Article title: Pitfalls at Chemistry of Adenoviral Vector Vaccine against COVID-19 and how to Circumvent It.

Authors: Amr Kamel Khalil Ahmed[1], Abdullah Alkattan[2]

Affiliations: 1Department of Public Health, Tuberculosis Program, First Health Cluster, Ministry of Health, Riyadh, Saudi Arabia.[1], 3Biomedical sciences department, Faculty of Veterinary Medicine, King Faisal University, Saudi Arabia.[2]

Orcid ids: 0000-0003-3477-236X[1], 0000-0001-5294-8088[2]

Contact e-mail: drmedahmed@gmail.com

License information: This work has been published open access under Creative Commons Attribution License <http://creativecommons.org/licenses/by/4.0/>, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Conditions, terms of use and publishing policy can be found at <https://www.scienceopen.com/>.

Preprint statement: This article is a preprint and has not been peer-reviewed, under consideration and submitted to ScienceOpen Preprints for open peer review.

Links to data: 10.31219/osf.io/anqcp

DOI: 10.14293/S2199-1006.1.SOR-PP96ICB.v1

Preprint first posted online: 27 June 2021

Keywords: : ChAdOx1 COVID-19 vaccine; EDTA; zinc; adenovirus; polyethylene glycol

Pitfalls at Chemistry of Adenoviral Vector Vaccine against COVID-19 and How to Circumvent It.

Amr Ahmed¹, Mohammad Nezami², Abdullah Alkattan³

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

²President and CEO of Sahel Oncology LLC, Orange Coast Medical Center of Hope
Inc. 496 Old Newport Blvd. #7 Newport Beach, CA 92612, USA

³Biomedical sciences department, Faculty of Veterinary Medicine, King Faisal University, Saudi Arabia.

Corresponding to

Amr Ahmed - email: drmedahmed@gmail.com – ORCID: 0000-0003-3477-236X

Abdullah Alkattan – email: abdullahalkattan@gmail.com – ORCID: 0000-0001-5294-8088

Keywords: ChAdOx1 COVID-19 vaccine; EDTA; zinc; adenovirus; polyethylene glycol

Dear Editor,

The coronavirus disease of 2019 (COVID-19) is a pandemic outbreak that causing number of deaths reached over 3 million since December 2019. Despite the approval and production of several vaccines against SARS-CoV-2 infection, many countries still suffering from daily deaths due to COVID-19. Although the vaccination's success in reducing the cases infected with SARS-CoV-2, many people globally still not immunized against COVID-19 due to their doubts about the risks and benefits of the vaccines. Here, we would like to spot light on vaccines' excipients and how to evade the hurdles of some COVID-19 vaccines.

ChAdOx1 nCoV-19 vaccine is an adenovirus vector vaccine that designed to provoke immunity against SARS-CoV-2. This vaccine contains several inactive ingredients, including sodium chloride, magnesium chloride hexahydrate, ethanol, sucrose, and Ethylenediaminetetraacetic acid (EDTA) ^[1].

EDTA is a very potent zinc chelator which is used commonly in protein interaction studies. Exposure to EDTA even in lower concentrations may cause extreme stripping of zinc from many proteins, including zinc-binding proteins that described as a component of the largest and most complex gene superfamily in metazoans and the most common class of transcription factors. The zinc dissociation rates can vary greatly among these proteins. Furthermore, zinc-binding domains may not refold into their true native structure after EDTA removal (2). (3,4). A Study by Matt et al revealed that EDTA treatment of native Cysteine/Histidine rich region 1 (C/H1) leads to irreversible denaturation and aggregation, and that particular concern their finding that unfolded C/H1 results in non-specific protein-protein interactions. (4) The addition of extra zinc did not restore C/H1 to its original structure, and the binding between C/H1 and HIF1alpha is abolished, (2).

On the other hand, the EDTA-induced thrombocytopenia is a risk phenomenon caused by EDTA-dependent anti-platelet auto-antibodies that identify antigens modified by EDTA. few case reports indicating that thrombocytopenia and thrombosis events after receiving the vaccines containing EDTA cannot be ruled out [5,6] The cation chelation by EDTA causes changes in the shape of the platelet membrane glycoprotein IIb-IIIa complex and showed an obscure epitope that becomes available for autoantibodies. (7) This leads to platelet clumping and aggregation in vitro lead to low platelet counts which result in slightly increased in white blood cells. (8)

Another issue is the adenovirus in the vaccine. It can induce thrombocytopenia, a potentially serious complication of gene therapy protocols using this type of vector. While studying the interaction between adenovirus and platelets, we proved adenovirus platelet attachment by detecting Cocksackie adenovirus receptor. We concluded that the interaction between adenovirus and platelets leads to platelet activation and quick appearance of P-selectin on the platelet's surface; this triggers the formation of platelet-leukocyte aggregates. This interaction is essential since it promotes leukocyte rolling on the endothelium and slows platelets that excrete the ligand in an inflammatory setting. The appearance of thrombocytopenia within 24 hours of adenovirus administration shows that this is a response to the virus. Studies of the administered adenovirus show that liver macrophages clear most of the virus within 24 hours, the same amount of time that platelets take to be cleared. According to this observation, we can conclude that it is possible that the adenovirus and the activated platelets are cleared with the same mechanism. For further evidence, we noticed the appearance of adenovirus particles in the liver macrophage 10 minutes after the injection. (9)

Many interactions are acknowledged to take place between Ad capsid and cellular and non-cellular blood components, in addition to the complement system. For instance, Ad vectors interact with platelets, causing thrombocytopenia. (10,11)

The most difficult obstacle for Ad vectors comes from the induction of an intense immune response, possibly caused by reacting with antigen-presenting cells like macrophages and dendritic cells; this causes the release of pro-inflammatory cytokines/ chemokines such as interleukin 6 (IL-6), tumor necrosis factor α - interferon γ inducible protein-10, and RANTES. (12)

The spleen is the most important site of cytokine and chemokine production like interleukin-6 so reduced uptake of PEGylated vectors by the spleen might explain why lower IL-6 levels observed after delivery of PEGylated Ad vectors. De Geet et al observed that PEGylated Ad vectors showed a significantly lower transduction of spleen (3-fold lower) and kidney (2.5-fold lower). PEGylation also inhibited the development of thrombocytopenia. In conclusion, PEGylation of adenoviral vectors combined with methylprednisolone administration improves the therapeutic index of adenoviral gene transfer. (13)

PEGylation already induced significantly lower serum il-6 levels by 70% (14)

We noted that the study by Katie et al studied immune response by a single dose of ChAdOx1 nCov-19 not included test of IL-6 (15)

We conclude from previous studies that the PEGylating of adenoviral vectors can be promise technology as safety profile as significantly reduced IL-6 and liver toxicity and how avoiding the pitfalls of chemistry and virology so the PEGylation since first time at 1999 introduced by O’Riordan,C.R et al (16) need more advancements.

Conflict of interest: The authors declare they have no conflicts of interest.

References:

- 1- https://www.ema.europa.eu/en/documents/product-information/covid-19-vaccine-astra-zeneca-product-information-approved-chmp-29-january-2021-pending-endorsement_en.pdf
- 2- Nyborg JK, Peersen OB. That zinging feeling: the effects of EDTA on the behaviour of zinc-binding transcriptional regulators. *Biochem J.* 2004 Aug 1;381(Pt 3):e3-4. doi: 10.1042/BJ20041096. PMID: 15270700; PMCID: PMC1133908.
- 3- Dial, R., Sun, Z. Y. and Freedman, S. J. (2003) Three conformational states of the p300 CH1 domain define its functional properties. *Biochemistry* 42, 9937–9945
- 4- Matt, T. D., Martinez-Yamout, M. A., Dyson, H. J. and Wright, P. E. (2004) The CBP/p300 TAZ1 domain in its native state is not a binding partner of MDM2. *Biochem. J.* 381, 685–691
- 5- Pitkin F (2017) Ethylenediaminetetraacetic Acid (EDTA)-Induced Thrombocytopenia: A Case Report. *J Tradit Med Clin Natur* 6: 209.
- 6- Greinacher, A., Thiele, T., Warkentin, T. E., Weisser, K., Kyrle, P. A., & Eichinger, S. (2021). Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination. *The New England journal of medicine*, 384(22), 2092–2101. <https://doi.org/10.1056/NEJMoa2104840>
- 7- Bizzaro N (2013) Pseudothrombocytopenia. *Platelets. Amsterdam: Academic Press* 3: 989-997.
- 8- Keller NP, Siegrist D, Alberio L (2014) “A case of EDTA-dependent pseudothrombocytopenia: simple recognition of an underdiagnosed and misleading phenomenon,” *BMC Clin Pathol* 14: 14-19.

- 9- Othman M, Labelle A, Mazzetti I, Elbatarny HS, Lillicrap D. Adenovirus-induced thrombocytopenia: the role of von Willebrand factor and P-selectin in mediating accelerated platelet clearance. *Blood*. 2007 Apr 1;109(7):2832-9. doi: 10.1182/blood-2006-06-032524. PMID: 17148587.
- 10- Morral, N, O'Neal, WK, Rice, K, Leland, MM, Piedra, PA, Aguilar-Cordova, E et al. (2002). Lethal toxicity, severe endothelial injury, and a threshold effect with high doses of an adenoviral vector in baboons. *Hum Gene Ther* 13: 143–154.
- 11- Wolins, N, Lozier, J, Eggerman, TL, Jones, E, Aguilar-Cordova, E and Vostal, JG (2003). Intravenous administration of replication-incompetent adenovirus to rhesus monkeys induces thrombocytopenia by increasing in vivo platelet clearance. *Br J Haematol* 123: 903–905.
- 12- Iorian Kreppel, Stefan Kochanek, Modification of Adenovirus Gene Transfer Vectors With Synthetic Polymers: A Scientific Review and Technical Guide, *Molecular Therapy*, Volume 16, Issue 1, 2008, Pages 16-29, ISSN 1525-0016, <https://doi.org/10.1038/sj.mt.6300321>.
- 13- De Geest, B.; Snoeys, J.; Van Linthout, S.; Lievens, J.; Collen, D. Elimination of innate immune responses and liver inflammation by PEGylation of adenoviral vectors and methylprednisolone. *Hum. Gene Ther.* 2005, 16, 1439–1451
- 14- Mok, H, Palmer, DJ, Ng, P and Barry, MA (2005). Evaluation of polyethylene glycol modification of first-generation and helper-dependent adenoviral vectors to reduce innate immune responses. *Mol Ther* 11: 66–79.
- 15- Ewer, K.J., Barrett, J.R., Belij-Rammerstorfer, S. *et al.* T cell and antibody responses induced by a single dose of ChAdOx1 nCoV-19 (AZD1222) vaccine in a phase 1/2 clinical trial. *Nat Med* 27, 270–278 (2021). <https://doi.org/10.1038/s41591-020-01194-5>
- 16- O'Riordan, C.R.; Lachapelle, A.; Delgado, C.; Parkes, V.; Wadsworth, S.C.; Smith, A.E.; Francis, G.E. PEGylation of adenovirus with retention of infectivity and protection from neutralizing antibody in vitro and in vivo. *Hum. Gene Ther.* 1999, 10, 1349–1358.