What is the potential risk of the cationic lipid in the Pfizer/BioNTech vaccine?

The RNA – ribonucleic acid – being used in the mRNA vaccines would dissipate immediately after being injected if not encapsulated. The encapsulation technique used by Pfizer/BioNTech is a lipid nanoparticle composed of four lipids: ALC-0315 = (4-hydroxybutyl) azanediyl)bis (hexane-6,1-diyl)bis(2-hexyldecanoate), ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), and cholesterol. These lipids all have various roles in stabilising the lipid nanoparticle (LNP), aiding cell entry, and enabling the nanosphere to burst open when it is inside the cell.

This article will focus on that first lipid, the opaquely named ALC-0315. A previous piece on this page focused on polyethylene glycol (PEG, with the designation ALC-0159 in the BioNtech vaccine). The PEG is used to prevent the LNPs getting stuck to each other or other surfaces: it "is preferably inserted at the LNP surface as a steric barrier to interactions with surfaces or other LNPs to avoid aggregation during storage". It is possible to react allergically to PEG, or for it to be attacked by antibodies one already has to it: the manufacturers have already warned not to have this vaccine if you have suffered allergic/anaphylactic reactions to PEG in the past. But the cationic lipid ALC-0315 harbours very great risks of its own – possibly far greater – that have not so far received any coverage at all.

What is a cationic lipid?

ALC-0315 is a cationic lipid, which means it is able to form an aggregate complex with anionic genetic materials such as DNA or RNA. Cationic lipids typically have a positively charged head group followed by hydrophobic tails of varying composition. Anionic means negatively charged. The electrostatic interaction between the negatively charged RNA backbone and the positively charged heads of the cationic lipids keep the mRNA "tethered" until the LNP gets to its intended location (see the figure in this article as an example: https://www.mdpi.com/2076-393X/9/1/65).

Once the entire LNP slips into the cell (enabled by the liposome interaction with the membrane), the cationic lipid interacts with negatively charged phospholipids in the endosomal membrane – the endosome being the "sorting station" that meets the LNP when it has entered the cell – "resulting in membrane destabilization and release of the payload to the cytosol".

What happens to the fragments inside the cell?

So what happens to the lipid fragments of the LNP inside the cell once the mRNA "payload" has been released to perform its mission of forming the Spike protein of SARS-CoV-2?

First of all we have to remember that this is happening very many times and in various cells of the body, not just in the muscle tissue into which the vaccine was first injected. Animal trials have shown that LNPs when injected tend to naturally flow with the bloodstream. No biodistribution studies via radiotracker (e.g. Luciferase) appear to have yet been conducted on human beings with the Pfizer/BioNTech vaccine, but it is likely that – as in animal trials – the LNPs distribute throughout the body. A study published in 2017 analysed the biodistribution of LNPs after intramuscular administration in mice. It found delivery of the LNPs to 17 different compartments of the mice apart from muscle within varying periods of time, including heart, testes, kidney, bone marrow and even the brain.

One also has to remember that it is not in the plasma that the lipid fragments are left to be broken down and disintegrated, but inside the body's cells, assuming they have been attracted in through the cell membrane as intended, where many other sensitive organelles are to be found.

The possible toxicity of this lipid

The formulation of Pfizer/BioNTech's LNP is proprietary, but sources cite the proportion of the cationic lipid ALC-0315 in the lipid envelope at approx. 50%. We do not know how many of these LNPs are being injected per 30 mcg (one dose), but this is a point that needs investigation. These cationic lipids have been investigated for over 30 years since cationic lipid-based gene delivery (lipofection) was first published by Felgner's group in 1987, but have not found their way into general application because of their toxicity. It is not just cellular biologists with experience of this field who state that cationic lipids are "very, very toxic". A peer-reviewed paper in Toxicology Research from April 2018 states in the opening sentence of its abstract: "cationic lipids still have the problem of toxicity, which has become one of the main bottlenecks for their applications." This finding is reflected throughout the literature on cationic lipids, across the entire three decades. An article as recent as May 2019, "Lipid Nanoparticles for Delivery of Therapeutic RNA Oligonucleotides" says "A major drawback with the use of cationic lipids for gene delivery is the high net positive charge associated with the headgroup as well as induction of immune response … Furthermore, particles of cationic nature are known to undergo accumulation in the liver, lung, and spleen."

Previously only used in cancer therapy

So what is the exact mechanism of this cytotoxicity? "Cellular toxicity of cationic lipids has been linked to increased production of reactive oxygen species." (Reactive oxygen species, also called free radicals, are unstable molecules that contain oxygen and that easily react with other molecules in a cell; a build-up of these in cells may cause damage to DNA, RNA, and proteins, and may cause cell death.). A 2016 article explains: "Several previous reports have suggested that cationic liposomes induce reactive oxygen species (ROS) and ROS-mediated toxicity in cells." This study goes on to test whether liposomes containing cationic lipids might perform apoptosis of cancer cells alone, without any cargo. It finds this to be true. Nanoparticles containing cationic monovalent lipids "are able to induce cancer cell death through production of ROS in the absence of any therapeutic cancer reagents."

This was indeed the field that BioNTech specialises in: using lipid nanoparticles in cancer therapy. The company had never produced a vaccine before this mRNA Covid-19 one in association with Pfizer. Placing cationic lipids inside tumour cells to induce cell death is one thing, as apoptosis is intended, but is this acceptable collateral damage when the same technology is used in a vaccine?

The potential mechanism of damage is that once the LNP is in the cell and has released its mRNA, the cationic lipid fragments with their positive charge react with the negatively charged organelles and membrane substances in the cell, creating porosity and calcium influx.

Mechanism of action on liver cells

A detailed analysis of the effect of cationic lipids on liver cells from December 2017 explains the mechanism as follows (CLs meaning cationic lipids): "Pathway analysis showed significant changes in pathways involving amino acid metabolism, energy metabolism, lipid metabolism and oxidative stress in the CLs exposure group vs the control group. Metabolites related to the above-mentioned pathways included phenylalanine, methionine, creatine, oxalacetic acid, glutathione, oxidized glutathione, choline phosphate and several unsaturated fatty acids, indicating that cells were disturbed in amino acid metabolism, energy and lipid supply when CLs exposure-induced injury occurred. It is concluded that CLs may induce cytotoxicity by enhancing reactive oxygen species *in vitro*, affect the normal process of energy metabolism, disturb several vital signaling pathways and finally induce cell death.

Exponential effects due to mitochondrial damage?

Every cell (apart from red blood cells) contains multiple mitochondria, our cells' powerhouses. These process oxygen to make energy, so there is a huge load of oxygen radicals in mitochondria that need to be contained and neutralised. The outer membrane of the mitochondria contains cardiolipin, which is negatively charged and would soon be compromised by cationic lipids reacting with it. Once the delicate mitochondrial membrane becomes porous from attack by the cationic lipids, the radical oxygen species from inside the mitochondria are no longer contained and the reaction is likely to be exponential, as cells often contain many thousands of mitochondria.

Dopaminergic neurons in the substantia nigra have around 2 million mitochondria each The implications depend on which cells the LNPs happen to land inside, how many of them there are, and which pathways are being impaired by the cell death this free radical attack causes. But certainly a putative link can be made with severe neurological sequelae if cationic lipids should find their way into the basal ganglia, for example, or the substantia nigra.

Considerable marker dysregulation noted

Around 60% find their way to the liver, as mentioned in the EMA report: “The applicant has estimated the percent of dose distributed to the liver to be ~60% for ALC-0315”. In an animal trial submitted as evidence for authorisation of the vaccine by BioNTech to the EMA, this resulted in high elevations of liver enzymes GGT – “exposure generated increased levels of gGT (>200%)” – indicative of liver damage, and AST, which rises in liver and cardiac inflammation, as well as “slight to moderate increases in ALT and ALP levels, possible indicative of liver effects.” “Cationic lipids show in vitro toxicity toward phagocytic cells and inhibit in vitro and in situ NO and TNF-a production by activated macrophages.”

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Surely it would be prudent for trials to examine and exclude the possibilities of this oxidative and other damage occurring before continuing to roll out this particular vaccine? The official MHRA summary of adverse reactions to the Pfizer/BioNTech vaccine for the UK for just one month, from 19th January to 11th February, lists 70,314 adverse events, ranging from cardiac, hepatic and gastrointestinal disorders through to polyneuropathies, 978 eye disorders (including 9 cases of blindness) and 173 deaths. The mode of action of cationic lipids is just one of the possible risks of these novel genetic therapeutics, but damage via the pathways described in thirty years of literature surely deserves targeted questions and answers. Too much is at stake.