Immunoregulatory Effects of Glutathione During Mesenchymal Stem Cell Differentiation to Hepatocyte-Like Cells

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ABSTRACT

Background: The role of mesenchymal stem cell in cellular therapy is the subject of interest for many researchers. The differentiation potential of MSCs and abilities in modulations of the recipient's immune system makes them important cells in tissue regenerative studies. MSCs by releasing the proinflammatory cytokines play important role in immunomodulatory systems; however the signaling pathways for releasing of these mediators are not well understood. Glutathione has been shown to play a role in modulation of cytokines in hepatogenic differentiation. **Objective:** In the current study we aimed to investigate the effects of buthionine sulfoximine (BSO, inhibitor for glutathione synthesis) and N-acetylecystin (NAC, an inhibitor for ROS generation) on proinflammatory cytokines production in a hepatogenic differentiation model. **Results:** BSO and NAC significantly decreased IL-6 and TNF-α levels at 14 days of differentiation, whereas, NAC decreased the levels of IL-8 at days 2 and 14 of differentiation. Moreover, intracellular glutathione level during the differentiation was depleted. **Conclusion:** Our current study suggests a novel role of GSH as an immunopharmacological regulatory molecule during hepatogenic differentiation. Finally, this information may shed some light on the understanding of MSCs responses in transplantation and cell therapy in diseases such as chronic hepatic diseases.

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INTRODUCTION

The role of glutathione in the regulation of cytokines releases of stem cells during the differentiation is not well described. Thus, understanding the mechanisms of

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cytokine releases of activated-mesenchymal stem cells mediated immunosuppresion during differentiation will be considered as tools for cellular therapy. MSCs are known for ability to self-renew, undergo clonal expansion, and differentiate into the multiple musculoskeletal tissues and posses the potential to regulate the immune system (1-6). It has been shown that isolated MSCs from the bone marrow have potential to differentiate to the multiple connective tissues such as bone, cartilage, liver, muscle and adipose tissues (1-2). In this line, new observations demonstrated that MSCs may have immunosuppressive capacity and considered as a unique candidate for regenerative studies (3). Immunosuppressive function of MSCs has accounts by high capacity to release IFN and proinflammatory cytokines (7). Basically, with high capacity of differentiation, MSCs considered as the ideal cells in the therapeutic approaches. The differentiation of MSCs is regulated by various signals from microenvironment, which is consisting of many biological molecules and biomechanical forces. These factors play a vital role in determining the efficacy of MSCs biology (6). The cytokines play important role in regulation of MSCs differentiation (5,8-10). Moreover, it has been shown that reducing GSH regulates the production of cytokines (11,12) Besides, evidence indicated that differentiation is under control of ROS generation (13).

ROS is considered as potent oxidants which are produced firstly by the mitochondria in cells as a by-product of normal metabolism during conversion of molecular oxygen (O_2) to water (H_2O) (14). One of the antioxidant defenses in cells as endogenous is glutathione (15). GSH is an important low molecular weight thiol in cells and plays a central role in controlling cellular thiol/disulfide redox state, which is essential for normal redox signaling in normal status (16).

It has been reported that L-buthionine-(S, R)-sulfoximine (BSO, a glutathione depleting agent) is able to suppress osteoclast differentiation by depleting glutathione. In contrst N-acetylcysteine, thiol compounds (which deacetylates to increase GSH level), or GSH, can increase the osteoclast differentiation (17). In this connection, it has been reported that in glial precursor cells, BSO increases differentiation with low ratio cell proliferation, whereas NAC as a glutathione precursor induces cell proliferation and differentiation (18). Cytokines are redundant secreted proteins with growth, differentiation, and activation functions that regulate the nature of immune responses. Cytokine may have a completely different function depending on the cellular source, target and playing important role in the immune response. Some cytokines have proinflammatory potential such as interleukin-1β, IL-6, IL-8, and TNF-α and others have anti-inflammatory effects such as IL-10 (19). Oxidative stress has been shown that activate NF-κB, for upregulating of inflammatory cytokines in pancreatic acinarious cells (20). Moreover, generation of ROS in endothelial cells increases proinflammatory cytokine releases (7).

It is now clear that BSO induces generation of intracellular ROS and the release of IL-1β, IL-6 and TNF-α, whereas GSH precursor; NAC, abrogated cytokine releases by suppression of ROS generation in the alveolar epithelium (11). In this line, Redondo et al. has shown that NAC inhibits production of TNF-α and IL-1β (12).

Thus in this study possible involvement of proinflammatory cytokines in the hepatogenic differentiation of human MSCs has been investigated. For this purpose, MSCs were subjected to hepatogenic differentiation process in presence of glutathione modifiers and the changes in the release of proinflammatory cytokines (IL-6, IL-8, TNF-α) during a two-week differentiation was studied.

MATERIALS AND METHODS

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 obtained from Gibco BioCult (Paisley, UK). L-buthionine-SR-sulfoximine (BSO), L-NAC, Dexamethasone (DEX), growth factor (HGF), Oncostatin M (OSM), mouse anti human antibodies for albumin, α-fetoprotein, rabbit anti mouse FITC-conjugated immunoglobulin G (IgG) and oil red O-staining kit were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Antibodies for flow cytometric assay wereobtained from Dako (Glostrup, Denmark) and Oxford Biomedical Research (Oxford, UK). RNA extraction kit, cDNA Synthesis Kit and materials for PCR amplification were purchased from Qiagen (Valencia, CA, USA) and Fermentas (Hanover, MD, USA). Glutathione quantification kit was obtained from Alexis Biochemicals (BV-K264, San Diego, CA, USA). Human TNF-α, IL-6 and IL-8 ELISA kits were purchased from Quantikine (R&D Systems Inc, Minneapolis, USA).

MSCs Isolation and Culture. Bone marrow aspirates (10 ml) were obtained from iliac crests of human donors (three samples from three individuals were used in this study) at the Bone Marrow Transplantation Center, Shariati Hospital, and Tehran, Iran. The samples were collected after informed consent was taken from the patients according to guidelines of the Medical Ethics Committee. MSCs were isolated from the bone marrow as described in our previous publication (21). Briefly, the aspirates were diluted with phosphate buffer saline (PBS). Cell solution was gently overlaid on the Ficoll-Hypaque (1.077 g/ml) 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 at room temperature. The isolated mononuclear cell layer was then washed in PBS, re-suspended in growth medium containing DMEM-low glucose supplemented with 15% FBS, 2 mM glutamine, 100 mg/ml streptomycin, 100U/ml penicillin and plated in polystyrene plastic 75cm^2 tissue culture flasks. The cell cultures were maintained at 37°C in a humidified 5% CO_2 incubator. After 3-4 days of incubation, the non-adherent cells were washed away leaving behind the adherent cell population that was growing as fibroblastic cells in clusters. Hematopoietic stem cells and non-adherent cells were removed with every 3-4 days changes in medium. When cells reached 70-90% confluence, the cells were harvested with 0.25% trypsin-EDTA solution and plated in 25-cm plastic cell culture flasks at a density of 10^4 cells.

Cell Viability and Proliferation. Trypan blue exclusion assay was used to MSCs in third passage (1×10^4 cells/cm^2). The Brdu assay was used to estimate the cell proliferation rate in control and treated cells. The cells were labeled with 10 μM Brdu for 2 h at RT and fixed and made permeable with the FixDenat solution for 30 min, prior to incubation with the anti-Brdu peroxidase-conjugated antibody for 90 min. The peroxidase activity was measured using tetramethyl benzidine (TMB) as substrate. Finally the reaction was stopped by adding H_2SO_4 and absorbance was recorded at 450 nm.

Osteogenic and Adipogenic Differentiation of MSCs. For osteogenic differentiation, the MSCs were induced for two weeks differentiation in α-MEM supplemented with 10% FBS, 0.1mM DEX, 10mM β-glycerophosphate, and 50 mM ascorbate-phosphate. The culture medium was changed twice weekly for two weeks. The cells were fixed with methanol for 10 min at RT and identified by specific isocheimal staining for calcium with alizarin red staining kit and visualizing with a phase contrast microscope (Nikon, Tokyo, Japan). In case of adipogic differentiation, the MSCs were incubated in

DMEM supplemented with 10% FBS, 1 mM dexamethasone, 200 mM indomethacin, 1.7 mM insulin, 500 mM isobutyl-methylxanthine, 0.05U/ml penicillin and 0.05 mg/ml streptomycin for two weeks. After 14 days, cultured cells were detected for adipocyte identification, using oil red O-staining. Briefly, the cells were fixed in 10% solution of formaldehyde in aqueous phosphate buffer for 1h, washed with 60% isopropanol, and stained with oil red O-solution for 10 min, followed by repeated washings with distilled water before being destained in 100% isopropanol for 15 min. Control cells without the differentiation stimuli were stained in the same manners.

Hepatic Differentiation Protocol. MSCs at passage three reached to confluency were seeded on 24-well plastic cell culture plates. Hepatic differentiation was performed using a 2-step protocol employing HGF, DEX and OSM based on the previous reports (21). Briefly, in the first step which lasted for seven days, the cells were cultured in a culture medium consisting of DMEM-low glucose supplemented with 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 and hepatocytes-like cells were obtained after two weeks of differentiation. During this period, the culture media was changed twice weekly.

Characterization of Hepatocytes-like Cells Using Liver Specific Markers. Differentiation of the cells was confirmed by showing the differentiation potential of the progenitor cells to adipocyte and oesteogenic cells. Also the hepatocyte-like cells were characterized *by* detection of different liver-specific markers. Expression of albumin, alpha fetoprotein (AFP), cytokeratins (8,18,19), tryptophan 2, 3-dioxygenase and cytochrome 3A4 were considered markers of hepatocyte-like cell differentiation as described in our previous publications (21,22).

Treatment of MSCs with NAC and L-BSO. MSCs at third passage were treated with BSO (1 and 5 μM) or NAC (0.1 and 1 mM) for 7-17 days. Then the cells were recovered at different time intervals (2, 7 and 14 days after differentiation) and processed for the assays as described below.

Cytokines Assays. Culture supernatants were collected and analyzed by Quantikine human TNF-α, IL-6 and IL-8 releases by Immunoassay kits (R&D Systems Inc, Minneapolis, USA, Cat# DTA00C, D6050 and D8000C). The assays were performed according the kit instructions.

Determination of GSH in Differentiating MSCs. The levels of GSH, GSSG and total glutathione were measured in MSCs using Glutathione Quantification kit (BV-K264, Alexis Biochemicals, San Diego, CA, USA). The assay was carried out according to the instructions provided by the manufacturer. Briefly, the glutathione levels were measured in cells treated for 2, 7 and 14 days with either BSO (1 and 5 μM) or NAC (0.1 and 1 mM) during differentiation. The biochemical assays were carried out in a set of cell samples before any treatment and considered as control.

Statistical Analysis. The valued indicated were obtained from at least 3 samples carried out in duplicate. The significant levels for differences were considered at $p<0.05$ calculated by Student *t*-test. The cell proliferation (Brdu assay) data are presented and mean ± SD. The results of cell counts and cytokines and GSH levels are presented as mean ± SEM percent of cytokine/GSH level in undifferentiated cells.

RESULTS

Differentiation of MSCs to Osteoblasts and Adipocytes. The differentiation potential of human bone marrow MSCs to osteoblasts and adipocytes was approved by showing the specific staining using alizarin red and oil red O-staining on day 14 of differentiation. As shown in Figure 1, alizarin red staining and oil red o-staining indicate the presence of calcium deposits, characteristic of osteogenic cells and lipid droplets in the differentiated cells, respectively, whereas undifferentiated MSCs were negative in both staining.

Differentiation Status		Parameters				
		Urea (mg/dl)	SGPT (IU/l)	SGOT (IU/l)	Transferrin (mg/ml)	Albumin (µg/ml)
Before Differentiation		0.07±0.01	0.03±0.009	0.04±0.008	0.003±0.001	0±0
After Differentiation (Day 14)	Control	12.5±1.9**	5.3±0.8**	6.7±0.7**	3.1±0.5**	5.8±0.8**
	BSO (1µM)	12.8±1.2**	6.5±0.5**	7.5±0.8***	3.8±0.5***	7.3±1.2**
	BSO (5µM)	13.4±1.6**	6.8±0.7***	8.3±0.9***	3.9±0.5***	7.2±1***
	NAC (0.1mM)	10.5±1.5***	5±0.7**	6±0.6**	2.8±0.4***	4.4±0.8***
	NAC (1mM)	10±1.7***	4.5±0.6**	6.3±0.6***	2.7±0.4**	4±0.2***

Figure 1. Transdifferentiation of human bone marrow derived MSCs into adipocytes and osteoblasts.Cells differentiated into osteogenic and adipogenic lineages were positive for Alizarin red staining (A) and oil red O-staining (B). Undifferentiated MSCs as control were negative for both staining. C= alizarin red staining and D= oil red O-staining.

Furthermore the hepatocytes-like cells recovered on day 7 and 14 of differentiation expressed liver specific markers were routinely measured in our laboratory. The accumulation of albumin and AFP expression in cells during hepatogenic differentiation was demonstrated by Immunocytochemistry (ICC) assay. In addition, expression of cytokeratin-18 (CK-18), CK-19 and cytochrome P450 (CYP3A4) in differentiating hepatocytes-like cells were observed using reverse transcription polymerase chain reaction (RT-PCR). The details about the characterization of hepatocytes-like cells have been presented in our previous publications (21,22).

Under normal condition the number of MSCs in a 25 cm^2 flask reached to about 20000 cells on day 14 days of differentiation (Figure 2A). BSO treatments resulted in a significant decrease in cell numbers estimated on day 2 of differentiation exhibited a 13-26% decrease in presence of 1.0 and 5.0 µM of BSO. The cell numbers was further

decreased to ~50% on day 7 of differentiation, which reached to minimum (~85% decrease) on day 14 of differentiation due to BSO (1.0 and 5.0 µM) as compared to controls (Figure 2B).

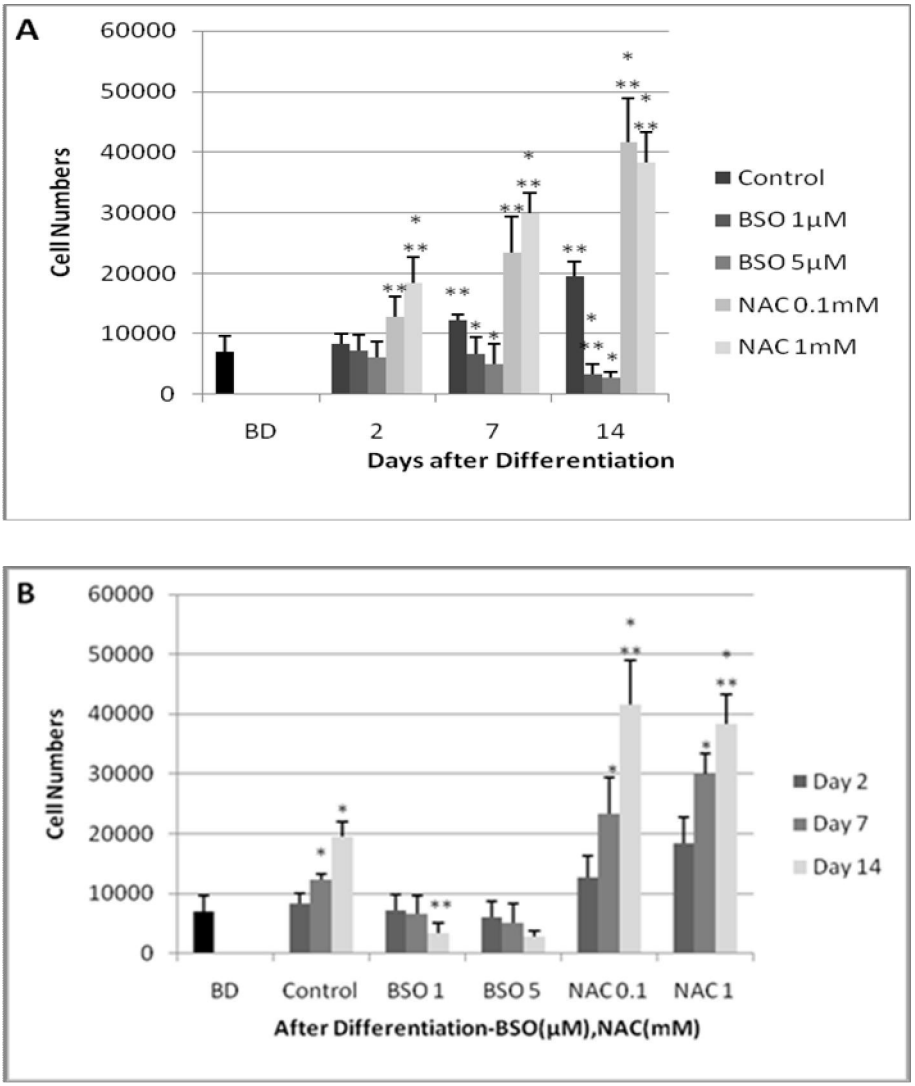

Figure 2. Effects of buthionine sulfoxide and N-acetylcysteine on cell counts during differentiation of human bone marrow mesenchymal stem cells into hepatocyte-like cells. A: Time-course changes in cell numbers during differentiation. The cell counts before differentiation (BD) and after differentiation (2, 7 and 14 days) was calculated in trypan blue staining method. B: The effects of BSO and NAC on the cell numbers during differentiation of MSCs. BSO (1.0 and 5.0 µM) and NAC (0.1 and 1.0 mM) dissolved in medium and added to the culture media. *Indicates significant difference between the untreated (control) and treated cells ($p<0.05$, n=4). ** shows statistically difference between the cells before and after differentiation induction.

Treatment of MSCs with NAC (0.1 and 1.0 mM) increased the cell numbers as compared to untreated cells (Figure 2A). Approximately 53 and 120% increase in the cell numbers was observed in cells treated with 0.1 and 1.0 mM of NAC respectively.

As shown in fig. 2B, NAC treatment resulted in further increase (90-145%) in cell counts on days 7 and 14 at ~114 and 97% respectively. The rate of MSC proliferation was declined when exposed to higher concentration of NAC (1.0 mM).

Effects of BSO and NAC on cell proliferation. The proliferation data obtained by Brdu assay showed that pre-treatment of MSCs with BSO (1.0 and 5.0 µM) resulted in a significant decrease in cell proliferation estimated at different time intervals when compared to control group (Figure 3A).

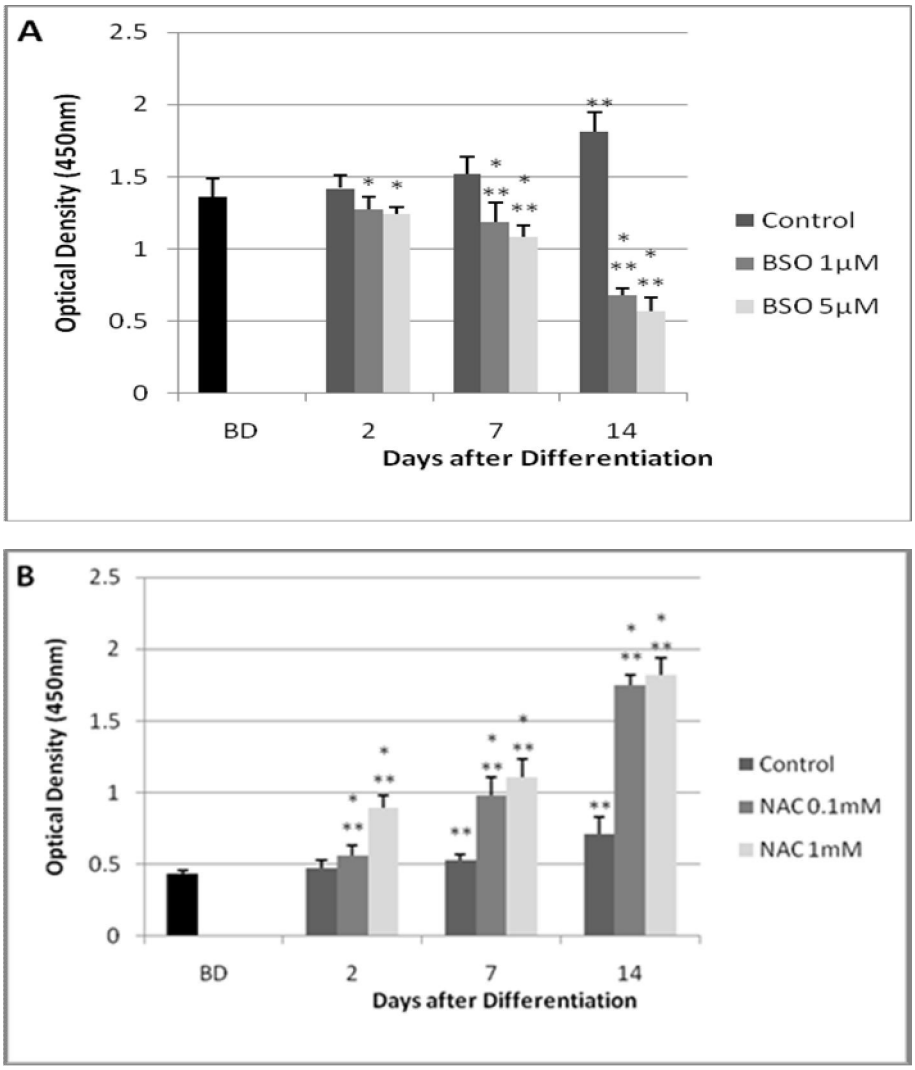

Figure 3. The rate of cell proliferation in human bone marrow mesenchymal stem cells during differentiation into hepatocyte-like cells. A: The rate of cell proliferation (Brdu assay) measured before differentiation (BD) and after differentiation (2, 7 and 14 days) in presence of BSO or NAC. **B**: The rate of cell proliferation measured in presence of BSO (1.0 and 5.0 µM) and NAC (0.1 and 1.0 mM) dissolved in medium and added to the culture media. The results are presented as mean±SD and * indicates significant difference between the treated and untreated (control) cells ($p<0.05$, n=4). **indicates significant difference between the cells before differentiation (BD) and their differentiated counterparts.

BSO caused about 10-13% decrease in cell numbers recovered on day 2 of differentiation compared to untreated cells. The cell numbers was decreased to 22-28% on day 7 of differentiation, which was further decreased (62-68%) in hepatocyte-like cells recovered after two weeks (Figure 3A). The number of MSCs on day 2 of differentiation was increased by 19 and 89% in cells treated with 0.1 and 1.0 mM of NAC respectively. There was further increase in cell numbers by 85-109% on day 7 after NAC treatments (Figure 3B). The number of NAC-treated cells was elevated by 146-156% after two weeks of differentiation induction.

The Effects of BSO and NAC on the Cytokine Releases During MSCs Differentiation. The effect of GSH modifiers on the cytokine release in cells during hepatogenic differentiation is as shown in fig. 4. In case of untreated cells (control), the level of IL-6 was decreased due to differentiation induction and it was reached to lowest levels on day 7 of differentiation. BSO (1 and 5 μM) resulted in a significant increase in IL-6 level as compared to the respective controls. There was a small decline in the IL-6 levels in cells recovered on day 14 of differentiation. Unlike BSO, NAC treatment to differentiating cells resulted in a significant decrease in the levels of cellular IL-6 when compared to corresponding controls (Figure 4).

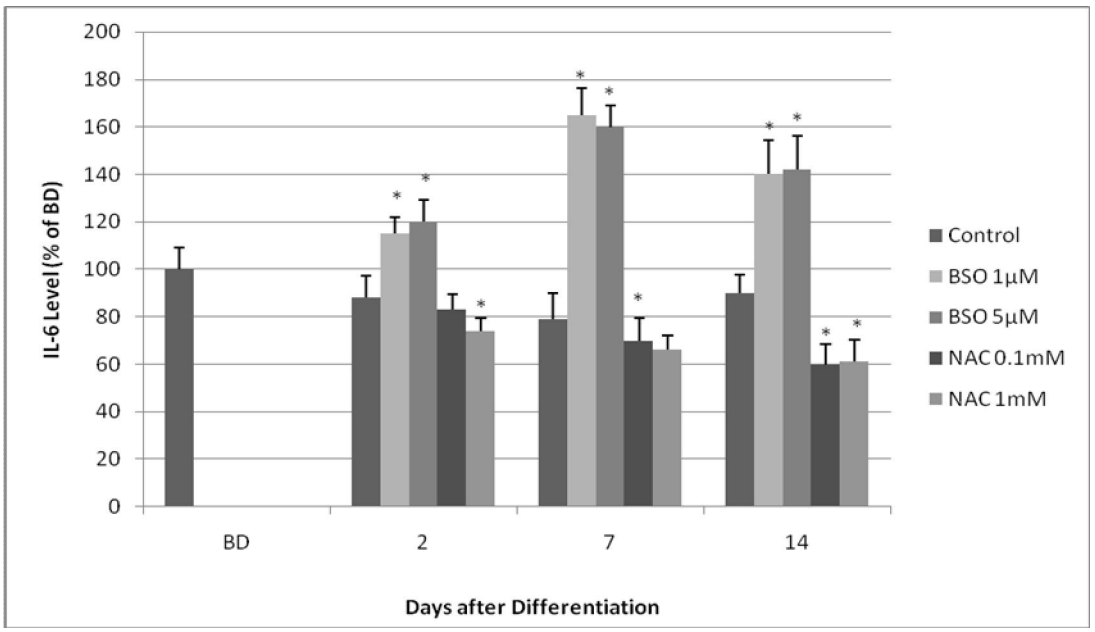

Figure 4. Effects of BSO and NAC on interleukin-6 level in MSCs before differentiation (BD) and after differentiation. MSCs were culture and incubated with BSO or NAC as described in 'material and methods' section for 14 days. The amounts of IL-6 were determined by supernatant of treated and untreated cells. The results are presented as mean± of IL- 6, of three experiments. *Indicates significant difference from the control (untreated) cells ($p<0.05$, $n=4$) and ** Indicates significant difference from the undifferentiated cells (BD) ($p<0.01$, $n=4$).

The results of IL-8 assays showed that there was a small but significant decrease in IL-8 in cells treated with BSO when compared to the cells collected before differentiation

(non-differentiated MSCs) (Figure 5). The IL-8 release from the cells treated with BSO was depending on the dose and the time of exposure of the cells to BSO.

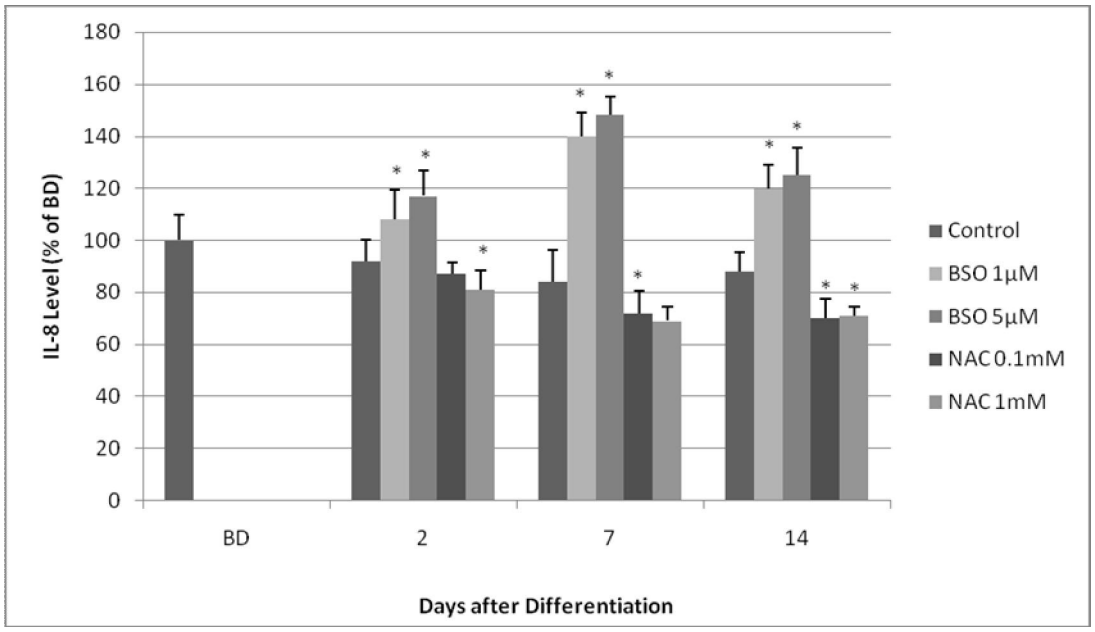

Figure 5. Effects of BSO and NAC on interleukin-8 during hepatogenic differentiation. Experimental procedures are as described under figure 4. *Indicates significant difference from the control (untreated) cells ($p<0.05$, n=4) and ** Indicates significant difference from the undifferentiated cells (BD) ($p<0.01$, n=4).

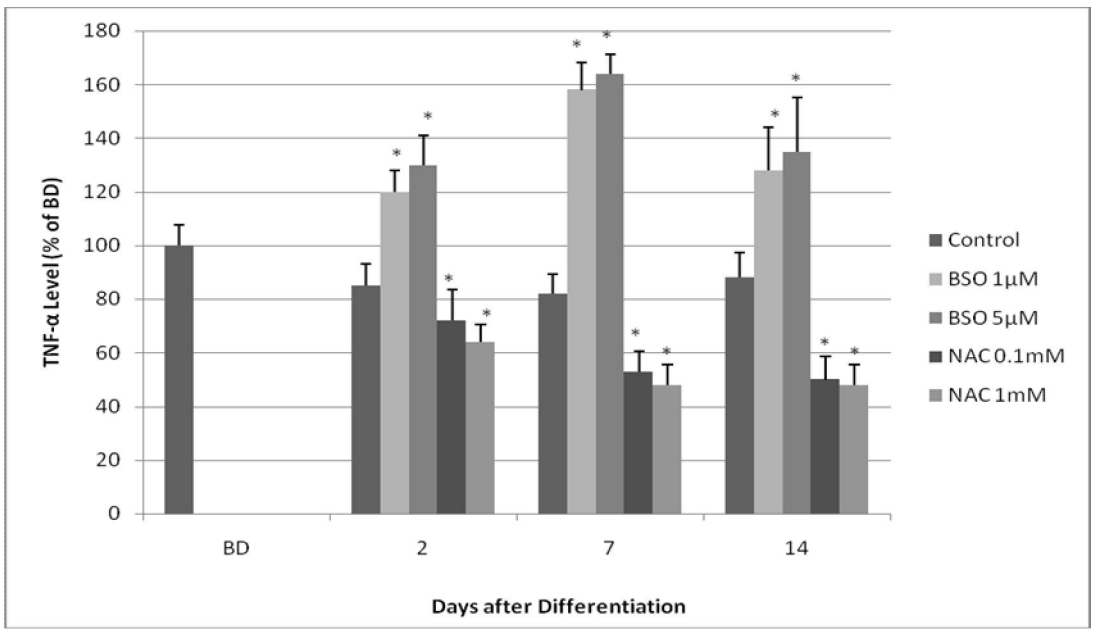

Figure 6. TNF-α change in MSCs and differentiated cells in presence of BSO. Experimental procedures are as described under figure 4. *Indicates significant difference from the control (untreated) cells ($p<0.05$, n=4) and **Indicates significant difference from the undifferentiated cells (BD) ($p<0.01$, n=4).

The results of TNF-α analysis during hepatogenic differentiation show that there was a significant decrease in TNF-α in cells before differentiation (non-differentiated MSCs). Whereas during differentiation, TNF-α was elevated significantly due to BSO treatments (Figure 6). These changes were more obvious in cells collected on day 7 of differentiation.

BSO treatments (1 and 5 μM) caused a significant increase in TNF-α in cells recovered on day 2 and 7 of differentiation. In contrast, treatment of cells with NAC (0.1 and 1 mM) suppressed the release of TNF-α depending on the time and dose of treatments.

The Effects of BSO and NAC on GSH Levels. As shown in Figure 7 there is a small but significant decrease in cellular GSH due to induction of differentiation. BSO treatment (1 μM) resulted in a significant depletion in GSH during differentiation process, and there was a gradual decline in GSH in cells collected on days 7 and 14 after differentiation. In contrast, NAC treatments (0.1 and 1 mM) resulted in a time and dose-dependent elevation of cellular GSH during differentiation period (Figure 7). The GSH level was induced to highest levels (>4 folds increase) due to NAC treatments in hepatocyte-like cells on day 14 of differentiation.

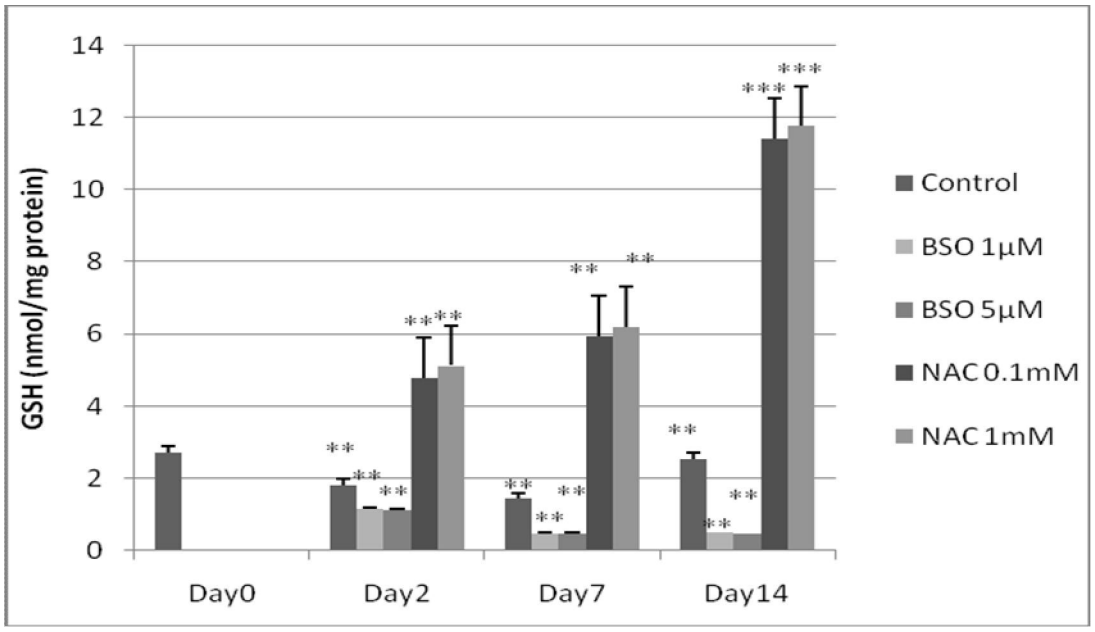

Figure 7. Effects of BSO and NAC on GSH level in MSCs before differentiation (BD) and after differentiation. MSCs were culture and incubated with BSO or NAC as described in 'material and methods' section. The amount of GSH was determined in supernatant of treated and untreated control cells. The results are presented as mean ± SD percent of GSH level in undifferentiated cells of three experiments. *Indicates significant difference from the control (untreated) cells ($p<0.05$). ** Indicates significant difference from the undifferentiated cells (BD).

DISCUSSION

Mesenchymal stem cells (MSCs) are currently under intense investigation to determine their role in cellular therapy. MSCs differentiate into mesenchymal and non-

mesenchymal cell lineages. The differentiation potential of the stem cells in conjunction with their ability to secrete various immunomodulatory factors which modulate the immune system makes them a promising cell source in regenerative medicine (6). It has been reported that that MSCs has anti-inflammatory and immunomodulatory properties and could be considered in cellular therapy for graft-versus-host disease (GVHD) and autoimmune diseases (23).

Evidences presented in the present study revealed that the levels of IL-6, Il-8 and TNF-α undergo changes during hepatogenic differentiation of human bone marrow MSCs. Moreover, these factors were regulated by glutathione (GSH) levels in cells during the differentiation. Induction of hepatogenic differentiation of MSCs was associated with a significant decrease in cellular TNF-α (Figure 6). The ratio of intracellular thiol reductants and reactive oxygen species (ROS) appears to play a major role in determining the cell growth or apoptosis. Imbalance of the redox status, either by high levels of oxidation or increase of reducing agents, favors decreased growth and increased apoptosis (24,25). Glutathione imbalances in the cell have been linked to specific changes in signal transduction downstream of growth factors or cytokines, ultimately leading to changes in the overall outcome of signaling. The effect of NAC on cell growth is probably due to its ability to reduce the ROS generation and related signaling pathways.

The relationship between cellular GSH and hepatogenic differentiation was demonstrated by assessing the biochemical and metabolic functions of liver specific markers such as albumin, transferrin, and urea as well as specific enzyme markers. The involvement of GSH in hepatogenic differentiation was further confirmed when differentiated cells were treated with GSH modifiers (NAC and BSO). GSH depletion in cells treated with BSO was associated with a significant increase in TNF-α suggesting an adverse relation between GSH and TNF-α. This was further confirmed by showing that increase in GSH biosynthesis in presence of NAC (GSH precursor) resulted in suppression of TNF-α, these findings indicated a regulatory function of GSH in hepatogenic differentiation and immune properties of MSCs.

The limitation of this study was the cytotoxicity effects of BSO which caused a significant decrease in the proliferation of MSCs in culture and the number of hepatocyte-like cells differentiated from them. Perhaps the cytotoxicity effects of BSO are often associated with cell death and production of inflammatory mediators such as TNF-α and related factors. Considering the cytotoxic effects of BSO, a less toxic concentration was chosen with GSH depleting potential to enable us performing biochemical assays.

The consequences of GSH depletion was shown in TNF-α, IL-6 and IL-8 levels which were more evidently elevated in cells collected after the first week of differentiation. It was assumed that elevation in IL-6 and IL-8 in cells treated with BSO is due to cell death, but the levels adjusted to the biologically active cells based on cell count results (Figure 2) show the role of GSH in immuno-inflammatory reactions during a two-week differentiation process.

The active role for GSH in redox regulation of different processes has been reviewed (26). Based on this, GSH depletion is believed to be a hallmark in the progression of cell death in a variety of apoptotic stimuli in different cell types (27,28).

Hepatogenic differentiation of MSCs performed in presence of NAC (precursor of GSH biosynthesis). The cytotoxic effects of NAC under the experimental conditions used, is ruled out. Hence, the relationship between GSH variation and interleukins and TNF-α

levels is considered as an important finding. Perhaps the stimulatory effects of NAC on the rate of the stem cell proliferation and differentiation to hepatocyte-like cells could be assigned to the GSH system.

It was found that induction of MSCs into the differentiated hepatocytes-like cells caused a transient decrease in GSH that was correlated with the inhibition of cytokines. Induction of GSH synthesis which elevated GSH levels during differentiation prevented the increase in interleukins. In contrast, addition of BSO that depleted GSH was associated with increase in interleukins and TNF-α during hepatogenic differentiation. Under this circumstances, the changes in cell counts and the rate of cell proliferation imply that glutathione play a major role in regulation of interleukins which are activators of cell growth and differentiation. The role of ROS which is linked to GSH redox system in cell proliferation and differentiation of MSCs cannot be ruled out. Similar results were reported showing that GSH can directly modulate proliferation and function of lymphocytes (26).GSH affects ROS levels and activate or inactivate specific redox sensitive targets at cell cycle checkpoints, and signaling pathways thereby influencing cell fate in lymphocytes GSH affects signaling pathways and cell cycle progression and probably the NF-κB pathway that regulates the expression of various inflammatory genes including cytokines and chemokines (29).

GSH as an important antioxidant is linked to ROS generation and relevant signaling may suggest that NAC-related changes in GSH can differentially later proliferation and differentiation of MSCs. GSH elevation by NAC on one hand increases MSC proliferation in culture, on the other hand can modulate the levels of liver specific markers (such as, albumin and α-fetoprotein) measured in hepatocytes derived from these cells (Unpublished data). This preliminary study clearly show that GSH changes during a long-term in vitro differentiation play important role in regulation of stem cell proliferation and differentiation to biologically active hepatocyte-like cells and its implication in cell therapy in chronic liver diseases (30). Additional studies warranted to confirm immunoregulatory effects of glutathione on biological activity of growth factors and cytokines for inducing differentiation.

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