Article title: Are babies of vaccinated Pregnant and lactating mothers with ChAdOx1 AstraZeneca covid vaccine more risk for zinc deficiency and SCID disease.

Authors: Amr Kamel Khalil Ahmed[1], Mahmoud Elkazzaz[2]

Affiliations: Department of Public Health, Tuberculosis Program, First Health Cluster, Ministry of Health, Riyadh, Saudi Arabia.[1], Department of chemistry and biochemistry, Faculty of Science, Damietta University, Egypt[2]

Orcid ids: 0000-0003-3477-236X[1], 0000-0003-3703-520X[2]

Contact e-mail: drmedahmed@gmail.com

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Pregnant and breastfeeding mothers who have received the ChAdOx1 AstraZeneca covid vaccine may have infants with an increased risk of zinc insufficiency and SCID disease

Amr Ahmed ^{1*} , Mahmoud El Kazzaz ²

¹ Department of Public Health, Tuberculosis Program, First Health Cluster, Ministry of Health, Riyadh, Saudi Arabia.

² Department of chemistry and biochemistry, Faculty of Science, Damietta University, Egypt

Keywords: ADA, EDTA, ChAdOx1 COVID-19 vaccine, Zinc, SCID

Corresponding author: drmedahmed@gmail.com

Introduction

The extracellular and intracellular adenosine levels are maintained by the adenosine deaminase (ADA). It acts totally like an allosteric modulator, a catalyst, a cell-to-cell connector, and a co-stimulator. The irreversible deamination of 20-deoxyadenosine or adenosine to 2-deoxyinosine or inosine is enhanced via adenosine deaminase (ADA, EC 3.5.4.4), respectively. There are two distinct enzymes in humans: ADA1 and ADA2. ADA act as a monomeric enzyme for purine metabolism. ADA function grabs the attention of scientists because around 15% of inherited immunodeficiencies are due to a mutant version of ADA. Severe combined immunodeficiency (SCID) is known with severe lymphopenia, and malfunctioning of the natural killer (NK), B, and T cells. ADA inactivation results in a buildup of lymphotoxin deoxyadenosine triphosphate, inducing immature thymocytes apoptosis. X-ray crystallography has revealed various crystal structures of ADA, all of which feature a firmly bound Zn²⁺ required for the native protein's stability and catalytic function.¹

ADA has a significant role in purine metabolism. Lymphopenia and SCID may develop due to ADA inactivation. ADA-SCID patients have been administrated retroviral vectors through gene therapy; however, insertional oncogenesis represents a potential issue. Zinc Finger Nucleases (ZFNs) have been alternatively used to induce genome repair by targeted homologous recombination at the ADA gene. ZFNs potent for human ADA (hADA) were generated, then evaluated for the capacity to promote homology-directed repair.²

Around 30% of proteins in cells form complexes with metals. Metal ions are essential not just for regulating biological functions but also for stabilizing proteins. (ADA, EC 3.5.4.4) has a molecular mass of 40 kDa and contains a firmly attached zinc ion essential for its activity. The lack of its enzymatic activity leads to malfunction B and T cells, and SCID development.³

ADA is a critical purine metabolic enzyme required for adequate immunological competence. The current study examines the effect of Zn^{2+} on the maintenance of ADA. After eliminating Zn^{2+} , the protein's crystallographic structure has retained a high degree of order and similarity to the original one, with some alterations limited to the areas surrounding the pocket. However, the protein's stability has been dramatically reduced.³

A study has demonstrated the binding capabilities of transcription factors of the zinc-binding module C/H1 (cysteine/histidine-rich region 1) discovered in p300 and the transcriptional co-activator proteins CBP (CREB-binding protein). Their significant findings have shown that treating native C/H1 with EDTA results in irreversible denaturation and aggregation. Moreover, they have discovered that the released form of C/H1 has engaged in non-specific protein-protein interactions. EDTA is a highly effective zinc-chelating agent employed in the investigations of protein interactions. The potential damage of the structure and interactions of zinc-binding proteins by EDTA treatment must be considered. Excess Zn^{2+} has not returned C/H1 to its normal structure, highlighting the irreversible denaturation induced by the EDTA. EDTA's remarkable affinity for these metals is due to a high-speed and essentially diffusion-limited association rate paired with a prolonged dissociation rate. Additionally, EDTA strongly binds with Zn^{2+} compared to other divalent metals like Mg^{2+} .⁴

Using EDTA to eliminate endogenous zinc seems to provide a technique for investigating the consequences of short-term zinc supplementation during crucial stages of pregnancy in the zinc-deficient rat.⁵

PEGylation of adenoviral vectors may be a viable technique in terms of safety profile because it has lowered IL-6 and liver toxicity significantly; however, it requires some optimization.⁶

AstraZeneca's covid-19 vaccine demonstrates some concerns regarding its excipients, such as polysorbate 80, which serves as a stabilizer, and EDTA, which serves as a binding agent.⁷

EDTA was found to kill breast milk cells, disrupt the milk fat globule membrane, disturb the membrane-bound protein, free fatty acids, and decrease pH. Additionally, it resulted in false-positive hemolytic tests.⁸

EDTA is widely used in modern pharmacy and medicine. However, few investigations have been conducted to determine the cytotoxic consequences of EDTA on biological samples.⁸

It was hypothesized that EDTA bound free zinc, preventing it from being exchanged for integrated zinc. EDTA caused a zinc deficiency, which has teratogenic implications.⁹

The teratogenic activity of disodium EDTA might be due to metal chelation, which resulted in a metal reduction, depriving the growing embryo of minerals.⁹

These findings ensure that DNA synthesis was hindered because of the impairment of replication enzymes by EDTA. In rabbit hepatocytes, thymidine kinase and DNA polymerase activities were decreased, whereas adenosine deaminase and dehydrogenases activities

remained stable. EDTA-treated cells have demonstrated a reduced diadenosine tetraphosphate concentration. Zinc chloride increased diadenosine tetraphosphate production, then hindered the hydrolase. Zinc has been thought to be a probable second messenger for inducing mitogenicity, as evidenced by the cation's reversal of DNA synthesis inhibition.⁹

Pregnant rats which were administrated 3% Disodium EDTA at different pregnancy stages developed severe deformities when administered 100 ppm zinc rather than 1000 ppm zinc. There was no evidence of fetal or maternal toxicity with ZnCaEDTA (8 mmol/m²/day) or ZnEDTA (8 and 20 mmol/m²/day). EDTA was supplied via gavage and in feed to rats on GDs 7–14 at 1000mg/kg/day and 1250mg/kg/day, respectively, and resulted in progeny abnormalities and maternal poisoning. However, in mice, offspring of dams fed orally 1000 mg/kg/day EDTA on GDs 8–12 exhibited no abnormalities. It has been proposed that zinc inadequacy due to EDTA caused teratogenic consequences.⁹

Conclusions

EDTA is an effective zinc chelator that can impair adenosine deaminase stability. Around 30% of cell proteins form complexes with metals. Although the teratogenic effect of Disodium EDTA may be due to metal chelation, clinical trials are needed to monitor infants born for mothers who have received the ChAdOx1 AstraZeneca covid vaccine.

Abbreviations:

adenosine deaminase (ADA)

Severe combined immunodeficiency (SCID)

Ethylenediaminetetraacetic acid (**EDTA**)

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