Retinoic Acid & Experimental Design Laboratory

Motivation – Vitamin A deficiency claims the lives of over 600,000 children under the age of 5 annually\(^1\). Maternal vitamin A deficiency leads to significant birth defects, resulting in elevated mortality of both fetus and mother\(^2\). Similarly, over exposure to vitamin A can cause birth defects, and lethal toxicity in adults. In order to develop vitamin A deficiency rescue strategies for infants, over expression effects on developing embryos need to be investigated.


Research Question: What is the effect of excessive vitamin A exposure on embryonic development? State an initial hypothesis, complete background research activity, and then state revised hypothesis.

I initially hypothesize ....

After background research, I hypothesize....

Activity # 1 Background Research Analysis

One of the first steps in scientific research is surveying the current body of research literature to determine what is already known. In the following exercise, you will read excerpts from three research articles on vitamin A induced birth defects in animal models and human clinical studies. Answer the questions following each excerpt, then return to the above “Research Question” and formulate a revised hypothesis.
Excerpt #1 – Gene Regulation and Synthesis of Vitamin A

“Vitamin A acts through one of the most powerful and pervasive biological systems that exist for the regulation of gene expression. Many genetically encoded proteins are required for retinoid uptake, metabolic conversion, storage, transport, intracellular binding, and transcriptional regulation (Fig. 1). RA binds to 14 different RA-receptor proteins that regulate transcription either as heterodimers with each other or with other members of the nuclear receptor gene family, including the receptors for thyroid hormone, vitamin D, and peroxisome proliferator–activated receptor (PPAR). Many of the retinoid-linked nuclear receptors are widely expressed throughout the brain in an overlapping fashion. Because of the large number of receptor [forms] and the enormous number of different [pairing] combinations, RA-dependent processes can be influenced by a vast range of combinatorial receptor constellations. The expression of many brain proteins is known from in vitro studies to be potentially regulated by RA. Because specific RA actions in vivo depend on the cellular and developmental context, however, one cannot infer from the in vitro data which of the many possible [pathological] RA regulation[s] take place at a particular site and developmental stage in the brain.

The effects of perturbations on the elaborate retinoid system underline its importance. For instance, vitamin A deficiency during early development leads to severe malformations of different organs, which tend to phenocopy genetically caused or sporadically occurring deformities, such as inborn heart, lung, kidney, skeletal, and eye defects. This indicates that RA exerts a critical regulatory role on many of the genes that establish the basic body plan in the early embryo. One of the oldest questions in retinoid research concerns what determines the sites of RA actions and the locations of the defects caused by vitamin A deficiency. Most components of the retinoid-signaling pathway were believed at some time to be the critical spatial determinants of RA function, until experimental elimination of their genes revealed inconsistencies in this interpretation of their role. For the most part, mice lacking a single one of the retinoid genes (RA receptors, retinoid-binding proteins, or retinoid converting enzymes) appear more or less normal. When different knockout mice are bred together, however, the compound mutants exhibit all the symptoms and malformations found in vitamin A–deprived embryos and known collectively as the fetal vitamin A–deficiency syndrome. The effects of different retinoid mutations are additive and are exacerbated by nutritional vitamin A deprivation. The retinoid genes whose elimination causes the most severe vitamin A–deficiency syndrome are the retinaldehyde dehydrogenases (RALDHs), the enzymes that create high RA concentrations locally. In contrast to the widespread expression of the RA receptors, expression of the RALDHs is restricted to a few sites, with most intervening tissue being totally free of any RA synthetic enzymes.”
Fig. 1. Very simplified schematic of the many molecules involved in retinoid biosynthesis and handling, and of the transcriptional processes influenced by binding of RA to its nuclear receptors. These include the various retinoids, the enzymes implicated in their biosynthesis, the retinoid-binding proteins that help regulate their location and activity, and the nuclear receptors and cofactors through which the retinoids act to regulate transcription. (From Drager et al. 2006)

- Drager, U. Retinoic acid signaling in the functioning brain. Science STKE. 2006 (324) pe10
Excerpt #1 Reading Questions

Q#1.1) How many receptors does retinoic acid bind to? Name one.

Q#1.2) Vitamin A deficiency induces embryo abnormalities similar to genetic based defects in what organ systems?

Q#1.3) What retinoid acid gene results in the most severe forms of vitamin A deficiency syndrome when removed? Identify it in the schematic by circling it.

Q#1.4) From the schematic, what Retinoid and what nuclear receptor, bind to the DNA to regulate mRNA transcription?

Q#1.5) Mice missing only one gene from the vitamin A synthesis network appear normal, but the absence of multiple vitamin A synthesis genes cause symptoms of vitamin A deficiency. Why do you think this is?
Excerpt #2 – Summary of birth defects related to vitamin A

“Vitamin A is an essential nutrient required for normal embryonic development. Both an excess and deficiency during gestation will result in abnormalities. Vitamin A (in its more common dietary form, retinyl palmitate [RP]) is teratogenic when orally administered to pregnant rats, mice, hamsters, guinea pigs, rabbits, and dogs. The induced malformations occur in all body systems including the central nervous system (CNS), the face, the cardiovascular system (ventricular septal defect, aortic arch anomalies), the limbs, the urogenital system, the respiratory system, and the gastrointestinal system. The ear malformations are of particular interest as similar malformations have been observed in humans, exposed to another retinoid, 13cRA. The question then arises, would similar malformations be induced in humans exposed to RP? Teratogenic outcomes have reportedly been associated with exposures >40,000 IU [IU – international unit of biological activity] or >10,000 IU. Equally reports have also suggested that supplemental RP intakes of <10,000 IU have no increased risk of major malformation. This contradictory data in humans highlights the importance of laboratory animal data in risk estimation.”


Excerpt #2 Reading Questions

Q#2.1) What is the common dietary form of vitamin A?

Q#2.2) What body systems are affected by vitamin A deficiency or excess during development?

Q#2.3) In what year did the earliest study cited occur (see footnotes)? Given that vitamin A is still a current research topic, what does that say about its complexity?

Q#2.4) What is a teratogen? (consult dictionary if necessary)

Q#2.5) The author indicates that animal models are needed to investigate a “contradiction” in the human data. What is the contrary/conflicting data? Why is the contradiction important to settle?
Excerpt #3 – Clinical study of the effects of vitamin A administration on newborns

“It is well accepted that both clinical and subclinical vitamin A deficiency is associated with increased mortality in preschool-aged children and that universal supplementation of populations with endemic vitamin A deficiency can significantly reduce total mortality between 19 and 54% in children 6 mo–5 y old. More recently, it has been demonstrated that vitamin A supplementation in the first few days of life can reduce early infant mortality (0–6 mo) between 16 and 64% in South and Southeast Asia, although this effect is not observed if supplementation is delayed until after the first month of life during infancy or if provided during pregnancy.

“A variety of studies, including those trials that have shown an effect on mortality, have demonstrated that vitamin A supplementation to young children has little, if any, impact on the incidence of illnesses such as diarrhea, measles, and acute respiratory infections, which are the most common killers of young children in developing countries. As a result, it has been assumed that the mechanism of action of vitamin A on mortality is mediated through a reduction in case fatality. The direct evidence for this effect on case fatality is limited except for measles, where case fatality in hospitalized measles is reduced by ~50% with vitamin A supplementation. To directly address this question for other common childhood illnesses, we collected daily morbidity information during a community-based trial of vitamin A supplementation of newborn infants in south India.”

Excerpt #3 Reading Questions

Q#3.1) What are two vitamin A deficiency treatment strategies described in the opening paragraph?

Q#3.2) Up till when after birth was vitamin A supplementation of infants effective in reducing mortality? What was the range of % reduction observed?

Q#3.3) What illnesses are the most common killers of young children in developing countries?

Q#3.4) The authors state that vitamin A supplementation does not reduce the incidence of these illnesses, but does reduce their mortality rate. What do you think this means about the role of vitamin A in illness prevention/mitigation?

Q#3.5) What data did the authors collect to study this connection between vitamin A treatment and illness related morbidity? What are the advantages/disadvantages of this human clinical study versus an animal study?