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ORIGINAL ARTICLE

The effect of different doses of retinyl palmitate (vitamin A) on placental volume in rats

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Abstract

Vitamin A and its derivatives are essential for embryonic development, but an overdose of vitamin A is toxic to the offspring. The placenta is an interface that nourishes and protects the embryo. Although there are numerous publications on the effect of vitamin A on the placenta, there is insufficient information on the changes in the morphology of this organ caused by different doses. The aim of our study was to demonstrate the effect of retinyl palmitate administered at different doses on the volume of the placenta and its component, the decidua. Pregnant rats were divided into 6 groups between gestation day 10 and 12. The first group received 10.000 IU/kg, the second group 20.000 IU/kg, the third group 50.000 IU/kg, the fourth group 100.000 IU/kg and the fifth group 200.000 IU/kg oral vitamin A. The control group received 1 ml of corn oil on the same days. On day 19 of gestation, placentas were collected and 5 µm sections were stained with *Massons's trichrome*. The volumes of total placenta and decidua were estimated using the *Cavalieri* volume estimation method. All placental volumes of the experimental groups were larger than those of the control groups. The decidual volume increased abruptly at a dose of 50.000 IU/kg and remained higher than the control volume at higher doses. The ratio between the decidual and placental volumes increased at 50.000 IU/kg and was smaller than the control at 200.000 IU/kg. A large placental volume is thought to be an indicator of placental insufficiency. Although our results suggest that an increase in placental volume above 10.000 IU/kg may have a negative effect on placental function, we conclude that the imbalance between decidual and placental volume above 50.000 IU/kg strengthens the suggestion that placental insufficiency may have increased after this dose.

Keywords: Vitamin A, placenta, volume

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Introduction

Vitamin A and its derivatives (retinoic acid (RA), retinoids) are essential for the regulation of important biological functions such as cell division, differentiation, growth, development and immunity. Vitamin A is essential for the maintenance of healthy growth [1,2]. Multivitamin supplements are routinely given to pregnant women to prevent congenital malformations [3]. Retinoids are very important for maternal health; they are absolutely necessary for the development of the placenta and the developing embryo. RA the retinoid synthesized in embryonic tissues essentially controls the expression of numerous important target genes for development. Alterations in the levels of RA result in abnormal embryonic development. Studies in animal models have shown that severe deficiency of maternal vitamin A leads to early embryonic death [4]. Fetal developmental abnormalities are observed when vitamin A deficiency occurs at lower levels [5]. On the other hand, vitamin A overdose is teratogenic in the embryo and has membranolytic and hepatotoxic effects in adults [6]. Studies conducted in recent years have shown that vitamin A doses that do not cause macroscopic morphological abnormalities can lead to behavioral disorders and mental insufficiency [7]. Since the embryo cannot synthesize vitamin A on its own, the developing mammalian embryo receives vitamin A through a maternal-fetal barrier, the placenta [8]. The presence of measurable fetal liver vitamin A at birth demonstrates the effectiveness of the placenta in transporting retinol during pregnancy [9,10]. It is evident that retinol crosses the placenta, but the mechanism of transfer to the fetus and how it is transferred is not fully understood [11].

The ability of retinoids to cross the placental barrier in humans, mice, rats, and monkeys has been investigated in previous studies [10,12-14]. These studies show that the transfer of each vitamin A derivative to the embryo is supported in a particular way. It has also been found that the placenta is capable of metabolizing some retinoids and producing retinoids from maternal precursors [15]. It has been suggested that the placenta serves as a vitamin A depot until the embryonic liver becomes functional. In addition, it has been suggested that the placenta acts as a retinoid buffer by releasing retinoids when maternal retinoid uptake is insufficient and stores retinoids to protect the embryo from the potential toxic effects caused by excess maternal retinoids [16].

The placenta is known to harbor nuclear retinoid receptors. In addition, RA inducible genes (stimulated by retinoic acid or Stra) are expressed in placental regions involved in maternal-embryonic exchange [4]. Therefore, it is important to study the effects of retinoids on the placenta.

Understanding the differentiation and morphology of the placenta in various experimental animals is undoubtedly necessary to understand human development. The rat placenta consists of three main parts: the basal zone, the decidual zone, and the labyrinth zone. The basal zone is located near the decidual layer and contains various cell types. These cells are trophoblast stem cells, trophoblast giant cells, and glycogen cells. The labyrinthine zone contains the maternal cavities and blood vessels. Although the uteroplacental compartment in rats has similarities and differences to the hemochorial placental species, they are most similar to mice. This has led to the use of many different terms to describe the components of the uteroplacental compartment [17]. The direction of the uteroplacental compartment is determined by the place where the blood enters the uterus. This area is called the mesometrial compartment and the other end is called the anti-mesometrial compartment. The mesometrial compartment of the uterus is composed of stromal cells, blood vessels (endothelial cells, smooth muscle cells), immune/inflammatory cells (macrophages), myometrial smooth muscle cells, and trophoblast cells [18]. The cellular composition of this compartment varies depending on the stage of gestation and species-specific characteristics. After implantation, natural killer (NK) cells proliferate and infiltrate into the mesometrial decidua adjacent to the developing chorioallantoic placenta. Decidual cells are derived from uterine stromal cells and have different functions depending on their location [19-21].

The development and function of decidual cells of rats and other species have been the subject of curiosity of researchers for many years. The decidua generally provides trophoblastic nutrition and serves as an immunological barrier, but its specific functions are not known. The cell division and cell cycle of the decidua have not been studied in detail. However, little is known about how decidua cells compensate for survival, stress, and other adverse conditions [22].

There are few morphological studies on the effects of vitamin A on the placenta and decidual layer. Therefore, we aimed to investigate the effects of doses of vitamin A, considered teratogenic and non-teratogenic to the fetus, on the placenta and decidual layer using stereological techniques.

Materials and Methods

This study was approved by the local animal experimentation ethics committee (#951-172). Female *Wistar* albino rats weighing 250-300 g were divided into 6 groups. Rats were housed in cages with a capacity of 5-6 rats and access to food and water ad libitum at constant room temperature (21°±3°C) and a cycle of 12 hours of darkness and 12 hours of light. All animals were obtained from Eskişehir Osmangazi University Medical and Surgical Experimental Animals Research and Application Center (TICAM). Menstruation of rats was determined by vaginal swabs. Females in estrus were kept with males for copulation. The next morning, females with sperm in the vaginal smears were considered pregnant and on the first day of gestation (P0).

On days P10-P12, the first group received 10.000 IU/kg, the second group 20.000 IU/kg, the third group 50.000 IU/kg, the fourth group 100.000 IU/kg, and the fifth group 200.000 IU/kg oral vitamin A (retinyl palmitate, Merck, Darmstadt-Germany) diluted in corn oil. [23]. 0.5 ml of the dose-adjusted vitamin A mixture was administered to the subjects with a gavage needle. The control group received 1 ml of corn oil on the same days. At P19, the rats were anesthetized, the placenta was removed by cesarean section, and the mothers were sacrificed. Ten placentas were randomly selected from each group. After histological procedures sections of 5 μm were stained with *Masson's trichrome* (Fig. 1). The volumes (Vp) of the placenta and the volume of the decidua basalis (Vd) were estimated using the *Cavalieri* volume estimation method, and their ratios to each other (Vd/Vp) were calculated.

The groups' data series were subjected to the *Kolmogorov-Smirnov* test, and after ensuring that the series were normally distributed, the groups were compared using a one-way ANOVA. Jamovi 2.3.21 software was used for the calculations.

Results

When the calculated placental volumes (Vp) were compared, it was found that the placental volumes of the experimental groups were higher than those of the control group. While this difference was significant when comparing with the 10.000 IU/kg group (p<0.05), it was very highly significant when comparing the control group with the other groups (p<0.001). When the volumes of the 10.000 and 20.000 IU/kg groups were compared with those of the other experimental groups, they were found to be smaller than the volumes of the 50.000 and 200.000 IU/kg groups (p<0.001 resp. p<0.01). At

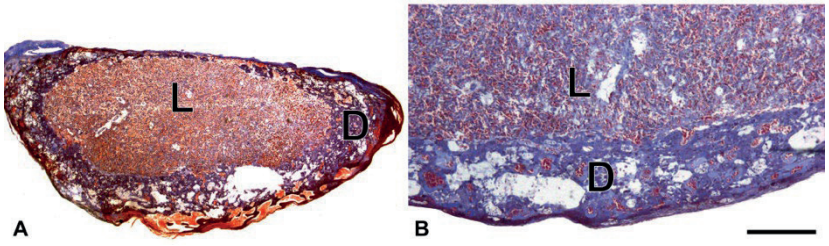

Figure 1. Massons's trichrome staining of rat placenta. The left image shows the placenta at low magnification (A). The red-brown area is the labyrinth, and the purple-red area is the decidua (scale 1.000 μm). The right image is a higher magnification of the left image (B). The red-purple area is the labyrinth, the purple area is the decidua (scale 160 μm). L: labyrinth, D: decidua.

the same time, it was calculated that the placental volume of the 100.000 IU/kg group was greater than that of the 10.000 IU/kg group (p<0.05) (Fig. 2).

When decidual volumes (Vd) were compared, it was found that the decidual volumes of the groups receiving retinyl palmitate at a dose of 50.000 IU/kg and more were higher than those of the control group. While this difference was significant when comparing the control group and the groups receiving 200.000 IU/kg group (p<0.05), it was very highly significant when comparing the control group with the other experimental groups (p<0.001). When comparing the experimental groups with each other, the highest decidual volume was calculated for the 50.000 IU/kg group (p<0.001). While the decidual volume of the 10.000 IU/kg group, which was not different from the control group, was smaller than the decidual volumes of the 100.000 IU/kg

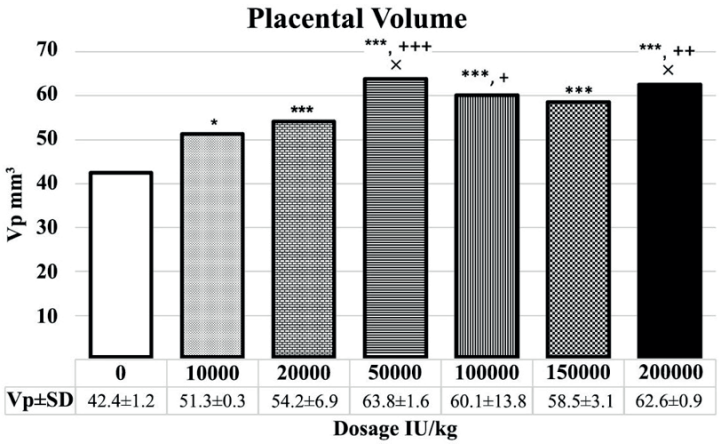

Figure 2. Graph and table showing total placental volume and statistical significances between groups (n=10)**.** The comparison of the control group with the other groups *; the comparison of the group given 10.000 IU/kg with higher doses + and the comparison of the group given 20.000 IU/kg with higher doses are represented by ×. One symbol means p<0.05, two means p<0.01, three means p<0.001.

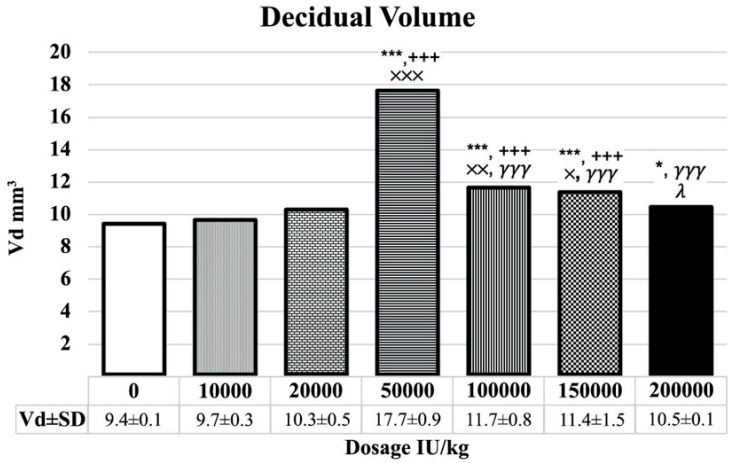

Figure 3. Graph and table showing decidual volumes and statistical significances between groups (n=10). The comparison of the control group with the other groups *; the comparison of the group given 10.000 IU/kg with higher doses +; the comparison of the group given 20.000 IU/kg with higher doses ×; the comparison of the group given 50.000 IU/kg with higher doses γ; and the comparison of the group given 100.000 IU/kg with higher doses are represented by λ. One symbol means p<0.05, two means p<0.01, three means p<0.001.

and 150.000 IU/kg groups (p<0.001), no difference was calculated for the 200.000 IU/kg group. Another group whose decidual volume did not differ from the control group was the 20.000 IU/kg group. While the decidual volume of this group was smaller than that of the 100.000 (p<0.01) and 150.000 IU/kg (p<0.5), there was no difference between the decidual volume of the 200.000 IU/kg group. No difference was calculated between the decidual volumes of the 100.000 and 150.000 IU/kg groups. However, although there was no difference between the decidual volume of the 200.000 IU/kg group and that of the 150.000 IU/kg group, the decidual volume of the 200.000 IU/kg group was smaller than that of the 100.000 IU/kg group (p<0.05) (Fig. 3).

When the ratio obtained by dividing the decidual volume by the placental volume (Vd/Vp) was examined, it was found that the ratio was significantly higher in the 50.000 IU/kg group in all groups (p<0.001). While no difference was generally observed between the other groups, the Vd/Vp ratio of the 200.000 IU/kg group was lower than that of the control group (p<0.001) and the 100.000 (p<0.05) group (Fig. 4).

Discussion

Our results show that placental volume increases with the dose of retinyl palmitate. A highly significant increase in placental volume is observed compared to the control group, especially above a dose of 20.000 IU/kg. Studies have shown that the placenta is a target organ for retinoids [24]. Wen et al (2011) noted that a large placental volume is an indicator of both low vascular resistance (low density) and placental insufficiency [25]. We are unable to obtain direct data on whether the increase in volume at doses below 50.000 IU/kg is related to placental insufficiency and whether it adversely affects fetal nutrition. Considering that the nervous system is the first organ affected by fetal malnutrition, our previous study may shed light on this problem. In this study, pregnant rats were given different doses of oral retinyl palmate, and it was observed in the study that the number of hippocampal neurons decreased from 20.000 IU/kg. However, an increase in the number of dividing cells was observed up to 50.000 IU/kg, while the number of apoptotic cells increased after this dose [26]. Also, in a study we conducted on the effect of vitamin A on the fetal liver, we observed that cell death

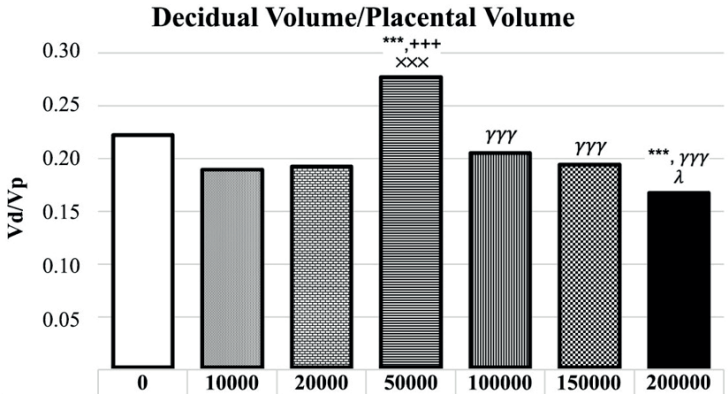

Figure 4. Graph and table showing the ratio of decidual volume and placental volume and statistical significance between groups (n=10)**.** The comparison of the control group with the other groups *; the comparison of the group administered 10.000 IU/kg with higher doses +; the comparison of the group administered 20.000 IU/kg with higher doses ×; the comparison of the group administered 50.000 IU/kg with higher doses γ; and the comparison of the group administered 100.000 IU/kg with higher doses is represented by λ. One symbol means p<0.05, two means p<0.01, three means p<0.001.

in the liver increased at doses above 10.000 IU/kg [27] there are few studies on the microscopic effects of these doses on the organism. Based on the information in the above two articles, the increase in placental volume at doses of 10.000 and 20.000 IU/kg supports the notion that vitamin A promotes cell proliferation. However, when the effect of vitamin A on the fetal liver is examined, it is found that cell death increases above 10.000 IU/kg. It is not known whether this effect is due to compensation of a toxic effect of vitamin A or to malnutrition of the placenta. However, considering the results of the two studies, we think that the reason for the high placental volumes observed after 50.000 IU/kg could be placental insufficiency associated with vitamin A toxicity.

When examining the decidual volumes in our findings, the observed values support this suggestion. The decidual volumes peaked at 50.000 IU/kg and then abruptly decreased. However, decidual volumes were higher at doses higher than 50.000 IU/kg than in the control group. Ozaki et al (2017), concluded that decidual cells are very sensitive to retinoids. In their study, they showed that the amount of active retinoid in the placenta is kept under control by the RDH12 (retinaldehyde dehydrogenase 12) and DHRS3 (short chain dehydrogenase/reductase 3) genes of decidualized endometrial cells. The authors concluded that despite these compensatory mechanisms, high doses of retinoids are toxic to decidualized cells [28]. The fact that the volume of placental and decidual cells in our study was higher than in the control group, especially after 50.000 IU/kg, suggests that cells may have proliferated here to compensate for the high dose of retinoids. Comparing the volumes of decidual and placental cells determined in our study (Vd/Vp), the fact that this rate is lower in the 200.000 IU/kg group, supports the view that this dose is toxic to decidual cells. However, to support this view, stains with markers for cell division and cell death must be used.

Lee et al (2012), observed that high doses of RA caused the same malformations in some organs as RA deficiency. They attributed this effect to the increase in levels of enzymes that degrade retinoids in teratogenic doses of RA [29]. A study three years later showed that high doses of retinoids increase the synthesis of molecules that degrade retinoids in the placenta and embryo [30]. A study on the effects of vitamin A deficiency on the placenta found that deficiency of this vitamin induces cell death in the placenta. They concluded that this effect is caused by infiltration of neutrophils [31]. Deepak et al (2019), concluded that low placental levels of RA downregulate the vascular endothelial growth factor-1 (VEGF-1) receptor. They also showed that this increased TFAP2 (transcription factor AP-2-alpha [activating enhancer-binding protein 2 alpha]) levels [32] It was concluded that increased TFAP2 levels contributed to preeclampsia and inadequate placental development [33]. In our study, the decrease in Vd/Vp at doses higher than 50.000 IU/kg and especially in placental volume at a dose of 200.000 IU/kg suggests that this decrease in placental volume is due to cell death, especially due to the increase in enzymes that degrade retinoids.

Conclusion

As can be seen from the literature, the effect of retinoids on the placenta is complex and multifactorial. Our study showed that oral retinyl palmitate at different doses caused an increase in placental volume, except for the decidual volume of the 200.000 IU/kg group, which was even lower than the decidual volume of the control group. Although an increase in placental volume was observed from 10.000 IU/kg, the ratio between the decidual volume and the placental volume differed from that of the control group after 50.000 IU/kg, suggesting that the placenta began to lose its optimal functionality from this dose.

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Conflict of interest

The authors have no competing interests to disclose.

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