parameters in the cell-supernatant. The results are mean ± SD of 3 independent experiments, *, $P < 0.05$ compared with control group (non-differentiated). #, $P < 0.05$ compared with control at the same day. BD: before differentiation, ELISA: enzyme-linked immunosorbent assay, MSCs: mesenchymal stem cells, NAC: N-acetyl cysteine.

differentiation as compared to undifferentiated cells. However, the levels of cellular TNF-α were depleted in the cells differentiated in presence of NAC. The pattern of changes in TNF-α was similar in the cells treated either with low or high concentration of NAC (0.1, 1.0 mM), but high NAC levels resulted in more TNF-α depletion. In case of the cells treated with high concentration of NAC, there was a significant decrease in TNF-α on day 2 which was recovered on day 7. The cells collected after 14 days of differentiation experienced a further decrease (up to 75%) in TNF-α level as compared to the cells collected at the same time in absence of NAC (Control).

The changes in IL-6 during differentiation is as demonstrated in Figure 5B. IL-6 shows an increasing pattern in the cells collected after 2, 7 and 14 days of hepatic differentiation in absence of NAC treatments. Under this condition, there was about 3-fold ($P < 0.05$) increase in IL-6 levels in cells recovered after 14 days as compared to the undifferentiated cells. However, NAC treatments at lower concentration (0.1 mM) resulted in a significant decrease in cellular IL-6 in hepatocyte-like cells after 7 and 14 days. Likewise, IL-6 was decreased in cells treated with a higher NAC levle (1.0 mM). A sharp decrease in cellular IL-6 was observed in the cells treated with higher NAC levels after 2 days, which was partially recovered in cells after 7 and 14 days of differentiation.

The data showing changes in IL-8 during hepatic differentiation of MSCs are demonstrated in Figure 5C. The IL-8 level was significantly decreased in cells pre-treated with NAC for 7 and 14 days during the differentiation as compared to undifferentiated cells (Figure 5). A decrease in IL-8 in cells treated with NAC (1.0 mM) for 7 days was more obvious in the cells collected on day 7 of differentiation.

The results of IL-10 as a pro-inflammatory factor is presented in Figure 6. The IL-10 showed an increasing pattern in cells treated with NAC (0.1 and 1.0 mM). The increasing pattern of IL-10 was observed in cells during the 3-weeks hepatic differentiation.
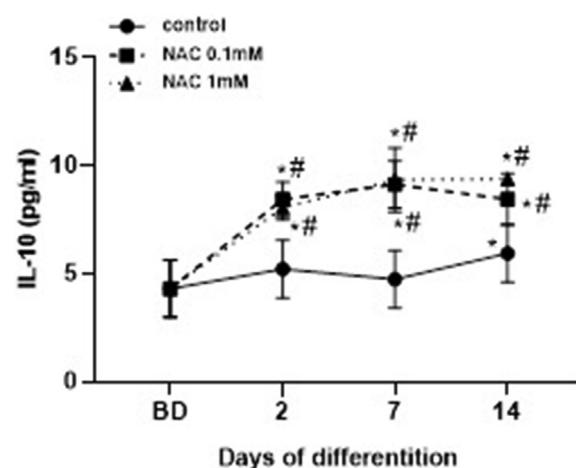
Figure 6. Effects of N-acetylcystein treatments on cellular interleukin-10 changes in mesenchymal stem cells during differentiation into hepatocyte-like cells. MSCs were cultured and incubated with or without NAC (0.1 or 1.0 mM) for 2, 7 and 14 days. IL-10 was assayed in cell-supernatants by ELISA technique. The results represent mean ± SD of 3 independent experiments, *, $P < 0.05$ compared with day 0 (BD). #, $P < 0.05$ compared with control at the same day. BD: before differentiation, ELISA: enzyme-linked immunosorbent assay, MSCs: mesenchymal stem cells, NAC: N-acetylcysteine.

4. Discussion

Using hepatocytes differentiated from human MSCs is a feasible alternative to human adult hepatocytes transplantation (Christ and Stock, 2012). However, it is possible that the immune and inflammatory signatures of transplantable cells can affect the rate and severity of immune rejection. Therefore, priming the hepatic cells differentiated from stem cells with immunomodulatory and anti-inflammatory factors is believed to enhance the therapeutic potential of these cells (Irudayam et al., 2015). The evidences presented in this study showed that during the *in vitro* differentiation, the inflammatory signature of the stem cells can pass onto the hepatic cells. Evidences show that keeping the glutathione at high levels in cells vulnerable to oxidative damage can help in regulation of differentiation and regulation of inflammatory responses assumed to support overall differentiation process.

There are different approaches to improve the stemness of MSCs and their differentiation potential (Zeilinger et al., 2016). Despite the recent advancements in the cell culture techniques for producing transplantable hepatocytes from stem cells, the viability and the functionality of the of the hepatocyte after transplantation is still a challenging issue.

NAC is a well-known glutathione inducer which can keep the cellular glutathione at high levels, which indirectly protects the cells against oxidative damage factors, thereby supporting the cellular differentiation. The first evidence to show the action of NAC was observed by showing the NAC effects on the rate of stem cell proliferation (BrdU assay), and hepatic differentiation of MSCs (Figure 4).

The data presented also showed that NAC-related glutathione synthesis affected stem cell differentiation potential into hepatocyte-like cells. NAC could also improve anti-inflammatory status of the cells. Furthermore, the action of NAC-related glutathione on inflammatory reactions may add to the the immunomodulatory properties of progenitor MSCs which are basically considered as the guardians of inflammation (Prockop and Oh, 2012). However, the underlying mechanism of MSCs immunomodulation is still not fully elucidated. The immunomodulatory properties of MSCs is assigned to different factors such as their ability to suppress B- and T-cell proliferation, regulation of regulatory T-cells, inhibition of NK -directed cytotoxicity, and dendritic cell maturation and function through producing various chemokines and cytokines as well as cytokine receptors (Gao et al., 2016; Prockop and Oh, 2012; Spaggiari et al., 2009).

During the *in vitro* differentiation process of MSCs into functional hepatocytes, growth factors and cytokines produced are part of the regulatory system for controlling the inflammatory responses, modulation of apoptosis and enhancing metabolic and biochemical activities of the cells (Liu et al., 2015). Balancing the cellular glutathione system could be considered as an efficient and feasible approach to improve the antioxidant and anti-inflammatory potential of the stem cells. Therefore, the stem cells with higher glutathione levels are benefited from higher antioxidant status (Allameh et al., 2018). Under these circumstances, NAC-dependent increase in cellular glutathione could alter inflammatory reactions in MSCs induced for hepatic differentiation. It has been described that reduced glutathione (GSH) is essential for optimal activity of cells involved in immune responses, as well as cytokine production, and therefore for mounting successful immune responses. In a study on cirrhosis patients, *Pena et al.* showed that a dose of 70 mg/kg of a GSH prodrug for 3 times a day for 9 days, resulted in an increase in blood GSH content and a decrease in TNF-α, IL-8 and IL-6 production Pena et al., (1999). In line with this, in a recent double-blind clinical trial in a group of HIV-positive subjects with low CD4+ T cell counts, 3 months of GSH supplementation restored levels of intracellular GSH and balanced the production of cytokines by increasing IL-12, IL-2, and IFN-γ concentrations and decreasing the levels of IL-6 and IL-10 (Valdivia et al., 2017). However, there are limited number of studies investigating the effects of GSH on the cytokine production of stem cells. We have previously reported that GSH depletion by L-buthionine sulfoximine in MSCs during hepatic differentiation was associated with increased proinflammatory

factors such as TNF-alpha and IL-6 (Ahmadi-Ashtiani et al., 2012). In the current study, the role of glutathione in cytokine production during hepatic differentiation was further established by showing that, NAC-dependent increase in glutathione synthesis in MSCs undergoing hepatic differentiation resulted in decrease in proinflammatory factors. A time-dependent increase in cellular glutathione by NAC was associated with improvment in the cytokine balance in hepatocyte-like cells together with improvment in the process of hepatic differentiation of MSCs.

The data presented in this paper showed that a higher glutathione levels in NAC-treated stem cells was associated with a significant decrease in pro-inflammatory cytokines including TNF-α, IL-6 and IL-8. Moreover, these changes are likely to pass onto the hepatocytes derived from them. A major reduction in these factors which occurred 14 days after differentiation onset in presence of NAC could be crucial in protection of the cells destined for transplantation. Depletion in the pro-inflammatory cytokines may be linked to the reduction in ROS production in cells with elevated glutathione (Allameh et al., 2018). In line with our results, Haddad et al. reported that the glutathione depletion is associated with augmentation of an oxidative stress-related pro-inflammatory state through ROS-dependent mechanism (Haddad, 2000).

The protective role of NAC was further demonstrated when the increased glutathione level during hepatic differentiation of MSCs was associated with induction of IL-10 production. Perhaps IL-10 is considered as important anti-inflammatory cytokine which plays a pivotal role in the induction and maintenance of an anergic state, and the limitation of the host immune response to foreign antigens (Akdis and Blaser, 2001; Mosser and Zhang, 2008).

Overall, the experimental evidences presented in this paper suggest that cellular glutathione helps in restoring the inflammatory signs and signals which can improve the cytokine balance in liver cells differentiated from the MSCs.

5. Conclusion

The findings of the present study indicated that besides the positive role of glutathione on the rate of stem cell proliferation, there was a noticeable improvement in anti-inflammatory status of the cells which was passed on to the hepatocyte- like cells differentiated from them. The improvement in the anti- inflammatory strength of the transplantable cells can be useful in decreasing the rate and severity of immune rejection and decrease the need for immune suppression.

Declarations

Author contribution statement

Abdolamir Allameh: Conceived and designed the experiments; Wrote the paper.

Hamid Reza Ahmadi-Ashtiani: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Narges Maleki: Performed the experiments; Analyzed and interpreted the data.

Competing interest statement

The authors declare no conflict of interest.

Additional information

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