

HYPERVITAMINOSIS A-INDUCED CENTRAL NERVOUS SYSTEM DEFECTS

MEHDI JALALI, Ph.D., AND ALIREZA FAZEL, Ph.D.

*From the Department of Anatomy, School of Medicine, Mashhad University of Medical Sciences,
Mashhad, Islamic Republic of Iran.*

ABSTRACT

In this investigation the effects of excess vitamin A administration during the early embryonic period were studied. Intramuscular injection of a single dose of 15000, 20000 or 25000 IU/kg vitamin A to pregnant Balb/c strain mice on either day 7, 8, 9 or 10 of gestation (vaginal plug= day 0 of gestation) produced major malformations in the central nervous system (CNS) including exencephaly, hydrocephalus, microcephalia, spina bifida and myelocystocele and also a few other defects such as limb malformations. The incidence and severity of these malformations was positively correlated with the dosage and time of exposure. Among experimental groups, the most effective dose of vitamin A which produced a high incidence of CNS defects was 25000 IU/kg injected on day 8 of gestation (35%) compared to the control group (0%). Histological studies on 18 day old experimental fetuses revealed spina bifida with and/or without spinal cord defect. These studies showed excessive embryonic cell death localized in the neural tube region following vitamin A exposure. In conclusion, excess vitamin A exposure in the early days and critical periods of development may interfere with certain developmental phenomena, resulting in various detectable CNS defects among newborn infants.

MJIRI, Vol. 12, No. 3, 279-283, 1998

Keywords: Vitamin A, neural tube defect, neurotation.

INTRODUCTION

Vitamin A or retinol is a polyisoprenoid compound containing a glycohexenyl ring. This vitamin is essential for the proper maintenance of the functional and structural integrity of epithelial cells. It has also been used for treatment of skin disorders such as acne. Vitamin A and its derivatives such as retinoic acid have been shown to act as a morphogen,³ and so it may play an important role(s) in neurotation. The teratogenic effects of vitamin A upon the developing embryo were first reported by Cohlan in 1953.² Further investigations have revealed that both an excess and a deficiency of vitamin A during early pregnancy may result in various malformations.^{7-9,18} Vitamin A deficiency in the maternal

diet produced a spectrum of congenital malformations such as ear and eye, urogenital, cardiovascular, and CNS defects. In addition to these abnormalities, other defects were detectable after excess vitamin A uptake near developing fetuses and neurotation.^{8,9,11,19}

The purpose of the present study was to determine if only one dose of vitamin A can produce similar defects in experimental animals. And if so, at what time of development this might occur.

MATERIALS AND METHODS

Young Balb/c strain mice maintained on controlled conditions (room temperature (25±1°C) and 12 hour light-



Fig. 1. Lateral view of the experimental embryo whose mother received 25000 IU/kg vitamin A on day 8 of gestation, showing complete exencephaly (star). $\times 14$.



Fig. 2. Lateral view of experimental embryo whose mother received 20000 IU/kg vitamin A on day 9 of gestation, showing exencephaly (arrow). $\times 14$.

dark cycle) were used. Three females weighing 30-35 gr were placed with one male of the same strain for 3 hrs at the beginning of the light cycle. Females with the presence of vaginal plugs were separated and placed in a different cage. The time of plug detection was considered as day zero of gestation.

The pregnant females were divided into control and experimental groups. The experimental group was further subdivided, based on the dose of vitamin A administered (15000, 20000 or 25000 IU/kg) and the exposure time (7th, 8th, 9th or 10th day postmating) (Table I). Each mouse of the experimental group received only a single intramuscular injection of vitamin A (Table I) while the control mice remained untreated. Pregnant females were sacrificed by cervical dislocation on early day 18th of gestation. Their abdominal cavities were opened. Uteri were exposed and conceptuses harvested. The number of dead or absorbed fetuses was reported. The survivors were evaluated for gross malformations.

All specimens were fixed in bouin or 10% formalin solution. For histological studies, specimens were processed with alcohol and embedded in paraffin, and eight micron sections were cut serially and stained with hematoxylin and



Fig. 3. Lateral view of experimental embryo whose mother received 15000 IU/kg vitamin A on day 9 of gestation, showing exencephaly (arrow). $\times 13$.



Fig. 4. Histological transverse section of control embryo on day 18 of gestation. Arrow= lamina; star= pedicle. H&E \times 80.

eosin. A minimum of four specimens were randomly selected for light microscopy analysis.

RESULTS

Using the dissecting microscope, all experimental and control fetuses were examined on day 18 of gestation. Fetal characteristics, incidence and severity of malformations were determined as illustrated in Table I. External examination of fetuses revealed a high incidence of CNS malformations among vitamin A exposed fetuses as compared with control groups.

These data showed that the most effective dose which could produce a high percentage of CNS malformations was 25000 IU/kg of vitamin A and the time of exposure was early day 8 of gestation. The CNS defects included exencephaly, hydrocephalus, microcephalia, spina bifida and myelocystocele (Figs. 1-3).

Data obtained from histological studies of the lumbar vertebrae of control and experimental fetuses showed a thin layer of connective tissue covering the vertebral canal and spinal cord. The vertebral laminae and superficial tissues were all missing. In other fetuses the vertebral arc was affected and pedicles were displaced laterally (Figs. 6,7). Furthermore, in some specimens, the central canal of the spinal cord was dilated and an attenuated roof plate was seen (Fig. 6). In some samples the roof plate was ruptured and the

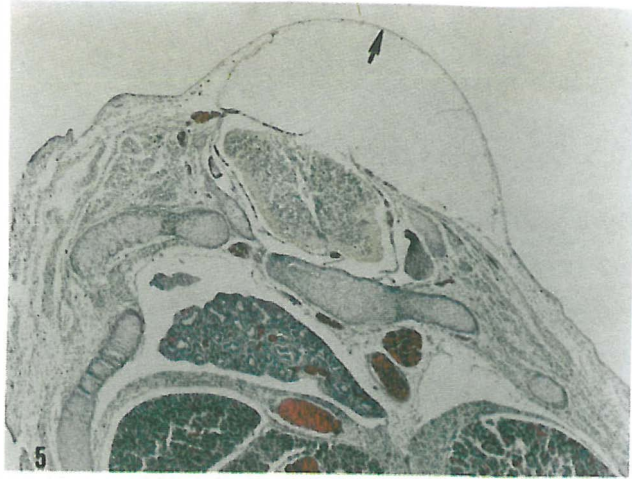


Fig. 5. Histological transverse section of experimental embryo whose mother received 20000 IU/kg vitamin A on day 9 of gestation, showing a meningocele. Arrow= skin and meninge. H&E \times 90.

affected area was covered by meningeal membrane and skin (Figs. 6,7). A low incidence of meningocele was observed among treated animals (Fig. 5).

Examination of control and treated litters on day 18 of gestation revealed that the incidence of resorption was increased significantly when the highest dose of vitamin A had been administered. Our data indicated that in all experimental groups the mean weight of fetuses was less than controls (1.27 g vs. 0.94 g for control and experimental fetuses, respectively).

DISCUSSION

The aim of this study was: (1) to determine the critical morphogenic period of neurulation in which excess vitamin A could produce CNS malformations, and (2) to find out the lowest teratogenic dosage of vitamin A which might produce a similar range of malformations among human infants. Based on our results, the lowest effective dose of vitamin A was 15000 IU/kg on early day 8 of gestation, while the most effective dose was 25000 IU/kg on early day 8 of gestation.

Previous studies have shown that excess vitamin A and its derivatives such as retinoic acid act as teratogens.^{9,11,19} It seems that when vitamin A enters the cell, it combines with cellular retinol binding protein (CRBP). This protein acts as an intracellular receptor which has a specific site for retinol. When binding occurs, the complex of retinol and protein receptor enters the nucleus. This complex has the ability to attack a specific site on DNA and alter the pattern of gene activities.

In this way it may activate or suppress the gene. Any change in transcriptional activity of the gene might lead to drastic changes in cytoskeletal proteins, surface receptors

Vitamin A-Induced CNS Defects



Fig. 6. Histological transverse section of experimental embryo whose mother received 25000 IU/kg vitamin A on day 8 of gestation, showing a myelocystocele. Dorsal arch and adjacent tissue are absent. Star= skin; circle= dilated central canal; arrowhead= attenuated roof plate. H&E $\times 70$.



Fig. 7. Histological transverse section of experimental embryo whose mother received 20000 IU/kg vitamin A on day 9 of gestation, showing a myelocystocele. Circle= dilated central canal; arrow= ruptured roof plate. H&E $\times 70$.

Table I. Effects of excess vitamin A treatment in different experimental as well as control groups.

Time of administration	Dose IU/kg	No. of litters	No. of fetuses	Spina bifida (%)	Exencephaly (%)
Day 7	15000	6	53	4	9
Day 7	20000	9	71	10	13
Day 7	25000	8	59	12	17
Day 8	15000	10	72	7	18
Day 8	20000	7	42	15	21
Day 8	25000	9	61	21	28
Day 9	15000	11	83	3	4
Day 9	20000	8	56	5	7
Day 9	25000	9	73	10	14
Day 10	15000	10	85	0	0
Day 10	20000	11	93	1	1
Day 10	25000	8	57	2	0
controls	0	17	183	0	0

and extra-cellular matrix (ECM) as well.⁹⁻¹¹ The ECM has an important role(s) during morphogenesis, therefore a decrease in its hyaluronate content might cause neural tube defects. Experimental studies have shown that administration of hyaluronidase inhibits the fusing of neural groove edges during early embryogenesis.^{4,13}

It is also known that contraction of the microfilaments in neuroepithelial cells forms the wedge appearance of the apical surface of the neuroepithelial cell and this leads to folding of the neural plate. Drugs such as vinblastine and cytochalasin inhibit the contraction of apical microfilaments which result in unfolding of the neural plate.^{12,20} Furthermore, Ca⁺⁺ is a necessary element for contraction of the apical microfilaments, since exposure to papaverin followed by Ca⁺⁺ deficiency has been shown to cause neural tube defects.¹⁶

Also, investigations have shown that retinoic acid administration to developing embryos causes ventral bending of the neural tube and changes the position of the posterior neuropore.¹⁸

Programmed cell death (PCD) has been recognized as an essential event(s) in morphogenesis. During the neurolation of the vertebrate nervous system, about fifty percent of the neuronal population undergoes PCD at the time of their functional connection to target tissues. Neurotrophic factors released by target or other cells are thought to be the principal agents controlling neuronal survival during and after PCD. Cell death also occurs in other parts of the developing embryo such as the primitive streak, the hindgut endoderm and the lateral plate mesoderm which may also induce growth imbalance that leads to failure of neurolation.^{1,5,6,16} In conclusion, it may be stated that excessive vitamin A and its derivatives may alter ECM contents during the critical stages of morphogenesis, resulting in excessive PCD in the neural tube and a different range of defects.

Women who are pregnant or who might become so should carefully control their intake of vitamin A both in regard to rich food sources and vitamin A supplements. The average intake for vitamin A is about 5000 IU per day. In cases where supplementation is advisable, especially during the critical period for exposure which appears to be from the third to the fifth week of human development, the total daily intake should not exceed 10000 IU (10 µmol) of vitamin A.^{13,17}

REFERENCES

- Alles AJ, Sulik KK: Retinoic acid-induced spina bifida: evidence for a pathogenetic mechanism. *Development* 108: 73-81, 1990.
- Cohlan SW: Excessive intake of vitamin A as a cause of congenital anomalies in the rat. *Science* 117: 535-536, 1956.
- Collins M, Eckhoff C: The teratogenicity of retinol and the reduced metabolic formation of all-trans-retinoic acid in the mouse. *Arch Toxicol* 66: 52-6, 1992.
- Copp AJ, Brook FA, Roberts HJ: A cell specific abnormality of cell proliferation in mutant mouse embryos developing spinal neural tube defects. *Development* 104: 285-295, 1988.
- Eastman A: Apoptosis: a product of programmed and unprogrammed cell death. *Toxicol Appl Pharmacol* 121: 160-164, 1993.
- Franklin JL, Johnson EM: Block of neuronal apoptosis by a sustained increase of steady state free Ca⁺⁺ concentration. In: Dexter TM (ed.), *The Role of Apoptosis in Development*. Manchester: Chapman and Hall, pp. 15-20, 1995.
- Gilbert SF: Development of the nervous system. In: Walbet V, Holder N, (eds.), *Developmental Biology*. New York: Sinauer Assoc., pp. 518-550, 1987.
- Ehlers K, Sturje H: Spina bifida aperta induced by valproic acid and by all-trans-retinoic acid in the mouse. *Teratology* 46: 117-130, 1992.
- Maden M: Distribution of cellular retinoic acid binding proteins I and II in the chick embryo and their relationship to teratogenesis. *Teratology* 50: 294-301, 1994.
- Malcolm M, Nigel H: The involvement of retinoic acid in the development of the vertebrate central nervous system. *Development (Suppl)* 2: 77-94, 1991.
- Moro-Balbos JA: Retinoic acid induces changes in the rhombencephalic neural crest cell migration and extracellular matrix composition in chick embryos. *Teratology* 48(3): 197-206, 1993.
- Morriss-kay G, Wood H, Chen W: Normal neurolation in mammals. *Ciba-Found Symp* 181: 51-69, 1994.
- Moore KL, Persaud TVN: The causes of human congenital malformation. In: Moore KL, Persaud TVN, (eds.). *The Developing Human*. 6th Edition, Philadelphia: W.B. Saunders Co., pp. 167-200, 1998.
- Morriss-Wiman J, Brinkley L: Changes in mesenchymal cell and hyaluronate distribution correlate with *in vivo* elevation of the mouse mesencephalic neural folds. *Anal Record* 226: 383-395, 1990.
- Mundle SD, Raza A: The two *in situ* techniques do not differentiate between apoptosis and necrosis but rather reveal distinct patterns of fragmentation in apoptosis. *Lab Invest* 72: 611, 1995.
- Nagele RG, Pietrolungo TF, Lee H: Studies on the mechanisms of neurolation in the chick. The intra-cellular distribution of Ca⁺⁺. *Experientia* 37(3): 304-6, 1981.
- Olson JA: Vitamin A, Retinoids and Carotenoids. In: Shild ME, Olson JA, Shike M, (eds.). *Modern Nutrition in Health and Disease*. 8th edition, Philadelphia: Lea & Febiger, pp. 287-306, 1994.
- Osmond MK, Butler AJ: The effects of retinoic acid on heart formation in the chick embryo. *Development* 113: 1405-17, 1991.
- Rothman KJ, Moore LL, Singe MR: Teratogenicity of high vitamin A intake. *N Engl J Med* 333(21): 1369-73, 1995.
- Smith JL, Schoenwolf GC, Quan J: Quantitative analyses of neuroepithelial cell shapes during bending of the mouse neural plate. *J Comp Neurol* 342(1): 144-151, 1994.

