THE EFFECTS OF ALL–TRANS RETINOIC ACID ON BLOOD CELLS IN RAT'S EMBRYO

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ABSTRACT
All-trans retinoic acid (ATRA) has beneficial and teratogenicity effects when used in a variety conditions. The objectives of this study were to determine the effects of ATRA on the Progenitors of red blood cell and platelets in rat's embryo. Single oral dose (100 mg/kg) of ATRA was administered to rat on gestation day (GD) 10 and fetuses were observed on GD 18 and compared with untreated group. In the experimental embryos of GD 18, the mean number of red blood cells (RBC, 10.5%) and platelets number (15%) were decreased. There was a significant relationship in RBC and platelets count. The mean diameter of RBC and nucleated red blood (NRBC) were compared in two groups. There was no significant relationship between experimental and control groups, except in NRBC diameter. Thus, the present data shows that ATRA may have negative effects on proliferation, differentiation and maturation of erythroid cells and platelets, without having any deleterious effects on the dimenation of RBC.

Keywords: Embryo, retinoic acid, blood cells.

INTRODUCTION

The first maturing blood cells and committed progenitors are provided by the yolk sac. It is now widely assumed that hematopoiesis in the yolk sac is primitive and that definitive hematopoiesis has its origins in the aorta/ gonad/mesonephros (AGM) region (McGrath *et al*, 2005). The commitment of embryonic cells to hematopoietic fates begins in proximal region of egg cylinder at the mid-primitive streak stage with the simultaneous appearance of primitive erythroid and macrophage progenitors (Palis *et al*, 1999). The generation of mature blood cells from pluripotent hematopoietic (lymphohematopoietic) stem cells involving highly regulated progression through successive stages. These involve commitment to a specific cell lineage, terminal differentiation of lineage-restricted progenitors, and growth arrest. It is apparent that a wide variety of external and internal stimuli influence and modulate lineage choice and differentiation (Bedi and Sharkis, 1995). Retinoids are a group of natural and synthetic vitamin A analogues, and exert important effects on the growth and differentiation of various cell types including hematopoietic progenitors (Douer and Koeffler, 1982; Tobler *et al*, 1986; Smeland *et al*, 1994; Rusten *et al*, 1996). Retinoids function as activating ligands for a class of nuclear receptors that control gene expression programs for a wide range of tissues and organs during embryogenesis and throughout life (Oren *et al*., 2003). 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). In contrast, considerable evidence from other *in vivo* and *in vitro* studies revealed the suppressive effects of retinoic acid (RA) on human mast cell progenitors (Kinoshita *et al*., 2000), growth of bone marrow mesenchymal stem cells (Oliva *et al*., 2003) and erythroid cell differentiation in mouse embryos (Yasuda *et al*., 1989). Mammals have two distinct erythroid lineages. The primitive erythroid lineage originates in the yolk sac and generates a cohort of large erythroblasts that terminally differentiate in bloodstream. The definitive erythroid lineage generates smaller enucleated erythrocytes that become the predominant cell in fetal and postnatal circulation (Kingsley *et al*, 2006). The erythroid cells in Yasuda *et al* study predominantly were primitive erythroid lineage (primitive erythrocytes, nucleated primitive erythroblasts and their progenitors).These progenitor's potential is extremely transient, persisting from Gestation Days (GD) 7.0 to 9.0 and were never detected at later stages. The definitive erythroid lineage differentiated from burst-forming unit erythroid (BFU-E) to colony-forming unit erythroid (CFU-E) which represent a more advanced stage of development within the definitive erythroid lineage and generate small colonies consisting of enucleate erythrocyte (Palis *et al.*, 1999).The results of previous studies are controversial. In addition, the effects of exogenesis All–trans retinoic acid (ATRA) during definitive hematopoiesis have not been described on the definitive erythroid lineage such as definitive erythrocyte and platelets. Therefore this study was performed on GD10 when the definitive erythroid line progenitors have the highest increase and proliferation rate in the yolk sac, bloodstream and the liver (Palis *et al*, 1999) and the mitotic index of yolk sac haematopoietic cells are increased (Kingsley *et al.*,

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2004). Hence, in this study, we have examined the effects of ATRA on the progenitors of red blood cells (RBC) and platelets and survey their outcomes.

MATERIALS AND METHODS

Animals and experimental groups

In this study Wistar strain of rats (about 3 months of age) were used. They were housed in light (12:12 light: dark cycle) and temperature-controlled (21°C) rooms and maintained on laboratory chow and tap water provided ad libitum. Adult virgin females (n = 10) (180-200 g in weight) were mated overnight with males of the same stock. A vaginal plug and smear observed indicated day 0 of pregnancy. Single dose of 100 mg/kg of ATRA (Sigma-Aldrich, USA) suspended in alcohol; corn oil (1:9) mixture (Padmanabhan, 1998) (light-proof containers under yellow light) was administered. The drug was given by oral intubations on the morning of (GD) 10 to experimental group (n=5). The control (n=5) were vehicle treated. The animals were euthanized on GD 18 by ether. The fetus's blood (control and ATRA treated each, n=23) were obtained directly from heart, as placental and mother circulation was continued and subsequently processed for staining (Gimsa) and followed by counting. The embryos weighed and fixed in 10% formalin, examined for external malformation. The blood cells were studied by light microscope and eye piece (x40 for RBC, platelet and x100 for diameter) using cytospin preparation.

STATISTICAL ANALYSIS

The values were expressed as Mean ± SEM. Comparison between two groups was made by student t-test, $p<0.05$ was considered as significant different.

RESULTS

The ATRA-treated group showed a significant decrease in body weight and placenta as compared to control group. All embryos in treated group showed marked malformation on external inspection.

In the treated fetuses on GD 18, the number of RBC per field was 216.8±4.03 with 10.5% decrease in experimental group, compared to control group (242.17±4.80) as was shown in fig. 1. The number of platelets per field was as following: in treated group 11.2±0.64 and in control 13.17±0.70 (fig. 2). There were significant relationships between two groups in RBC and platelet counts. The diameter of RBC and nucleated red blood (NRBC) for treated and control groups were: 6.16 ± 0.08 and 5.9±0.11; 9.000±0.23 and 7.37±0.09, (fig. 3) respectively. The ATRA significantly increased the mean diameter of NRBC.

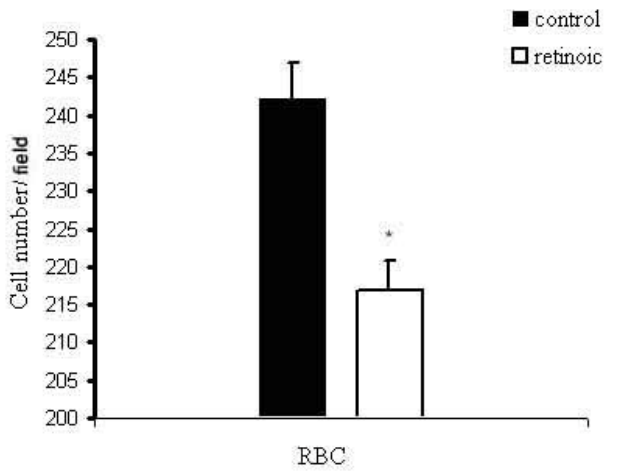

Fig. 1: Effect of ATRA (100mg/kg) on RBC number of rat embryo on GD 18. *P<0.0001 in comparison with control group.

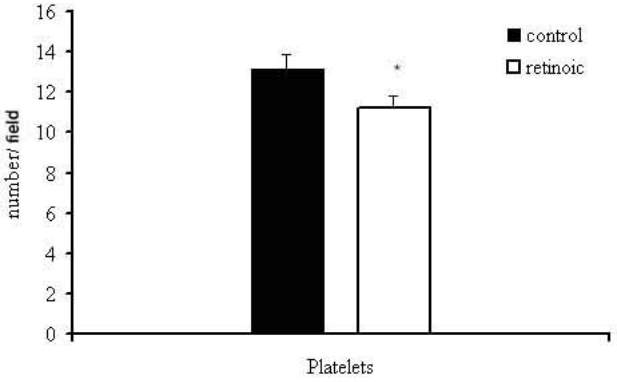

Fig. 2: Effect of ATRA (100 mg/kg) on platelets number of rat embryo on GD 18. *P< 0.04 in comparison with control group.

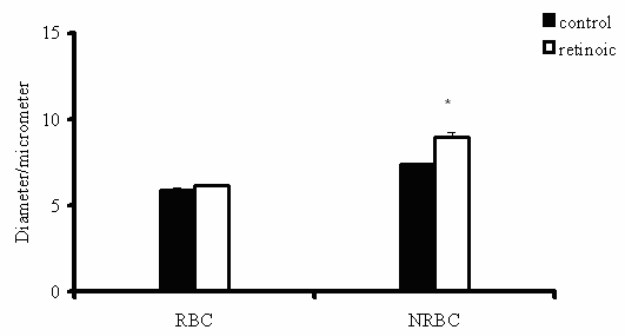

Fig. 3: Effect of ATRA (100 mg/kg) on RBC and NRBC diameter of rat embryo on GD 18, in comparison with control group. The mean diameter of NRBC (*P<0.0001) significantly increased in treated group.

DISCUSSION

Many studies have described that the developmental changes were caused by exogenous ATRA in various embryos (Kuratani *et al*., 1998). In this study, a single

dose of ATRA has been used to identify its effects during hematopoietic differentiation in rat's embryos. The data indicated that ATRA causes reduction in fetal body weight and growth retardation, congenital malformation, and suppression of proliferation and differentiation of the erythroid compartment and platelets.

Red blood cells

Despite the results of Douer and Koeffler *in vitro* study (1982), the present data show the decreased numbers of RBC in rat embryo on GD 18 when exposed to ATRA in utero on GD 10. This is in agreement with those of Yasuda *et al* (1989), in mouse embryos exposed to ATRA in utero on day 8 of gestation, revealed that ATRA suppressed the proliferation of immature erythroblasts, and differentiation of mature primitive erythrocytes. They suggested that ATRA destroys the messenger RNA system. The first blood cells in blood islands in embryo are produced by mesoderm cells of the yolk sac (Ferkowicz *et al*., 2003). Primitive red cells arise from unique primitive erythroid colony-forming cells (EryP-CFC) and definitive red cells arise from committed BFU-E and CFU-E progenitors (Kennedy *et al.*, 1997).

In this study, the samples were obtained on GD18, when 99% of the circulating cells were definitive erythrocytes. These cells first detected on GD11.5 and continue to be a minor component of the circulation on GD12.5. However, by GD 13.5; definitive red cells continue more than 50% of the circulating blood cells. This transition so that by GD17.5, 99% of the circulating cells are definitive erythrocytes (Kingsley *et al*., 2004).

When ATRA was given, the progenitors of definitive erythroid certainly were BFU-E, because they appear beginning on GD 8.25 (Palis *et al*., 1999; Wong *et al*., 1986) and expand in numbers over the next 48 hours in the yolk sac (Brotherton *et al*., 1979). Their numbers increase in the bloodstream between on GD 9.5 to 10.5 and within the liver soon after it emerges as a hematopoietic organs (GD10) (Brotherton *et al*., 1979; Kingsley *et al*., 2004).

In addition to BFU-E, in this time, the other progenitors of definitive erythroid are detected as CFU-E**.** These progenitors are first found in the yolk sac only after appearance of BFU-E [(Brotherton *et al*., 1979; Kingsley *et al*., 2004) on GD 9.5. By GD 10.5, high numbers of them are found in both the bloodstream (Palis *et al*., 1999) and the liver rudiment (Palis *et al*., 1999; Brotherton *et al*., 1979; Kingsley *et al*., 2004) along with maturing definitive erythroblasts [(Brotherton *et al*., 1979; Kingsley *et al*., 2004). Thus ATRA must suppress proliferation and differentiation of the definitive erythroid progenitors especially CFU-E during progressive increase in numbers in the yolk sac, bloodstream and embryo proper. CFU-E represents a more advanced stage of development within the definitive erythroid lineage and generates small colonies consisting of enucleate erythrocyte (Palis *et al*., 1999).

Moreover definitive erythroid lineage, on GD 10, it is likely that the nucleated primitive erythroblasts undergo progressive maturation, transitioning from proerythro-blasts on GD 8.5 to polychromatophilic erythroblasts by GD 11.5. They mature from proerythroblasts on GD 8.5 to orthochromatic erythroblasts on GD 12.5 to 15.5 (Kingsley *et al*., 2004), the vast majority of them lose their nuclei between on GD 12.5 to16.5 (McGrath *et al*., 2005) and terminally differentiate in the bloodstream and ultimately become enucleated primitive erythrocytes (Kingsley *et al*., 2004). Thus, probably the inhibition was occurred in progressive maturation of the nucleated primitive erythroblasts, not their progenitors. Because the progenitors of primitive erythroid cells emerge at mid to late primitive streak stages on GD 7.25 in the developing yolk sac, their numbers increase more than 7-fold, then declined sharply to undetectable levels by GD 9.0 (Palis *et al*., 1999).

Platelet

The decreased numbers of platelets on GD 18, probably results from inhibitory effects of ATRA on mega-karyocyte progenitors (Meg-CFC) on GD 10.Because megakaryocyte progenitors (Meg-CFC) expands in the yolk sac along with BFU-E between on GD 8.5 to 10.5. (Palis *et al*., 2006) and megakaryocyte cells are evident in the yolk sac beginning on GD 9.5, while circulating platelets are detected in the embryonic blood stream, as early as GD 10.5 (Tober *et al.*, 2007). This mean is that inhibition occurred before formation of platelet.

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 (primitive-MEP) that both emerge from hemangioblast precursors during gastrulation (Palis *et al.*, 2006). The Megakaryocyte lineage is closely associated with erythroid lineages throughout ontogeny (Tober *et al*, 2007).

Cells diameter

In this study, ATRA did not effect the cell size (except NRBC). This is in contrast to the study of Kinoshita *et al*. (2000) who indicated that RA diminished the mean diameter of culture of mast cells. The size of a cell depends on intrinsic and extrinsic (nutrients, temperature) factors. Cell size is also influenced by the number of genome sets. Ribosome biogenesis also appears to be an important regulator of cell size in multicellular organisms (Hafen and Stocke, 2003). Although the mechanisms responsible for the differences in cell size among different

red cell lineages are not well understood, they may relate to differential rates of cell division (Kingsley *et al.*, 2004).

In conclusion, this study shows that administration of exogenous ATRA (100 mg/kg) at 10^{th} day of GD may have negative effects on proliferation, differentiation and maturation of the definitive erythroid compartment and platelets, without having any deleterious effects on the dimenation of RBC.

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