Journal of Animal Science and Technology 53(3) 223~226, 2011
DOI:10.5187/JAST.2011.53.3.223

Effect of Retinoic Acid on Proliferation and Differentiation of Preadipocytes from Male and Female Pigs

Mi Yeon Song, Chang Gwon Dang and Chung Soo Chung*

Department of Animal Science, College of Agriculture, Life and Environment Sciences, Chungbuk National University, Korea

ABSTRACT

The current study was undertaken to determine the effect of retinoic acid (RA) on proliferation and differentiation of preadipocytes from male and female pigs. The preadipocytes were isolated from new-born male and female pigs by collagenase digestion and washed three times one day after seeding (designated as day 0 of culture). RA was included in the media at various concentratives from day 0 to 2. The cell number was measured on day 2 with hematocytometer after trypsin digestion. Cell differentiation was determined on day 6 by measuring glycerol-3-phosphate dehydrogenase activity. RA (0.1, 1 and 10 uM) showed no effect on proliferation of preadipocytes from both male and female pigs. However, RA significantly decreased differentiation of pig preadipocytes. Degree of differentiation with 0.1 uM, 1 uM and 10 uM of RA treatment was 80%, 41% and 29% respectively, compared with control. Similar inhibitory effect was found in the female pigs; 77%, 28% and 16% respectively. It is interesting that RA treated on cell proliferation stage had no effect on proliferation but had a strong inhibitory effect on differentiation which is happening in the late stage of cell culture.

(**Key words :** Retinoic acid, Pig, Preadipocytes, Proliferation, Differentiation)

INTRODUCTION

Recently many studies focused on the reduction of fat deposition in the livestocks as accumulation of excessive fat decreases efficiency of meat production and obesity is a serious risk to human health. However, at present there is no simple genetic predictor of obesity in livestock or human because of the complex polygenic origin of this condition (Herd et al., 2003). Nutritional manupulation can be one approach that has practical promise in preventing excessive fat deposition in the livestock.

Retinoic acid (RA) regulates cellular functions by binding to intracellular retinoic acid receptor (RAR) or retinoid X receptor (RXR). These two retinoid receptor families act via formation of either RAR-RXR heterodimer or RXR-RXR homodimer, both of which regulate the expression of RA target genes (Mangelsdorf and Evans, 1995; Berry and Noy, 2009). Suryawan and Hu (1997) reported that RA inhibited the differentiation of pig preadipocytes in the primary culture, suggesting that RA may have a role in regulating fat cell differentiation in growing animals. This view is supported by the negative correlation between intramuscular marbling fat and serum RA concentration in Japanese Wagu beef cattle fed vitamin-A deficient diets(Nakai et al., 1992; Torii et al., 1996).

Since fat cell is the major constituents of adipose tissue, it would be logical to manipulate differentiation of adipocytes for regulating fat deposition. The knowledge on the inhibitory action of RA on preadipocyte differentiation will be valuable for understanding the nature of adipose tissue development. There have been many studies with rodents cell or cell lines, but not many with pig adipocytes to investigate the roles of RA on preadipocyte differentiation. Suryawan and Hu (1997) reported the action of RA on differentiation of pig preadipocytes but not on proliferation. Kim and Chung (2008) measured the effects of RA with preadipocytes from only female pigs. The gender effect of sex steroids' action was investigated (Kim and Chung, 2010), but not on RA's action. Therefore, the present study was undertaken for further understanding of RA's action by measuring the effect of RA on proliferation and differentiation of preadipocytes from both male and female pigs.

* Corresponding author : Chung Soo Chung, Department of Animal Science, Chungbuk National University, Cheongju 361-763, Korea. Tel: 82-261-2549, E-mail: cschung@cbnu.ac.kr

MATERIALS AND METHODS

1. Isolation of pig preadipocytes

Pig preadipocytes were isolated from the stromal- vascular fraction of dorsal subcutaneous adipose tissue (Suryawan et al., 1997). Piglets were obtained from a commercial producer on the day of birth or the following day and killed by CO_2 asphyxiation. Their skin was immediately scrubbed and washed with an aqueous solution of 20% iodine and 70% ethanol. Dorsal subcutaneous adipose tissue was aseptically isolated, weighed and placed in 3ml per g of fat tissue of sterile Krebs Ringer bicarbonate buffer containing 20 mmol/l HEPES (4-[2-hydroxyethyl]piperazine-1-ethanesulfonic acid, Sigma, H-4034) and 2,000 U per g fat tissue of type-1 collagenase (Worthington Biochemical Corporation, Lakewood, NJ, LS004196) and finely minced with sharp sterile scissors. Minced adipose tissue was digested with collagenase for 40 min at 37ºC. Undigested tissue and suspended cells were separated by filtration through a 250 μm nylon mesh. Isolated cells were recovered in the filtrate and pelleted by centrifugation at 3,000 rpm for 10 min. The supernatant was discarded. The cells were washed by resuspension in Krebs Ringer bicarbonate buffer and centrifugation at 2,000 rpm for 10 min. The supernatant was discarded and the cell pellet was resuspended to 20 ml final volume with plating media consisting of 10% fetal bovine serum (FBS) (JR Scientific, Woodland CA, 43640) in a 50/50 mixture of DMEM(Sigma, D-5523) and Ham's F-12 (Sigma, N-6760) containing 12 mmol/l HEPES. The cells were again filtered through a 75 μm nylon mesh. Cells were counted using a hematocytometer and seeded in a 6-well plate (Corning Costar 3506) at a density of 1×10^6 cells(differentiation) in 2 ml per well and cultured at 37ºC under a mixture of 5% CO_2 and 95% O_2.

2. Proliferation and differentiation of preadipocytes

Twenty-four hours after seeding, primary cultures of pig preadipocytes were washed three times with DMEM/F-12 media. This was designated as day 0 of culture. Cells were subsequently cultured in the same media containing 0.6 μg/ml insulin, 1ng/ml transferrin, 0.5 μg/ml hydrocortisone and 10% FBS for measurement of cell differentiation. Media was replaced every second day for 6 days. Retinoic acid (RA) (all-trans retinoic acid, Sigma, R-2625) (0.1, 1, 10 uM) was included in the media at various concentrations from day 0 to day 2. Dimethyl sulfoxide (DMSO) was treated as control. The cell number for proliferation study was measured on day 2 with hematocytometer after trypsin digestion of cells in culture. The extent of cell differentiation was assessed on day 6 by spectrophotometric assay of glycerol-3-phosphate dehydrogenase (GPDH, EC 1.1.1.8) by measuring the disappearance of NADH during GPDH catalyzed reduction of dihydroxyacetone phosphate (Wise and Green, 1979). Four replicates and 6 replicates from 4 male and female pigs were used for measuring cell proliferation and differentiation respectively.

3. Statistical Analysis

A significant difference between control and retinoic acid - treated samples was determined when the P-value was less than 0.05. Statistical analysis were conducted by Dunnett's T-Test in SAS software (SAS. 2004).

RESULTS AND DISCUSSION

The effect of RA on proliferation and differentiation of preadipocytes of male and female pig has not been reported previously. In the current study we treated RA to pig preadipocytes in the early stage (day 0-2) of culture and measured cell proliferation on day 2 and cell differentation on day 6. When pig preadipocytes were exposed to the various concentrations of RA (0.1, 1 and 10 uM), no effect on proliferation was found in preadipocytes from male and female pigs (Fig. 1 and Fig. 2)

However, RA significantly decreased differentiation of pig preadipocytes. Degree of differentiation with 0.1 uM, 1 uM and 10 uM of RA treatment was 80%, 41% and 29% respectively compared with control (Fig. 3). Similar inhibitory effect was found in the female pigs; 77%, 28% and 16% respectively (Fig. 4). Two studies which investigated RA's action on differentiation of pig preadipocytes did not identify the gender of their experimental animals (Suryawan and Hu, 1997; Brandebourg and Hu, 2005). Thus, the possible differential effect of RA according to the gender has not been confirmed. The results of the present study suggest that there is no gender effect of RA on proliferation and differentiation of pig preadipocytes. Safonova et al. (1994) reported that RA at physiological concentrations (1 to 10nM) showed its action in rat preadipocytes and our study showed that 100 nM of RA reduced 20% in differentiation of male

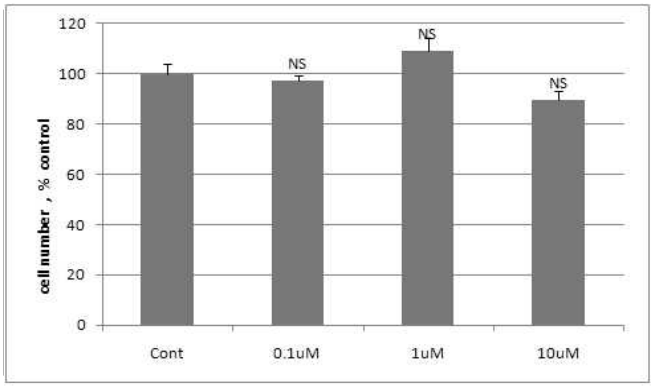

Fig. 1. Effects of retinoic acids (RA) on proliferation of preadipocytes from male pigs. The cells were treated with various RAs for two days during proliferation periods. Dimetyl sulfoxide (DMSO) was used as control (Cont). Cell proliferation was determined by counting cells by hemato-cytometer. Values are means + SE, difference from Cont; NS, non significant.

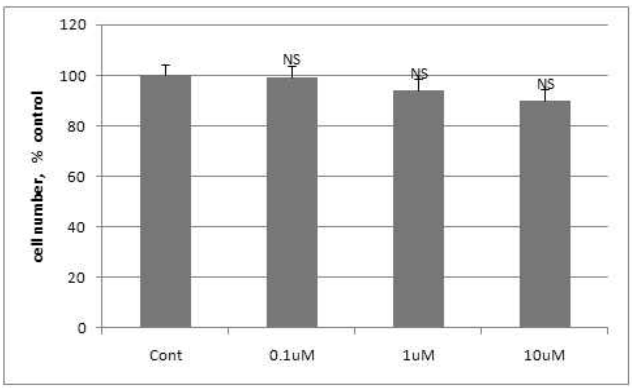

Fig. 2. Effects of retinoic acids (RA) on proliferation of preadipocytes from female pigs. The cells were treated with various RAs for two days during proliferation periods. Dimetyl sulfoxide (DMSO) was used as control (Cont). Cell proliferation was determined by counting cells by hematocytometer. Values are means + SE, difference from Cont; NS, non significant.

pig.

The inhibitory action of RA on differentiation in the current study was similar to the study of Suryawan and Hu (1997) showing that 10 uM of RA for 24 hr treatment significantly decreased differentiation of pig preadipocytes and they also reported that RA exerted its effect only in the early stage of preadipocyte culture. Regarding this observation it is quite interesting to note that RA treated on cell proliferation stage (day 0-2) did not have any effect on

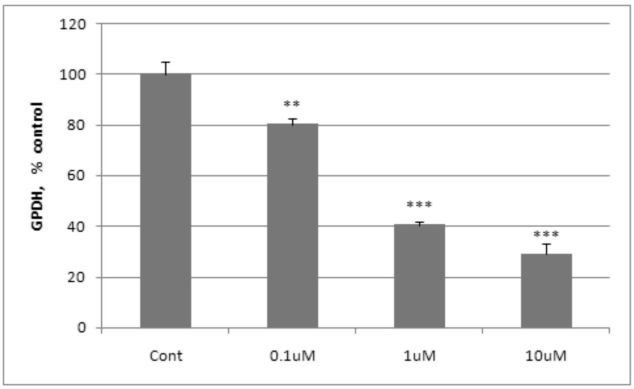

Fig. 3. Effects of retinoic acids (RA) on differentiation of preadipocytes from male pigs. The cells were treated with various RAs for two days during proliferation periods. Dimetyl sulfoxide (DMSO) was used as control (Cont). Cell differentiation was determined by glycerol-3-phosphate dehydrogenase (GPDH) activity. Values are means + SE, difference from Cont ** p< 0.01, *** p<0.001.

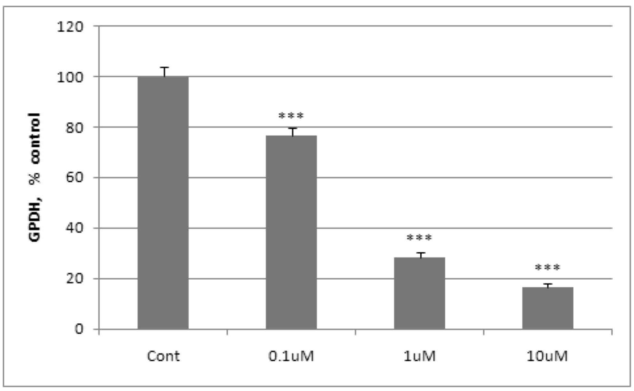

Fig. 4. Effects of retinoic acids (RA) on differentiation of preadipocytes from female pigs. The cells were treated with various RAs for two days during proliferation periods. Dimetyl sulfoxide (DMSO) was used as control (Cont). Cell differentiation was determined by glycerol-3-phosphate dehydrogenase (GPDH) activity. Values are means + SE, difference from Cont; *** p< 0.001.

proliferation but had a strong inhibitory effect on differentiation which is happening in the late stage of culture. This observation leads to the question of the mechanism by which RA exerts its inhibitory action on the process of differentiation of pig preadipocytes. Brandebourg and Hu (2005) reported that RA inhibits pig preadipocyte differentiation by a mechanism that involves activation of the RAR and

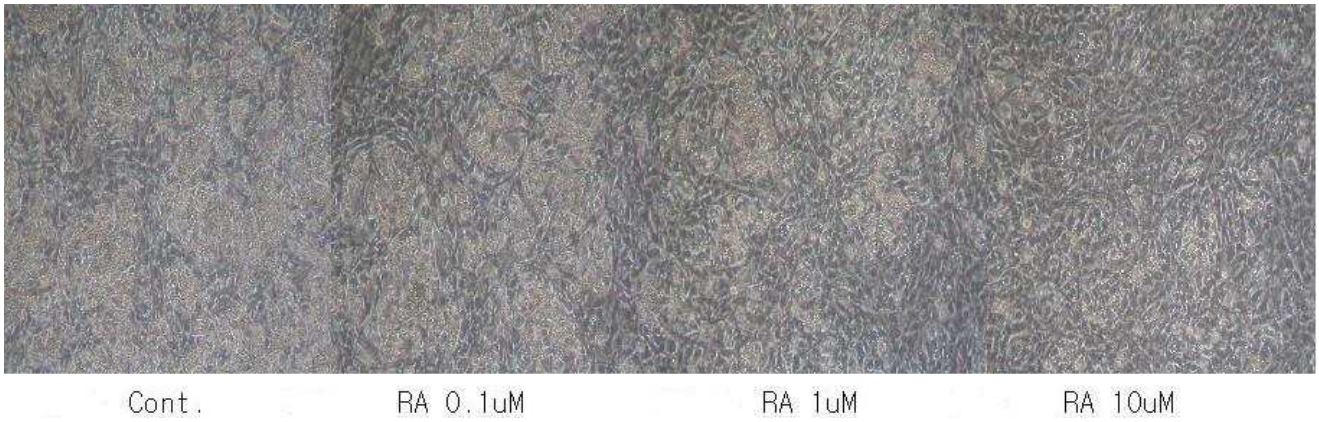

Fig. 5. Micrographs showing differentiation (day 6) of female pig preadipocytes treated with various concentrations of retinoic acid(RA). RA was added to the culture media. As RA concentration increased, differentiation was reduced.

downregulation of PPARγ on SREBP-IC mRNA. However, the exact mechanism of inhibitory action of RA remains unclear. Fig. 5 shows the degree of cell differentiation of preadipocytes treated with RA which reflects Fig. 4 quite well. The results of the present study suggest that the low intramuscular fat with high blood RA in Japanese Wagu (Torii et al., 1996) can be partly due to RA's inhibitory action on preadipocyte differentiation. Understanding RA's action will help us to devise an effective method to reduce adipose tissue deposition in pig.

In summary, RA did not have any effect on proliferation of adipocytes from male and female pigs, but exerted a strong inhibitory action on differentiation of adipocytes from both pigs. No gender effect was found in RA's action. It is interesting that RA treated in the cell proliferation stage did not have any effect on proliferation but showed a strong inhibitory effect on differentiation which is happening in the late stage of cell growth.

ACKNOWLEDGMENTS

This work was supported by the research grant of the Chungbuk National University in 2009.

REFERENCES

Berry, D. C. and Noy, N. 2009. All-trans-Retinoic Acid Represses Obesity and Insulin Resistance by Activating both Peroxisome Proliferation-Activated Receptor β/δ and Retinoic Acid Receptor. Mol. & Cell. Biol. 29:3286-3296.

Brandebourg, T. D. and Hu, C. Y. 2005. Regulation of differentiating pig preadipocytes by retinoic acid. J. Anim. Sci. 83:98-107.

Herd, F. M., Archer, L. A. and Arthur, P. F. 2003. Reducing the cost of beef production through genetic improvement in residual feed intake: opportunity and challenges to application. J. Anim. Sci. 81 (E Supplement 1):E9-E17.

Kim, H. R. and Chung, C. S. 2008. Effects of Retinoic acid on differentiation of pig preadipocytes. J. Anim. Sci. & Tech. (Kor.) 50:315-320.

Kim, W. Y. and Chung, C. S. 2010. Effects of sex steroid hormones on proliferation and differentiation of preadipocytes from female and male pigs. J. Anim. Sci. & Tech. (Kor.) 52:17-22.

Mangelsdorf, D. J. and Evans, R. M. 1995. The RXR heterodimers and orphan receptors. Cell 83:841-850.

Nakai, A., Hasegawa, K. and Hiramitsu, M. 1992. Vitamin a deficiency in Japanese black cattle. J. Clin. Vet. Med. 10:2143-2148.

Safonova, I., Amri, E. and Ailhaud, G. 1994. Retinoids are positive effectors of adipose differentiation. Mo. Cell. Endocrinol. 104:201.

SAS. 2004. SAS/STAT 9.1 User's Guide Volume 1. SAS institute Inc., Cary, NC., USA

Suryawan, A. and Hu, C. Y. 1997. Effect of retinoic acid on differentiation of cultured pig preadipocytes. J. Anim. Sci. 75:112-117.

Suryawan, A., Swanson, L. V. and Hu, C. Y. 1997. Insulin and hydrocortisone, but triiodothyronin, are required for the differentiation of pig preadipocytes in primary culture. J. Anim. Sci. 75:105-111.

Torii, S., Matsui, T. and Yano, H. 1996. Development of intramuscular fat in Wagyu beef cattle depends on adipogenic or antiadipogenic substances present in serum. Anim. Sci. 63:73-78.

Wise, L. S. and Green, H. 1979. Participation of one isozyme ofcytosolic glycerophosphate dehydrogenase in the adipose conversion of 3T3 cells. J Biochem. 254:273-275.

(Received May 31, 2011; Revised Jun. 10, 2011; Accepted Jun. 16, 2011)