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The Histopathalogical Effects of Retinoic Acid on the Tissues

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Abstract: The aim of this study is to sum up the important information that has emerged from the last 10 years of experimental investigations over the effects of retinoic acid (RA) on embryonic structure and adult tissues. Administration of exogenous RA can affect the connective tissues including enhancement of myeloid compartment and suppression of erythroid cells and conversion of hematopoietic stem cells to erythroid progenitors. Also, it is able to induce osteogenic differentiation of stem cells derived from adipose tissues and etc. Examining the neural tissue highlighted that disruption of RA signaling in the adult leads to degeneration of motor neurons and development of some diseases. *In vitro* administration of All-Trans Retinoic Acid (ATRA) increased dendritic growth and synaptophysin puncta intensity and increased expressions of neuronal nuclei, neuron specific enolase, synaptophysin. RA also promotes expression of a marker of mature astrocytes. On muscular tissue, it can inhibit proliferation of smooth muscle cells (SMC) while promoting differentiation of SMC *in vitro* instead. The ATRA stimulates skeletal myogenesis while inhibiting cardiomyogenesis and hypertrophy and proliferation of cultured neonatal cardiomyocytes and cardiofibroblasts. In addition, differences in levels of embryonic RA may contribute to variability in great artery anomalies. In epithelial tissue, the squamous epithelium exposed to ATRA showed the columnar differentiation independent to proliferation. Also RA seems able to rescue the regeneration process of injured gut and revealing a better wound healing of the intestine undergone intra-operative radiotherapy. It can interrupt the process of progressive fibrosis, enhancements of the langerhans islets, exocrine pancreas, modulate the health of the mammary glands and repairs the lung cell. Thus, differences in levels of endogenous RA in embryonic and adult tissues may contribute to anomalies and pathogenesis of disease, furthermore RA has paradoxical effects on the parts forming the connective and muscles tissue in equal conditions.

Key words: Histopathalogical, retinoic acid, tissues, embryo, effects

INTRODUCTION

Animal tissues can be grouped into four basic types: connective, muscle, nervous and epithelial. Multiple tissue types comprise organs and body structures. While all animals can generally be considered to contain the four tissue types, the manifestation of these tissues can differ depending on the type of organism.

The normal development of most embryonic structures depends mainly on an intricate but highly orchestrated interplay between spatiotemporally and differentially expressed activators and their antagonistic molecules (Wang *et al*., 2004; Dean *et al*., 2009). Among the activators that support the normal development of most embryonic structures, retinoic acid (RA) is well known as a very influential molecules that is interrelated in many developmental (Hoffman *et al*., 2006; Manolescu *et al*., 2010) and multiple biological processes. It is involved in regulation of growth, remodeling and metabolic responses in adult tissues (Noy, 2010) through regulation of a wide variety of pathways in cellular differentiation and homeostasis in various tissues and organs, such as head and teeth, by inducing a series of homeobox genes (Maden, 2006). Retinoic acid is essential for lymph node formation (Van de Pavert *et al*., 2009). In addition, RA can also suppress carcinoma cell growth and is currently used in treatment of some cancers. Growth inhibition by RA may be exerted by induction of differentiation, cell cycle arrest, or apoptosis, or by a combination of these activities. Paradoxically, in the

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context of some cells, RA not only fails to inhibit growth but, instead, enhances proliferation and survival (Noy, 2010). Unnaturally high quantities of RA, (hypervitaminosis A), as well as its insufficiency (hypovitaminosis A, which in fact is a worldwide problem of great magnitude) during embryonic development cause a great spectrum of congenital malformations (Nobakht *et al*., 2006) and as well as in pathological consequences for adult (Penniston and Tanumihardjo, 2006).

However, in the light of specific experimental attempts, roles of the RA revealed to be expanded and whereas, most of previous reviews focus on anti-neoplastic effects or only on a special tissue, then a need to an overall and new review on the basis of the other recent studies was seriously felt.

Connective tissue and RA

Blood (Myeloid): Hematopoietic development during embryogenesis involves the interaction of extrinsic signaling pathways coupled to an intrinsic cell fate that is regulated by cell-specific transcription factors. The RA has been linked to stem cell self-renewal in adults and also participates in yolk sac blood island formation (De Jong *et al*., 2010).

There are strong evidences that vitamin A (Kang *et al*., 2007) and the retinoids have immune regulatory functions (Kang *et al*., 2007; Chen *et al*., 2009) and improve the numbers of various immune cell types such as T helper- 2 (Iwata *et al*., 2003) and neutrophil (Yousefi and Samene, 2010). Exogenous all-trans retinoic acid (ATRA) enhances the proliferation and differentiation of myeloid compartment specially neutrophil during both late-yolk sac and fetal liver stages of hematopoiesis in rat embryo (Yousefi and Samene, 2010) and the patients who were treated with the ATRA (Leelasiri *et al*., 2005). Also, the findings of previous studies show that the ATRA enhances the hematopoietic progenitor cells *in vitro* (Collins *et al*., 2001; Yu *et al*., 2010) and directly enhances the number of T lymphocytes in the peripheral blood (Seguin-Devaux *et al*., 2005). A recent study shows that addition of the ATRA greatly enhances the generation of hematopoietic progenitors from human embryonic stem cells and the RA-treated cells highly express definitive hematopoietic genes that form large numbers of multilineage and myeloid colonies (Yu *et al*., 2010). The RA plays a key role in myeloid differentiation through their agonistic nuclear receptors (RAR alpha/RXR) to modulate the expression of target genes (Zhang *et al*., 2000). This receptor can bi-directionally modulate granulopoiesis as a differentiation factor when liganded to the RA or as an inhibitor in absence of ligand (Kastner *et al*., 2001).

Blood (Erythroid): Many *in vitro* studies have analyzed the effects of retinoic acid on hematopoietic progenitors and differentiation and in some cases, showed a positive role for clonal proliferation of progenitors (Ghatpande *et al*., 2002; Yu *et al*., 2010). In contrast, considerable evidence from other *in vivo* and *in vitro* studies revealed the suppressive effects of RA on proliferation and differentiation of human mast cell progenitors (Kinoshita *et al*., 2000), growth of bone marrow mesenchymal stem cells (Oliva *et al*., 2003), erythroid cell (Yu *et al*., 2010; Yousefi, 2009) and platelets in mouse embryo (Yousefi, 2009). Decreased numbers of both red blood cells and platelets could be that probably the primitive erythroid and megakaryocyte cells origin from previously unrecognized bipotential megakaryocyte/ primitive erythroid progenitors that both emerge from hemangioblast precursors during gastrulation. The megakaryocyte lineage is closely associated with erythroid lineages throughout ontogeny (Tober *et al*., 2007). The study of Kinoshita *et al*. (2000), indicated that the RA diminished the mean diameter of culture of mast cells, but our data shows that the RA dose not have any effect on the blood cell size (except NRBC) (Yousefi and Samene, 2010; Yousefi, 2009). The cyto-differentiating action of ATRA has led to its usage in the treatment of several malignancies, particularly acute promyelocytic leukemia (APL). There have been many reports regarding the cell biological effects of ATRA on human myeloma cells and a few clinical trials. Most of these reports have revealed growth inhibition by ATRA mediated by down-regulation of the IL-6/IL-6R auto/paracrine loop and upregulation of p21/Cip1 (Otsuki *et al*., 2003). However, it is concluded that this agent enhances the proliferation and differentiation of myeloid compartment while, inhibites erythroid cells, although the reported has shown that the ATRA were interfered with proliferation and differentiation of hematopoietic stem cells to the erythroid progenitors with 6×10^{-8} mol L^{-1} *in vitro* and up-regulate the expression of hoxb2 and hoxb4 significantly (Du *et al*., 2009).

Bone and cartilage: The multi-step process of cartilage tissue formation is comprised of prechondrogenic mesenchymal cells compaction prior to differentiation into matrix-producing chondroblasts (Hoffman *et al*., 2003). Also bone formation and remodeling require a meticulous orchestration of a number of signaling components (Ducy *et al*., 2000; Teitelbaum, 2000).

The retinoids, predominantly the RA, are among the numerous signaling molecules that have been concerned in this process (Hoffman *et al*., 2003). The RA has been shown to influence differentiation and proliferation of osteoblasts (Kawaguchi *et al*., 2005; Skillington *et al*., 2002; Song *et al*., 2005) and Chondrocyte (Kirimoto *et al*., 2005). Recent studies have demonstrated that the RA is able to induce osteogenic differentiation of stem cells derived from adipose tissues (Malladi *et al*., 2006), dental pulp and periodontal ligament (Chadipiralla *et al*., 2010) *in vitro*. It is shown that the ATRA strongly induces collagenase-3 expression in cultures of embryonic metatarsal cartilage rudiments and in chondrocytic cells. This effect is dose and time dependent and requires the renewed synthesis of proteins and is mediated by RAR-RXR heterodimers. The above-mentioned effect acts through a signaling pathway involving p38 mitogen-activated protein kinase (p38MAPK). RA treatment of chondrocytic cells also induces the production of matrix metalloproteinase MT1 and also a membrane-bound metalloproteinase essential for skeletal formation, which participates in a proteolytic cascade with collagenase-3. RA treatment also resulted in upregulation of Cbfa1, a transcription factor responsible for collagenase-3 and osteocalcin induction in osteoblastic cells (Jimenez *et al*., 2001). Also, it is reported that the RA can suppress the cell growth, cartilage nodule formation, accumulation of proteoglycan, alkaline phosphatase activity and mineralization while RA dose dependently upregulates the levels of type X collagen and matrix metalloproteinase-13 (MMP-13) mRNA which are marker proteins of hypertrophic chondrocytes of Chondroprogenitor (ATDC5) cells in culture instead (Kirimoto *et al*., 2005).

Another study was performed to examine the influence of retinoid signaling on skeletal development, the primary limb mesenchymal cultures from the transgenic mice that overexpressed a weak, constitutively active retinoic acid receptor (retinoic acid receptor-α) in their developing limbs were compared with cultures from wild-type mice. The finding indicates that retinoids maintain cells within condensations in a prechondrogenic, mesenchymal cell state, which prevents the cells from differentiating into chondroblasts and the inhibition of receptor-mediated retinoid signaling induces the expression of Sox9, a transcription factor that is considered a master switch for the differentiation of chondroblasts. These effects are largely mediated by the activation of the p38 MAPK signaling cascade (Hoffman *et al*., 2003). So, the RA induces a regulatory cascade involving Cbfa1 and MMPs and is coupled to the development of a perichondrial invasion and osteogenic differentiation process that occurs during endochondral ossification (Jimenez *et al*., 2001).

Neural tissue and RA: RA also is involved in the induction of neural differentiation, motor axon outgrowth and neural patterning. Like other developmental molecules, RA continues to play a role after development has been completed (Maden, 2007). It has recently come to light, however, that many of the same functions that RA directs in the embryo are involved in the regulation of neural plasticity, long-term potentiation in the hippocampus, regeneration in the adult brain (Mey, 2006), ATRA can be synthesized in discrete regions of the brain. Distribution of retinoid receptor proteins in the adult nervous system is different from what seen during development and suggests that retinoid signalling is likely to have a physiological role in adult cortex, amygdala, hypothalamus, hippocampus, striatum and associated brain regions (Lane and Bailey, 2005). Disruption of RA signalling in the adult leads to the degeneration of motor neurons (motor neuron disease), development of Alzheimer's disease and possibly, development of Parkinson's disease (Maden, 2007). The experiments on sciatic nerve lesions and spinal cord contusion injury demonstrate that the RA signaling cascade is activated by these traumatic events. Activation of RARbeta appears to be responsible for neurotrophic and neuritogenic effects of RA on dorsal root ganglia and embryonic spinal cord. While the physiological role of RA in the injured nervous system is still under investigation, three domains of functions are suggested: (1) neuroprotection and support of axonal growth, (2) modulation of the inflammatory reaction by microglia/macrophages and (3) regulation of glial differentiation (Mey and McCaffery, 2004). RA levels require precise regulation by controlled synthesis and catabolism and when RA concentrations deviate from normal, in either direction, abnormal growth and development occurs (McCaffery *et al*., 2003). In rodents, the control of neural patterning and differentiation would be disrupted when RA concentrations are lowered, whereas, inappropriately high concentrations of RA result in abnormal development of cerebellum and hindbrain nuclei. The latter parallels the malformations seen in the human embryo exposed to RA due to treatment of the mother with the acne drug Accutane (13-*cis* RA) and, in cases when the child survives beyond birth, a particular set of behavioural anomalies can be described (McCaffery *et al*., 2003). Investigations over the effects of vitamin A deficiency (VAD) on neurogenesis and memory and the ability of RA treatment in preventing VAD-induced impairments show that long-term VAD decreased neurogenesis and led to memory deficits.

More importantly, these effects were reversed to what manifested in 4 weeks of RA treatment (Bonnet *et al*., 2008).

Bonnet *et al.* (2008) have firstly demonstrated that the effect of vitamin A deficient diet on the level of hippoccampal neurogenesis would be reversible and that RA treatment is important for the maintenance of the hippocampal plasticity and function.

It is shown that activity blockade increases RA synthesis in neurons and that acute RA treatment enhanced synaptic transmission. The RA-induced increase in synaptic strength is occluded by activity blockade-induced synaptic scaling (Aoto *et al*., 2008). Also a study has shown that within 30 min ATRA increased dendritic growth approximately two folds and PSD-95 and synaptophysin puncta intensity approximately three folds, in cultured mouse hippocampal neurons, suggesting the increased synapse formation (Chen and Napoli, 2008). The differentiation property of RA was confirmed by showing an extensive outgrowth of neurites, increased expressions of neuronal nuclei, neuron specific enolase, synaptophysin and synaptic associated protein-97 and decreased expression of inhibitor of differentiation-1 (Cheung *et al*., 2009). The ATRA decrease cell viability but not neurite outgrowth (Radio *et al*., 2010). Showing that addition of exogenous ATRA to slice cultures can promote expression of a marker of mature astrocytes, glial fibrillary acidic protein (GFAP), while inhibitioning the endogenous RA synthesis reduces GFAP expression instead. The results suggest a role for retinoic acid in modulating glial differentiation (King *et al*., 2009).

Muscular tissue and RA: Evidences suggests that ATRA plays a key role in the development and differentiation of smooth muscle cells (SMC) *in vitro* (Su *et al*., 2010) and it's very important during normal embryonic development (Ross *et al*., 2000). The ATRA increased the expression of myocardin, caldesmon, 22 kDa smooth muscle cell-specific protein (SM22alpha) and SM-myosin heavy chains in Rabbit Bone Marrow-Derived Mesenchymal Stem cells (RBMSCs). Furthermore it significantly inhibits proliferation of RBMSCs in a concentration-dependent manner and up-regulates the expression of SMC specific proteins and promotes SMC differentiation (Su *et al*., 2010). *In vitro* ATRA treatment induces smooth muscle differentiation of P19 cells (Manabe and Owens, 2001). In addition, ATRA has been shown to inhibit the proliferation SMCs. by inhibiting the expression of cyclins and cyclin-dependent kinases (Kosaka *et al*., 2001). Also a quantitative morphometry of the pulmonary arteries has shown that ATRA treatment (30 mg kg^{-1}) has significantly reduced the percentage of muscularized arteries in peripheral pulmonary arteries only with an external diameter between 15 and 50 μm and also significantly reduced the medial wall thickness in small muscular arteries only with an external diameter between 50 and 100 μm (Zhang *et al*., 2010). As obtained conclusions, the ATRA showed to inhibit proliferation while increasing differentiation instead. By the way, types of ATRA behaviors under different doses still remain obscure and need more researches.

Recently, Kennedy *et al.* (2009) have reported that the low levels of RA stimulate skeletal myogenesis by accelerating and increasing the expression of Wnt3a, Pax3, Meox1 and MRFs. This early and enhanced activation of skeletal muscle is refractory to inhibitory signals from bone morphogenetic protein 4 (BMP4) but not from a dominant negative β-catenin. Furthermore, low levels of RA inhibit stem cell differentiation into the cardiac muscle lineage, as shown by the absence of GATA-4 expression. The inhibitory activity of RA on cardiomyogenesis can be abrogated by the presence of BMP4. Therefore, BMP4 and RA function antagonistically to regulate each other's inhibition of entry into skeletal and cardiac muscle lineages, respectively (Kennedy *et al*., 2009). In addition, it was demonstrated that embryonic RA synthesis is required for proper differentiation of ventricular myocytes in mice (Niederreither *et al*., 2001; Subbarayan *et al*., 2000). The ATRA inhibits hypertrophy and proliferation of cultured neonatal cardiomyocytes and cardiofibroblasts induced by angiotensin II (Wang *et al*., 2002). Lu *et al*. (2003), revealed an inhibitory effect of low-dose chronic ATRA treatment which prevented the hypertrophy of intramyocardial and intrarenal arteries and ventricular fibrosis in spontaneously hypertensive rats (SHR). These findings revealed that the ATRA can prevent medial thickening of intramyocardial and intrarenal arteries and perivascular and interstitial fibrosis in the heart without any change of the blood chemistry profile in any group and provides the first evidence for an inhibitory effect of the ATRA on intramyocardial and intrarenal arterial hypertrophy and ventricular fibrosis *in vivo* (Lu *et al*., 2003). The ATRA also has an inhibitory effect on the cardiac fibroblast *in vitro* (Wang *et al*., 2002). So, the ATRA stimulates skeletal myogenesis while inhibiting cardiomyogenesis and hypertrophy and proliferation of cultured neonatal cardiomyocytes and cardiofibroblasts at lower levels on the other hand the ATRA plays a paradoxical behavior about skeletal muscles compared with cardiac muscles. It is suggested that the differences in levels of embryonic RA may contribute to variability in great artery anomalies observed in DGS/VCFS patients, so that Loss of Tbx1

and decrease of RA synthesis result in DiGeorge/velocardiofacial syndrome (DGS/VCFS)-like phenotypes in mouse models including defects in septation of the outflow tract of the heart and anomalies of pharyngeal arch-derived structures including arteries of the head and neck, laryngeal-tracheal cartilage and thymus/parathyroid. Recent studies have shown that reduction of RA or loss of Tbx1 can alter the contribution of second heart field progenitor cells to the elongating heart tube (Ryckebusch *et al*., 2010).

Epithelial tissue and RA: Some epithelial cells cover the outside of organism; these often form multiple layers, such as the skin. Other epithelial cells form monolayers that line internal organs (Zegers *et al*., 2003), while others form internal three-dimensional organs consisting of tubules and cysts (Affolter *et al*., 2003) that infiltrate the whole organism, carrying liquids and gases containing nutrients, waste and other materials. These tubes can form elaborate networks in the lung, kidney, reproductive passages and vasculature tree, as well as many glands branching from the digestive system such as the liver, pancreas and salivary glands (Zegers *et al*., 2003).

Retinoids are essential for epithelial tissue growth and differentiation (Rexer *et al*., 2001) and regulate these functions through activation of specific nuclear RA receptors (RARs) (Stoppie *et al*., 2000).

Oral administration of R115866 (2.5 mg kg^{-1}) to rats induced a marked and transient increases of endogenous RA levels in the plasma, skin, fat, kidney and testis consistent with its ability of enhancing endogenous RA content in tissues, so it was found to exert retinoidal activities (Stoppie *et al*., 2000; Thomas *et al*., 2005) furthermore, it is shown that R115866 can also potentiate the effects of RA on epidermal keratinocytes when RA appears at low concentrations (Giltaire *et al*., 2009). It is also manifested that RA homeostasis could be controlled and stabilized by cytochrome P26-induced oxidation (Verfaille *et al*., 2008).

In Barrett's oesophagus, the level of RA biosynthesis was increased and morphological and molecular analysis of the squamous epithelium exposed to ATRA showed the columnar differentiation independent to proliferation (Chang *et al*., 2007). In HBE1 cells, a respiratory tract cell line, DUOX2 mRNA was increased six folds after ATRA treatment. Similarly, the ATRA induced a 19-fold increase in DUOX2 mRNA expression in primary tracheobronchial epithelialcells with parallel increases in DUOX protein and DUOX-mediated H_2O_2 production as Well. The data extracted from the study done with Linderholm *et al*. (2010), affirmed that the ATRA is important to regulate DUOX2 expression, function and rhinovirus-mediated DUOX2 induction in the respiratory epithelial cells. The results of other studies showed that RA is required for gut regeneration while inhibitors of RA synthesis could suppress formation of the gut and use of 13-*cis* RA seemed able to rescue the regeneration process from the inhibitor-induced hypoplasia (Kaneko *et al*., 2010). In order to clarify the reparative effects of pre- and post-treatment of the vitamin A on the jejunum, methotrexate firstly used to make the damages such as villus shortening, fusion, epithelial atrophy, crypt loss, inflammatory infiltrate in the lamina propria and goblet cell depletion of the gut and thereafter administration of vitamin A showed to diminish the severity of the uttered damages as well (Yuncu *et al*., 2004). Balkan *et al*. (2006), tried to assess preventive role of the RA over side effects of intra-operative radiotherapy on intestinal wound healing compared to the non-retinoic acid conditioned group in which the RA conditioned group statistically had a significantly higher tensile strength and lower intestinal elongation values, revealing better wound healing.

Natarajan *et al*. (2009), also performed a study over intestinal epithelium of cirrhotic rats indicating that altered retinoid metabolism might have an influence on changes in intestinal epithelial cell differentiation, seen in liver cirrhosis (Natarajan *et al*., 2009). Also RA treatment can interrupt some of the steps toward the progressive fibrosis in monocrotaline-induced Lung disease (Baybutt *et al*., 2007).

Furthermore, in regard to effects of retinoids on the alveolar cells the study showed that a vitamin A deficient diet can lead to a decreased number of alveoli and emphysema in rats as result (Nabeyrat *et al*., 2001). The RA has also been shown to protect against injury-induced cell-cycle arrest of the type II pneumocytes by preserving cyclin activities and down-regulating the cyclin-dependent kinase inhibitor $p21^{cip1}$.

There are many expanded reviews done over the anti-carcinogenic effects of RA on different cells but we tried to bring those specially indicating such effects on epithelial cells as following:

The KYSE30 cancerous cells were cultured on the nanoporous membranes to examine the effects of RA treatment (0.01 M) the RA showed to inhibit cancerous cell-substrate adhesion and motility and also induce morphological and functional terminal differentiation of the cell line. This experiment testified that the impedance amplitude decrease was really due to the cell morphology change after the treatment of RA (Yu *et al*., 2009). The RA is an important mediator that directs lung cell repair by stimulation of the type II pneumocyte proliferation in the case of type I pneumocyte injury. In addition, RA inhibits proliferation of the pneumocytes that are in contact with

each other thereby it is protective against abnormal proliferation as in carcinogenesis (Baybutt *et al*., 2007).

Examination of RA treatment impact (10 μM 9- and 13-*cis*-retinoic acid) on the intestinal epithelial cells, Caco-2 cells an intestitnal cell line derived from a human colorectal carcinoma that spontaneously differentiates under standard culture conditions, a well-characterized marker for functional differentiation of enterocytes) indicated that the RA acts as a differentiation factor since, at 60% preconfluence and at 0-day confluence, it (1) lowered labeled thymidine incorporation; (2) modulated D-type cyclins (cell cycle regulatory proteins) by down-regulating the mitogen-sensitive cyclin D1 expression (thereby blocking DNA synthesis and cell proliferation) and up-regulating cyclin D3 expression (thereby inducing cell differentiation) and (3) showed a trend of increase in (p38 MAPK), which triggers caudal-related homeobox 2 (CDX2), a central protein in cell differentiation (Grenier *et al*., 2007).

Glands (pancreas) and RA: The pancreas is composed of two different types of glandular tissues in intimate association with each other. The main mass is exocrine, embedded in which are pancreatic islets of endocrine cells or langerhans (Murtaugh and Melton, 2003). Previous studies have revealed that exogenous ATRA inhibits the bud morphogenesis and differentiation of Exocrine pancreas (acinus), while in same culture enhances endocrine cells formation, specially beta cells before normal times *in vitro* (Shen *et al*., 2007). Moreover, Colakoglu *et al*. (2005), have shown that a high dose of RA can decrease the exocrine pancreas (acinus) during pancreatic organogenesis. While our (Yousefi *et al*., 2010) data indicated that the ATRA has positive effects on proliferation, differentiation and maturation of pancreatic acinus and pancreatic islets of langerhans. Also, the mean diameter of pancreatic acinus and nuclei of acinar cells were decreased in rat's embryo on GD 18, when exposed to the ATRA in utero on GD 10. Colakoglu *et al*. (2005), found that the ATRA administration on the 8th day of gestation caused inhibition of acinar TGF-b2 immune reactivity while on GD 12, acinar expression of TGF-b2 in acid was similar to what found in the controls. Shen *et al*. (2007), reported that exocrine differentiation was found to be suppressed at all concentrations of the ATRA compared to controls *in vitro*. Also it was reported that exogenously added ATRA in tissue culture experiments stimulated differentiation of endocrine and duct cells and promoted apoptotic cell death of acinar tissue and inhibited acinar differentiation (Colakoglu *et al*., 2005; Tulachan *et al*., 2003). As result, it is not easy to determine a cause to account for such a high diversity. Although, it's suggested that the RA can suppress exocrine differentiation by a combination of two mechanisms (1) up-regulation of the extra-cellular matrix component laminin and (2) enhancement of apoptosis. It's also suggested that loss of exocrine cells following application of exogenous RA correlates with an inhibition of Notch signaling activity and can be rescued by FGF-10 (Chen *et al*., 2004) However, much more studies are needed to clarify this issue. The results extracted from *in vivo* and *in vitro* studies have shown that exogenous ATRA causes differentiation of endocrine cell (Yamaguchi and Igarashi, 2006; Kang *et al*., 2004; Erasmus *et al*., 2003; Tulachan *et al*., 2003). Also, it expands Islets of langerhans in the pancreatic organogenesis, when administered by oral intubations in the morning of GD 10 (Yousefi *et al*., 2010) These observations are to in contrast the results obtained from the study done by Colakoglu *et al*. (2005), who reported that if it is administered before neurulation, the development of endocrine component will be disrupted, while its administration after neurulation has had less adverse affects on pancreatic organogenesis. The RA also increased insulin-secreting cells by stimulation of extra progenitor cells production (Erasmus *et al*., 2003). It is reported that pdx1 genes (Li *et al*., 2004) have had important and main roles to increase proliferation of insulin-secreting cells, which is regulated by the RA *in vitro*. Recent evidence indicates that the enhancing effect of RA on formation of enlarged islets may be resulted from up-regulation of extra-cellular laminin (Shen *et al*., 2007). It also inhibits the inflammatory response or on the other hand, modulates the health of mammary glands by suppression of TLR4/NF-κB expression both *in vivo* and *in vitro* (Gu *et al*., 2010). It can be concluded that retinoic acid may help in development of new therapeutic methods to increase the Langerhans islets, exocrine pancreas and modulate the health of the mammary glands in mammals.

CONCLUSION

As this study shows, the endogenous RA plays an important role in normal embryogenesis and adult tissue health and any change over the normal level of RA can develop many types of anomalies and pathogenesis of different tissues. The exogenous RA is probably accepted to compensate the endogenous RA deficit, conclusively treatment by the RA may be beneficial on the normal and cancerous tissues but it's unfortunately associated to adverse effects *in vivo* that many of them have been revealed and some are still obscure that needs much more

studies, thus it's recommended that more tests to be done on the animal models with artificially created favorite diseases. Furthermore, the RA may have some paradoxical effects on the parts forming the connective and muscular tissues in equal conditions and can also be used as a differentiation-proliferation-provoking agent on the epithelial cells especially in the pancreas.

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