INTERACTION BETWEEN FIBROBLAST AND 3T3-L1 CELLS UNDER OXIDATIVE STRESS CONDITION

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Abstract –

The present study was carried out to understand the effects of co-culturing on fibroblast and 3T3-L1 preadipocyte cells under H_2O_2 induced oxidative stress condition. The cell growth, oxidative stress markers and antioxidant enzymes were analyzed. The mRNA expressions of caspase-3, caspae-7, TNF-α, NF-KB, adiponectin and PPAR-γ were analyzed in co-culture of fibroblast and 3T3-L1 cells. Cell viability and antioxidant enzymes were significantly increased in co-culture than compared to mono-culture under stress condition. The caspase-3, caspae-7, TNF-α, NF-KB, adiponectin and PPAR-γ were significantly altered in H_2O_2 induced co-culture of fibroblast and 3T3-L1 cells when compared with the mono-culture of H_2O_2 induced fibroblast and 3T3-L1 cells. From our results, we have suggested that co-culture of 3T3-L1 and fibroblast cells may influence/regulate each other and made the cells able to withstand against oxidative stress and aging. It is conceivable that the same mechanism may have occurring in cell to cell while animals are stressed by various environmental condition.

Key Words – connective tissue, meat quality, *longissimus Dorsi* muscle and *semimembranosus* muscle.

I. INTRODUCTION

Cell-to-cell interactions play a crucial role in both normal and pathological conditions [1]. Co-culture techniques have long been used to study the interactions between the cell populations (homotypic or heterotypic) and can influence the cell differentiation and gene expression. It has been used in various fields of experimental biology and medicine and it can be applied for elucidate the role of cellular communication in tissue formation and regeneration [2]. More specifically, co-culture between endothelial cells and C2C12, develops prevascularized tissue [3,4]. Our previous study, showed that the co-culture effect of C2C12 and 3T3-L1 preadipocyte cells could influence on calpain, caspase and HSPs [5]. Various environmental stresses lead to enhance the ROS production may cause inconsistency in meat tenderness and affect the meat quality [6]. Each cell has been capable to exchange of chemical signals, ions and enzymes with other neighboring cells into intracellular space through the pores in the membrane which can influence the behavior of neighboring cells [7]. The present study was carried out to understand the co-culturing effects on cell growth, apoptosis, inflammatory and adipogenic pathways in H_2O_2 induced fibroblast and 3T3-L1 cells also compare the variation with mono-culture. The present study will provide an understanding of molecular mechanisms between fibroblast and 3T3-L1 preadipocytes in oxidative stress condition, an essential for biological events in animals.

MATERIALS AND METHODS

All chemicals and laboratory wares were purchased from Sigma- Aldrich Chemical Co. (St. Louis, MO, USA) and Falcon Lab ware (Becton-Dickinson, Franklin Lakes, Nj, USA), respectively.

Isolation of Intramuscular connective tissue fibroblasts from Hanwoo cattle: The fibroblast cells were freshly isolated from *Semimembranosus* (SM) muscles of *Hanwoo* cattle (20-30 months old) by sequential enzymatic digestion and further purified by magnetic-activated cell sorting (MACS) with antibodies against fibroblast-specific protein-1 (FSP-1). Cell subsets obtained were cultured in parallel for further experiments.

Co-culture of fibroblast cells and 3T3-L1 preadipocytes: Cells were co-cultured using trans-well inserts with a 0.4-μm porous membrane to separate fibroblast cells and 3T3-L1 preadipocytes. Each cell type was grown independently on the trans-well plates. Following cell differentiation, inserts containing adipocytes were transferred to fibroblast separate plates and inserts containing fibroblast transferred to adipocyte plates. Following co-culture for 24 h and 48 h after the

incubation period the lower well cells only treated with H_2O_2 for 4 h then harvested for further analysis.

Experimental groups:

The experiment groups were designed as follows: Group 1: Fibroblast (mono culture); Group; 2: Fibroblast (co-culture); Group 3: Fbroblast (mono culture) + H_2O_2; Group 4: Fibroblast (co-culture) + H_2O_2; Group 5: 3T3-L1 (mono culture); Group 6: 3T3-L1(co-culture); Group 7: 3T3-L1 (mono culture) + H_2O_2; Group 8: 3T3-L1(co-culture) + H_2O_2. The H_2O_2 concentration was used at 40 µM, 4 h.

Estimation of oxidative stress markers and antioxidants enzymes: The oxidative stress markers such as lipid hydroperoxides (LHP) [8] and protein carbonyl (PC) [9] content were measured. The activities of enzymatic antioxidant such as superoxide dismutase (SOD) and catalase (CAT) were measured in all experimental group by standard methods presented elsewhere [10].

RT-PCR: mRNA expression of selected genes were estimated such as TNF-α,NF-κB, caspase-7 caspase-9, PPAR-γ and adiponectin.

II. RESULTS

Figure 1 shows the percentage of cell viability on mono and co-culture of 3T3-L1 and fibroblast cells under normal and oxidative stress conditions. The percentage of cell viability was significantly decreased up to 40.54% and 41.46% in H_2O_2 induced mono-culture of 3T3-L1 with fibroblast cells; fibroblast cells with 3T3-L1 cells respectively where as it was significantly increased up to 54.05% (3T3-L1 with fibroblast cells) and 53.65% (fibroblast cells with 3T3-L1 cells). The activities of enzymatic antioxidants and oxidative stress markers are given in Table 2. Decreased activity of SOD (50%) and CAT (48.07%) in mono-culture of fibroblast cell and the decreased activity of SOD (49.60%) & CAT (59.52%) in 3T3-L1 cells were observed in H_2O_2 induced cells as compared to control. In co-culture of both cells with H_2O_2, the levels were significantly increased compared to H_2O_2 induced mono-culture cells. The oxidative stress markers such as LHP and PC were significantly increased in H_2O_2 induced mono-culture of 3T3-L1and fibroblast cells. In H_2O_2 induced co-culture of those cells were significantly decrease when compared to mono-culture (data not shown).

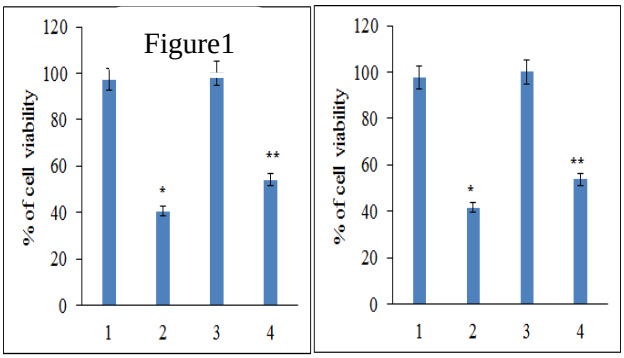

Fig.1 Co-culture of fibroblast cell and 3T3-L1 inhibits cell death in H_2O_2-induced cells. *Significant as compared with control; **significant as compared with H_2O_2-induced cells. Values are mean±SD of 3 independent determinations ($p<0.05$, ANOVA followed by DMRT).

Compared to control cells, the mRNA expression of caspase-3, caspase-7, TNF-α, NF-κB, were significantly up-regulated whereas it was increased in the co-culture of H_2O_2 induced fibroblast and 3T3-L1 cells (Fig.2, Fig.3 and Fig.5). The mRNA expressions of PPAR-γ and adiponectin were significantly down regulated in the mono-culture of H_2O_2 induced cells whereas it was increased in the co-culture of H_2O_2 induced fibroblast and 3T3-L1 cells at 24 h and 48 h, as recorded in Fig. 4.

III. DISCUSSION

Excessive ROS including H_2O_2 inherently involved in the pathogenesis of chronic cell death and different cell types may be differentially sensitive to H_2O_2. It is well known that H_2O_2 reduce the cell viability and apoptosis through activation of caspase-3 requires the activation of inflammatory pathways such as TNF-α, TGF-β, and NF-kB [11].

Figure 2

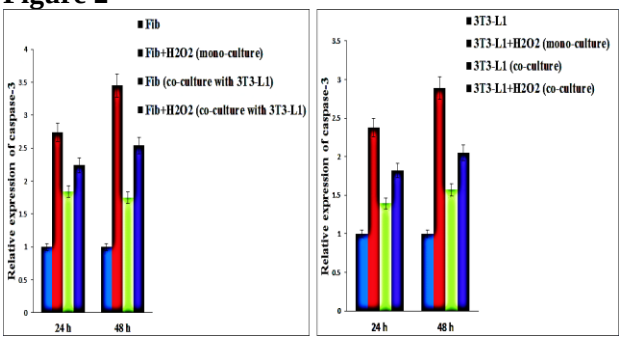

Fig.2 Representative RT-PCR of caspase-3 expression in co-culture of fibroblast (left) and 3T3-L1 (right) cell under basal and H_2O_2-stimulated oxidative stress conditions. Values are mean ± SD of 3 independent experiments. * Significant as compared to CON; ** Significant as compared to H_2O_2. ($p<0.05$, one-way ANOVA followed by DMRT). Similarly, the H_2O_2 induces cell death in adipocytes by inhibit antioxidant enzymes such as SOD and catalase. Adipose tissues are highly expressed antioxidant enzymes such as SOD and CAT comparable to the liver and kidney [12].

Figure 3

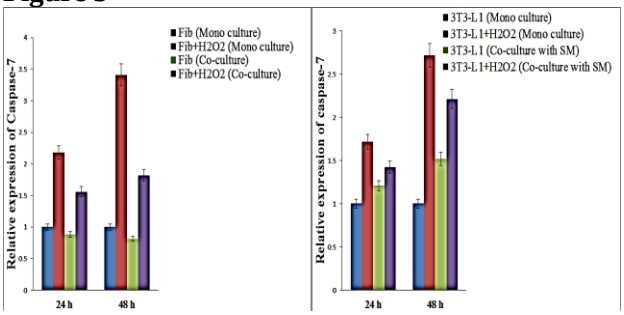

Fig.3 Representative RT-PCR of caspase-7 expression in co-culture of fibroblast (left) and 3T3-L1 (right) cell under basal and H_2O_2-stimulated oxidative stress conditions. Values are mean ± SD of 3 independent experiments. * Significant as compared to CON; ** Significant as compared to H_2O_2. ($p<0.05$, one-way ANOVA followed by DMRT).

Thus, co-culture of fibroblast cells with 3T3-L1 adipocytes cells may reduce the cell injury or cell death by localization of SOD and CAT from 3T3-L1 cell.

Figure 4

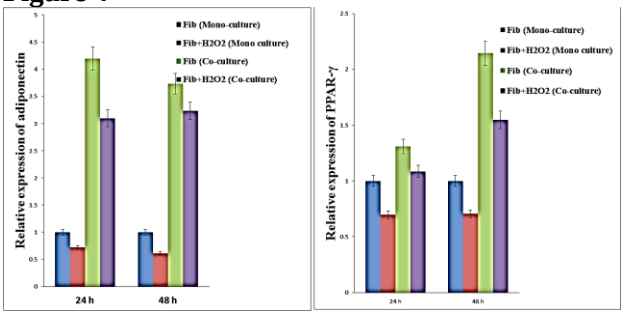

Fig.4 Representative RT-PCR of adiponectin (left) and PPAR-γ (right) expression in co-culture of 3T3-L1 cell under basal and H_2O_2-stimulated oxidative stress conditions. Values are mean ± SD of 3 independent experiments. * Significant as compared to CON; ** Significant as compared to H_2O_2. ($p<0.05$, one-way ANOVA followed by DMRT).

PPAR-γ is highly expressed in adipose tissue, and is considered to be the 'master switch' of adipocyte differentiation. Over expression of PPAR-γ in SIPS HDF cells reduced the levels of inflammatory molecules; by inhibiting the NF-kB signaling pathway also induced the cell survival capacity in both *in vitro* and *in vivo*. Several studies reported that PPAR-γ and PPAR-α inhibit the expression of inflammatory genes, such as TNF-α, TGF-β and MMPs [13].

Moreover, beneficial effects of adiponectin act as an endogenous antioxidant and anti-apoptotic agent against palmitate induced cells [14].

Figure 5

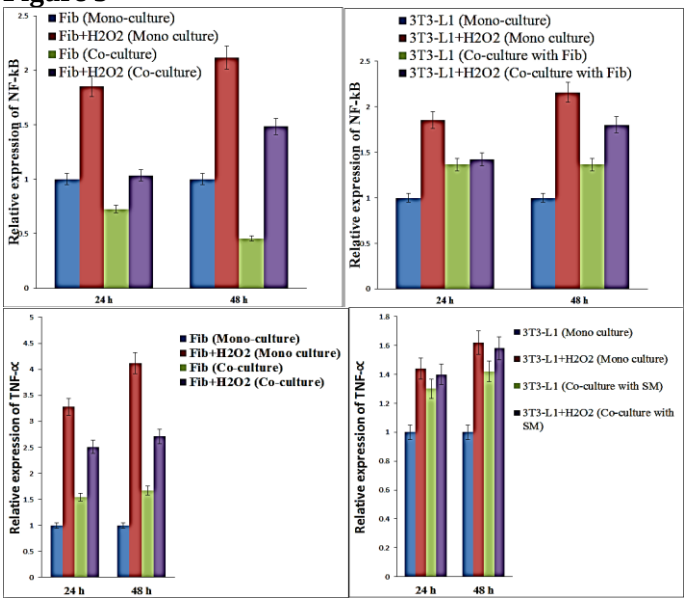

Fig.5 Representative RT-PCR of NF-kB and TNF-α expression in co-culture of fibroblast cell under basal and H_2O_2-stimulated oxidative stress conditions. Values are mean ± SD of 3 independent experiments. * Significant as compared to CON; ** Significant as compared to H_2O_2. ($p<0.05$, one-way ANOVA followed by DMRT).

Thus, during the co-culture 3T3-L1 cells secrets the PPAR-γ and adiponectin may voluntarily migrate into the fibroblast cells which could protects the cell from stress.

IV. CONCLUSION

H_2O_2, a free radical generator, significantly induced cell death and antioxidants also promote the inflammation by the activation of TNF-α and NF-kB. Our experimental results indicated that the cell viability, antioxidant such as SOD and CAT were significantly

increased in H_2O_2 induced co-culture of 3T3-L1 and fibroblast than mono-culture. In addition, the oxidative stress markers such as PC and LHP were altered in co-culture of 3T3-L1 and fibroblast cells. These inferences suggest that the co-culture between two cells exchange the secreted molecules are help each other for preventing cell death from oxidative stress condition. In addition, co-culture of 3T3-L1 and fibroblast cells can enhance the capability to fight with free radicals each other. The same mechanism would be occurring in vivo condition while animal affected by oxidative stress.

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