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Oxidative Damage to Lipids, Proteins, and DNA during Differentiation of Mesenchymal Stem Cells Derived from Umbilical Cord into Biologically Active Hepatocytes

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Abstract : Stem cells with therapeutic applications can be isolated from human placenta/umblical cord blood (UCB) as well as the cord tissue (UC). Stem cells in culture are vulnerable to oxidative stress, particularly when subjected to differentiation process. The aim of this study was to examine the chnages in the rate of oxidation that occurs to cellular macromolecules during hepatic differentiation of mononuclear cells (MSCs). In addition, the impact of the hepatic differentiation process of MSC on cellular and biological activity of the cells will be undertaken. For this purpose, first mononuclear cells (MNCs) were isolated from human UCB which was obtained from a healthy full-term infant. The cells were cultured at a density of 3×10^5 cells/cm^2 in DMEM- low-glucose culture media supplemented with 20% FBS, 2 mM L-glutamine, 100 µg/ml streptomycin and 100 U/ml penicillin. Cell cultures were then incubated at 37°C in a humidified 5% CO_2 incubator. After removing non-adherent cells by replacing culture medium, fibroblast-like adherent cells were resuspended in 0.25% trypsin-EDTA and plated in 25 cm^2 flasks (1×10^4/ml). Characterization of the MSCs was routinely done by observing their morphology and growth curve. MSCs were subjected to a 2-step hepatocyte differentiation protocol in presence of hepatocyte growth factor (HGF), dexamethazone (DEX) and oncostatin M (OSM). The hepatocyte-like cells derived from MSCs were checked every week for 3 weeks for changes in lipid peroxidation, protein carbonyl formation and DNA oxidation i.e., 8-hydroxy-2'-deoxyguanosine (8-OH-dG) assay. During the 3-week differentiation process of MSCs to hepatocyte-like cells we found that expression liver-specific markers such as albumin, was associated with increased levels of lipid peroxidation and protein carbonyl formation. Whereas, undifferentiated MSCs has relatively low levels of lipid peroxidation products. There was a significant increase ($p < 0.05$) in lipid peroxidation products in hepatocytes on days 7, 14, and 21 of differentiation. Likewise, the level of protein carbonyls in the cells was elevated during the differentiation. The level of protein carbonyls measured in hepatocyte-like cells obtained 3 weeks after differentiation induction was estimated to be ~6 fold higher compared to cells recovered on day 7 of differentiation. On the contrary, there was a small but significant decrease in DNA damage marker (8-OH-dG) in hepatocytes recovered 3 weeks after differentiation onset. The level of 8-OHdG which was in consistent with formation of reactive oxygen species (ROS). In conclusion, this data suggest that despite the elevation in oxidation of lipid and protein molecules during hepatocyte development, the cells were normal in terms of DNA integrity, morphology, and biologically activity.

Keywords : adult stem cells, DNA integrity, free radicals, hepatic differentiation
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