Short Communication

Expression of cytochrome P450 and glutathione S-transferase in human bone marrow mesenchymal stem cells

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Abstract
Currently several studies are being carried out on various properties of mesenchymal stem cells (MSCs) however there are a few investigations about drug metabolizing properties of these cells. The aim of this study was to measure the key factors involved in drug metabolism in human bone marrow MSCs. For this purpose, cellular glutathione (GSH), glutathione S-transferase (GSTs) and cytochrome P450 class 3A4 (CYP3A4) were detected in these cells. Results showed that CYP3A4 and GSTA1-1 mRNA are not detectable in MSCs however mRNA specific for GSTP1-1 was considerably expressed in MSCs. GSH content and GST activity was also detected in MSCs. These data suggest that human bone marrow MSCs possess the drug metabolizing activity which may be useful in handling drugs and chemotherapeutic agents passing to the bone marrow.
Keywords: Human bone marrow mesenchymal stem cells; Drug metabolism; Glutathione S-transferases, cytochrome P450

INTRODUCTION

Mesenchymal stem cells (MSCs) are multipotent stromal cells that originate from the mesenchymal and connective tissues. They function in tissue homeostasis and repair (Gregory *et al*., 2005) and express different surface markers (Reyes *et al*., 2001). These cells can give rise to different mesodermal cell lineages, including osteoblasts, chondroblasts and adipocytes (Wagnera *et al*., 2006; Talens-Visconti, 2006). Bone marrow has traditionally been seen as an organ composed of two main systems rooted in distinct lineages-hematopoietic stem cells (HSCs) and the associated supporting stroma (Weatherall, 1997). These cells not only coexist but also cooperate functionally (Bianco *et al*., 2001).

There are very few reports in the literature on the biotransformation enzyme expression in bone marrow stem cells. It has been reported that CYP2E1 is expressed in $CD34^+$ stem cells derived from human bone marrow (Czekaj *et al*., 2005). The drug metabolizing enzymes (DMEs) are a diverse group of proteins that are responsible for metabolizing xenobiotic compounds. These enzymes are divided into two groups: phase I includes oxidative drug metabolizing enzymes such as cytochrome P450s enzymes (CYP450) and phase II biotransformation enzymes such as glutathione transferases (GSTs). These enzymes are important factors for the elimination of toxic metabolites of the xenobiotic compounds (Guengerich, 1995). GSTs catalyze the formation of thioether conjugates

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between glutathione (GSH) and reactive xenobiotics and comprise a family of dimeric proteins whose subunits have been placed into five families namely, Alpha, Mu, Pi, Theta and Sigma, according to their amino-acid sequence identities (Mannervik *et al*., 2005).

GSTs are involved in the metabolism of toxic products of lipid oxidation generated by oxidative stress (Listowsky, 2005). GSTP is expressed in many tumors and over expression of this enzyme has been strongly associated with drug resistance (Lo and Ali-Osman, 2007; Hayes *et al*., 2005). Moreover, investigations have shown that expression of GST enzymes especially GSTPi have protective effects on HSCs against anticancer drugs such as cyclophosphamide (Matsunaga *et al*., 2000).

GSH acts as antioxidant via direct GSH-ROS (Reactive oxygen species) interaction and through glutathione peroxidaes (Meister and Anderson, 1983). Moreover, GSH serves as a necessary nucleophile in a number of detoxification reactions and GSH content of HSCs also is very important in anti cancer drug resistant (Tew *et al*., 1993).

In this study, attempts were made to study the major xenobiotic metabolizing factors in MSCs residing in human bone marrow. The results of this study is implicated in understanding the role of MSCs in supporting the bone marrow cellular functions and subsequent differentiation in situ or *ex vivo*. In this study human bone marrow MSCs were isolated and cultured and characterized as described in detail elsewhere (Kazemnejad *et al*., 2009). All samples (n=10) were obtained from Bone Marrow Transplantation Center, Shariati Hospital, Tehran, Iran and written informed consent was obtained from all the donors.

For Immunocytochemistry (ICC), the cells were fixed with 4% paraformaldehyde and permeabilized with 0.4% triton X-100 and then incubated overnight at 4°C with primary antibody; mouse anti-human total GST (1:1000). Subsequently, the cells were incubated with fluorescence labeled second antibody, FITC-labeled goat anti-mouse IgG at 37°C for 1 h in dark room. Then the cells were incubated with DAPI (4', 6-diamidino-2-phenylindole; 1:1000) for nuclear staining. The cells were visualized using a fluorescence microscope (Nikon, TE-2000, Tokyo, Japan). The GST specific activity was measured using CDNB (1-chloro-2, 4-ninitrobenzene) as the substrate according to Habig *et al*. (1974). Absorbance at 340 nm was monitored for 3 min using CECIL UV spectrophotometer. Protein concentration in the samples was determined using Bradford protein assay protocol (Bradford,1976).

Reverse transcription polymerase chain reaction (RT-PCR) was used to estimated the expression of *CYP3A4*, *GST A1* and *GSTPi*. Briefly, total RNA was extracted from the cells using the RNXTM (-PLUS) kit (CinnaGen Co, Tehran, Iran), then 11 microlitre of total RNA was reverse-transcribed using RevertAidTM First strand cDNA syntatase kit (Fermentas, Germany). PCR was conducted using approximately 50 ng cDNA to amplify genes. After initial denaturation at 95ºC for 2 min, PCR amplification was continued at 95ºC for 30 s, annealing temperature for 30 s, and 72ºC for 30 min for a total 30 cycles, and final extension at 72ºC for 5 min. The sequences of the primers used in this study are shown in Table 1. β-actin (as housekeeping gene) was used as

Table 1. Primers and annealing temperature used in RT-PCR.

Gene	sequence	Product size (bp)	Annealing temperature (˚C)	Accession Number
GST A1-1	F 5'- GTGCAGACCAGAGCCATTCT-3' R 5'- GCAAGCTTGGCATCTTTTTC-3'	170	58	AL590363.6
GST P1-1	F 5'- ACCTCCGCTGCAAATACATC-3' R 5'- GGCTAGGACCTCATGGATCA-3'	206	58	NM_000852.3
CYP3A4	F 5'-CCT TAC ATA TAC ACA CCC TTT GGA AGT-31.2 R 5'-AGC TCA ATG CAT GTA CAG AAT CCC CGG TTA-3'	382	62	
β-actin	F 5'- TTCTACAATGAGCTGCGTGTGG-3' R 5'- GTGTTGAAGGTCTCAAACATGAT-3'	119	58	

Table 2. Glutathione S-transferase activity and cellular GSH content in MSCs and HepG2 cells.

Cell type	Total GST (nmol/min/mg protein)	GSH level (μmol/10^6 cells)
MSCs	78±2.8	1.33±0.07
HepG2	31±1.5	0.32±0.01

Results are presented mean ± SEM of three samples carried out in triplicate. P<0.05 considered significant. HepG2 is served as positive control.

internal control.

The intracellular GSH was measured in the MSCs and HepG2 cells using 10% trichloroacetic acid (to precipitate proteins) and then the mixture was centrifuged at 15700 g for 1 min to remove the denaurated proteins and GSH was measured in supernatant using Ellman's protocol. GSH was expressed as μmoles/10^6 cells. In this study, all the samples and standards were run in triplicate. Data are presented as mean± SEM. The results were analyzed by Student's t-test. P<0.05 was considered statistically significant.

The results showed that total GST activity determined using CDNB as substrate in MSCs and in HepG2 cells (as positive control) is 78±2.8 and 31±1.5 nmol/min/mg proteins, respectively (Table 2). ICC analysis carried out on MSCs using mouse anti-human total GST showed that total GST enzyme is expressed to detectable levels. Likewise, total GST was expressed in HepG2 cells (Fig. 1).

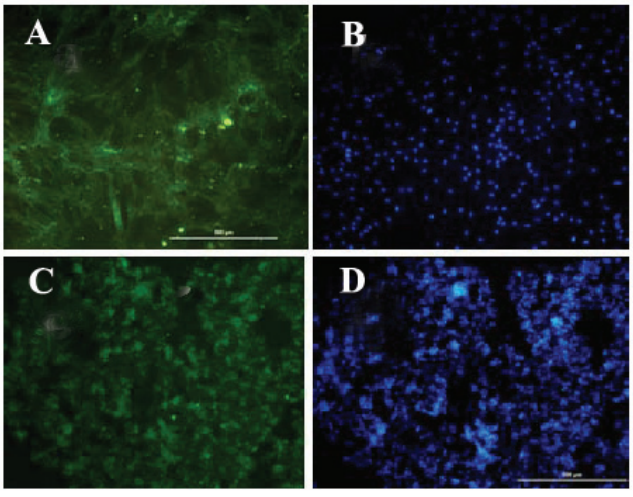

Figure 1. ICC staining of glutathione S-transferase in MSCs and in HepG2. A: Positive staining of GST was evidenced in MSCs. B: Nuclei stained with DAPI shown in parts. C and D: Positive staining of total GST and nucleus staining with DAPI in HepG2 were shown in parts.

As shown in Table 2, both cells (MSCs and HepG2 cells) contained detectable amounts of reduced GSH. Comparison of GSH levels indicates that GSH is significantly (P<0.05) higher in MSCs as compared to HepG2 cells. The level of GSH in MSCs and in HepG2 was 1.3±0.07 and 0.32±0.01 μmol/10^6 cells respectively (Table 2).

The expression of GST at protein levels in MSCs was further confirmed using RT-PCR technique designed for GST-P and GST-A specific mRNAs. The results presented in Figure 2 revealed that GST-p is well expressed in MSCs, whereas, GST-A as well as CYP3A4 were not expressed in these cell. Both the GST classes were expressed at mRNA levels in HepG2 cells which are considered as control (Fig. 2).

Over the past decade, chemotherapeutic agents have provided significant benefits and cures by eliminating tumor mass (Choti, 2009). One problem of cancer therapy is that the anti cancer agents also kill normal proliferating cells. Moreover cancer cells can resist to anti cancer drugs. This could be due to many factors such as individual differences of patients especially genes and proteins involved in DNA repair, apoptosis and over expression of enzymes that can detoxify the drugs such as GST enzymes (Johansson, 2010). For example, GSTP is over expressed in many tumors and over expression of this enzyme has been strongly associated with drug resistance (Lo and Ali-Osman, 2007; Hayes *et al*., 2005). Expression of biotransformation enzymes such as GSTs family especially GSTP in bone marrow cells may have protective effects on these cells against the toxicity of high dose chemotherapy with regimen comprised of anti cancer drugs (Niitsu *et al*., 1998).

Investigations have shown that MSCs and HSCs may have a poor ability to detoxify environmental chemicals via enzymatic biotransformation pathways, for example $CD34^+$ cells have capacity of biotransformation enzyme expression. Moreover it has been reported that bone marrow MSCs contain low basal levels of CYP450 isotypes such as CYP1A1, CYP1A2, CYP2B6, CYP2D6, CYP3A4 and CYP2E1 (Czekaj *et al*., 2005; Sa-ngiamsuntorn *et al*., 2011). The results of this study have indicated that undifferentiated bone marrow cells express very little CYP3A4 before differentiation. Whereas the cells can develop expression of CYP3A4 during hepatogenic differentiation induced in presence of hepatocyte growth factot (Allameh *et al*., 2009).

In the present study, we showed that MSCs derived

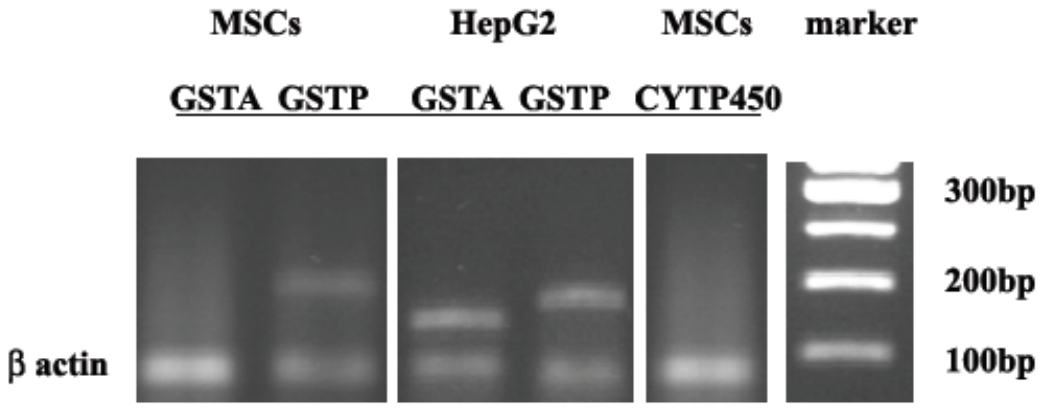

Figure 2. Expression of CYP3A4, GST A1 and GST Pi genes in MSCs and in HepG2 (as positive control) using RT-PCR. β-actin as a house keeping gene was used as internal control.

from bone marrow strongly express GSTP1-1 isoform at mRNA level; however expression of GSTA1-1 mRNA was not detectable in these cells. Investigation of functionality of total GST enzyme in these cells showed that total GST enzyme is functionally active in MSCs. Moreover, expression of total GST was confirmed using ICC. Although CYP3A4 was not detectable in MSCs derived from bone marrow. Expression of GSTP and relatively high level of GSH in MSCs suggest that drug metabolizing system is active in these cells. Some investigations also have shown that GST enzymes can be expressed in bone marrow stem cells. For instance, Shao and co-workers showed that HSCs derived from fetal liver expressed high levels of GSTP1 mRNA; however, were unable to express detectable level of GSTA1 (Shao *et al*., 2006). Also it has been reported that GSTA1 mRNA is not expressed in $CD34^+$ stem cells derived from human bone marrow (Czerwinski *et al*., 1997). Because the hGSTA proteins are the dominant GST isoforms that protect against oxidative damage (Hubatsch *et al*., 1998), hence lack of GSTA in MSCs implies that this class of GST may not be involved in protection of bone marrow cells against the byproducts of oxidative stress. Moreover, previous studies showed that MSCs have high level of GSH; therefore GSH content of MSCs can potentially protect bone marrow stem cells from the endogenous and exogenous xenobiotics (Allameh *et al*., 2009).

The expression of GSTP enzyme is believed to inhibit apoptosis in cancer cells and thereby make these cells resistant to anti cancer drugs (Bernardini *et al*., 2000). Hence GSTP expression in MSCs is involved in drug resistance which are transferred to bone marrow. Resistance of MSCs which are believed to support HSCs in bone marrow against chemotherapy drugs can reduce side effects of cancer therapy in bone marrow. The data presented hereby indicate that the presence of the basic enzymes necessary for drug metabolism in MSCs, as supporting tissue in bone marrow can offer partial protection to bone marrow cells. Further studies are required to better understand the role of xenobiotic metabolizing and conjugating enzymes in the susceptibility of MSCs to chemicals and anti cancer drugs.

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