STANDARD DEVIATIONS: pH. Small Word, Big Influence

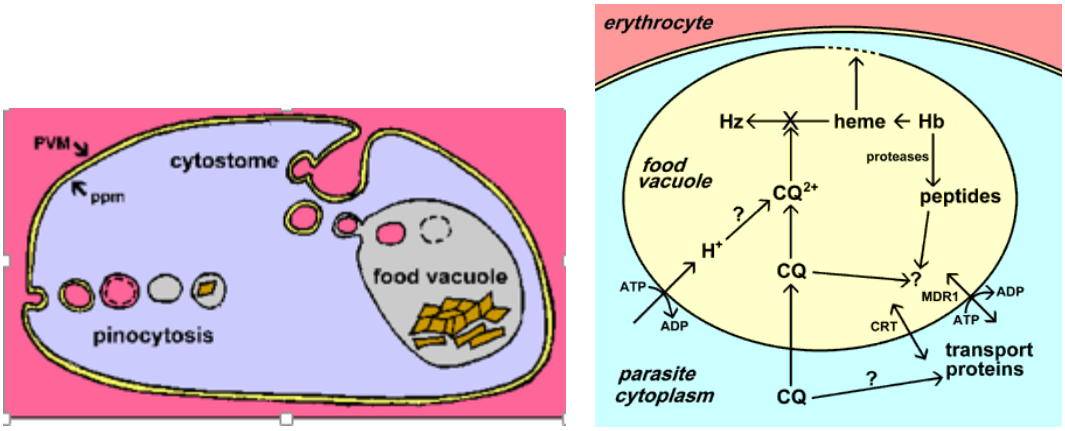
Greetings,

The anti-malarial properties of chloroquine and hydroxychloroquine (HCQ) have been known for about a century. The benefit for autoimmune disorders has helped people suffering from Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) for decades. Now, the drugs are being imagined as treatment for coronavirus infection. The mechanism that allows such a wide spectrum of utility can be described in two letters: **pH**.

In clinical laboratory science we recognize that the enzymatic reactions that drive the reactions and biochemistry we observe rely heavily on the balance of pH in an equation. Slight changes in hydrogen ion concentration accelerate or inhibit enzymes from catalyzing reactants; either creating a cascade of activity or resulting in the blockade of a pathway.

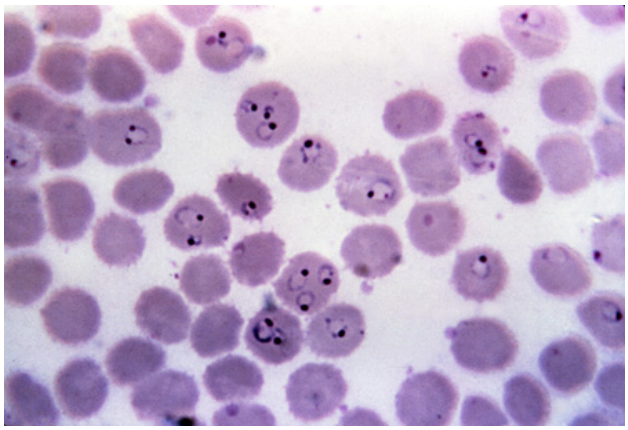
Chloroquine and its hydroxyl metabolite are alkaloids that raise pH and inhibit the production of certain products. They pass easily through lipid membranes of cells and are sequestered in lysozymes within cells. The **intracellular concentration can be thousands of times higher** than the concentration in plasma when steady-state dosages are reached.

In the malaria-infected cell, the *Plasmodium* parasite uses hemoglobin to as a source of amino acids for protein synthesis. Large quantities of heme are released as a result of hemoglobin digestion in the food vacuole. Within its specialized food vacuole, proteases are able to convert Heme into Hemozoin. Free **heme is toxic due to its ability to destabilize and lyse membranes** in its ferrous state (Fe^{2+}), as well as inhibiting the activity of several enzymes. The hemozoin complex is the non-toxic pigment formed by the *Plasmodium* proteases at acidic pH.



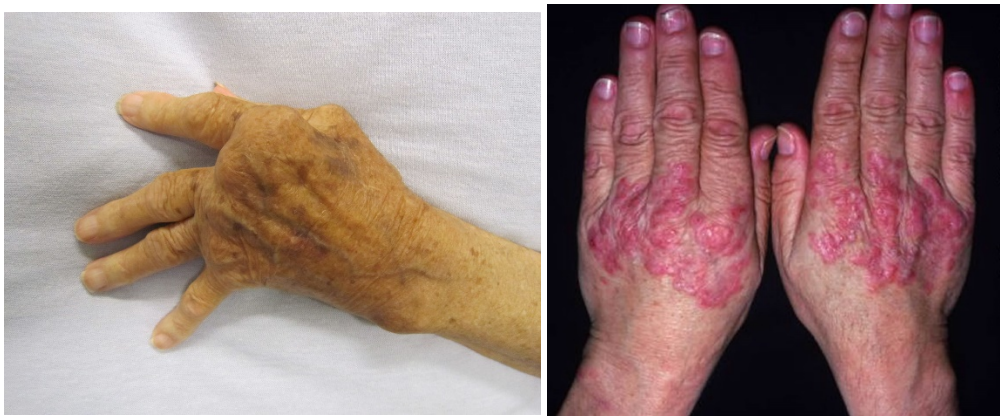
{Parasites digest hemoglobin, producing a toxic heme that is converted to hemozoin.}

Chloroquine and hydroxychloroquine inhibit pigment formation, as well as the heme degradative processes, and prevent the detoxification of heme. Chloroquine accumulate in the lysosome of the parasite, raise pH and prevent protease from converting heme to hemozoin. The free heme destabilizes the food vacuolar membrane and other membranes **and leads to the death of the parasite**.



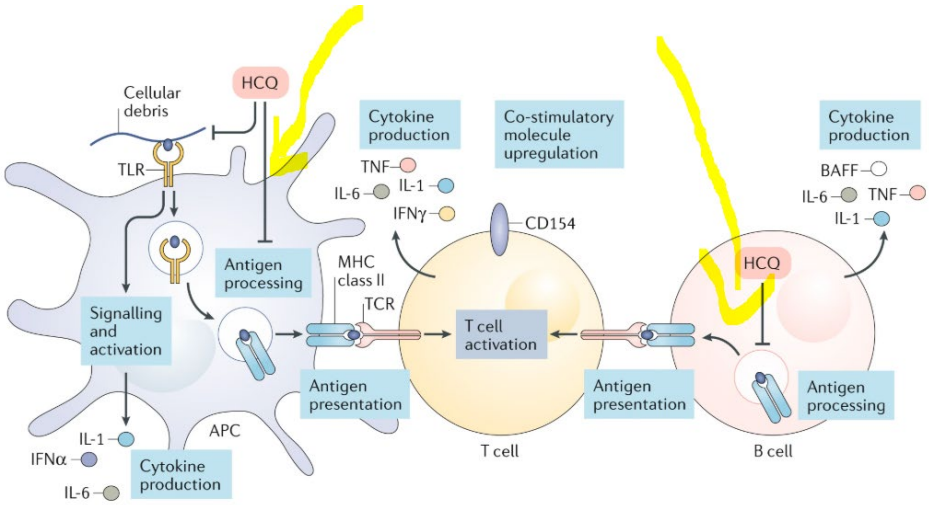
{Hemozoin is the pigment we observe in RBCs as hemoglobin is digested}

Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) are autoimmune disorders that cause hyper inflammation and trigger the immune system of the body to mistakenly attack healthy tissue. RA and SLE see tissue or DNA as antigen and respond by stimulating the production of signal proteins (cytokines) that cause proliferation of cells that are programmed to attack self. Joints, organs and blood vessels are targeted.



(Auto-immune reaction in RA (L) and SLE (R)}

These chloroquine drugs interfere with lysosome activity and autophagy by altering minute but critical pH dependent reactions. In RA and SLE, they inhibit immune activation by reducing Toll-like receptor signaling and cytokine production and, in T cells, reducing CD154 expression. It's this ability to interact with membrane stability and alter signaling pathways and transcriptional activity which results in inhibition of cytokine production and modulation of certain co-stimulatory molecules.

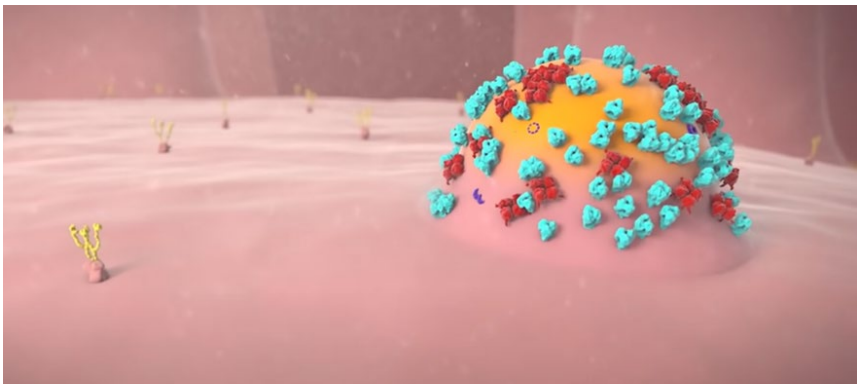


{By raising the pH in the endosome, these pathways are inhibited}

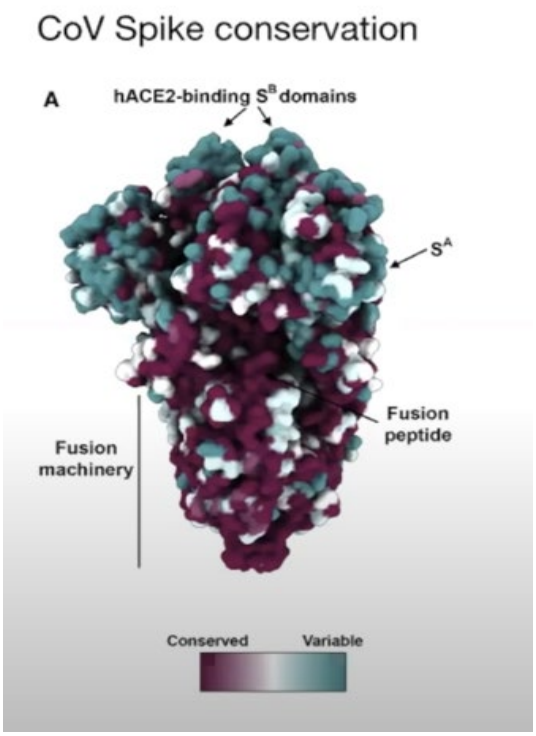
It's the ability of chloroquine to alter proteases by changing pH that makes it appealing in the fight against viral infection.

There are two promising paths for these molecules. They can alter the binding capability of the virus to the cell membrane and inhibit fusion. In addition, they can suppress the cytokine storm that causes damaging hyper inflammation in the immune response.

When the spike protein and ACE2 combine, an un-folding occurs that allows acidic protease cleavage to access the fusion machinery. If the pH is too high, that reaction is inhibited.



{Raising pH may block this fusion?}



{Cleavage is needed to access the fusion machinery; it's pH dependent.}

Recall that chloroquine concentrates inside acidic cellular compartments. It can get into *Plasmodium*'s food vacuole, but it also concentrates inside the cell's endosomes. Viruses hijack those endosomes for their own use. Both when they enter and exit a host cell, and also in the production of protein inside the cell. Chemically, chloroquine is a weak base, sufficient to raise the pH in these compartments. As endosomes become less acidic, the proteins inside are ruined — they unfold because they are no longer at the correct pH. What this means for coronavirus is that proteins are rendered nonfunctional because chloroquine changed the pH of the endosomes carrying them; binding may occur but the virus does not enter the cell.

In addition to ruining the viral invader's proteins, chloroquine can distort the shape of cytokine proteins in the endosomes of immune cells that fight infection. Sometimes, the immune system gets too excited and creates what is called a "cytokine storm," which has been a major complication of Covid-19. By quelling this cytokine storm, chloroquine provides a dual advantage in helping the body combat coronavirus infection.

Early studies of the anti-viral properties of the Chloroquine family have shown adverse effects in study patients, causing arrhythmias in some. Those findings have stopped the studies, for now.

Whether this drug has application for treating COVID-19 remains to be seen. The mechanism of its action and the principle of altering pH in suppressing protease activity is the essence of research into similar drugs and vaccine therapies.

Have a great week and be safe,

Bryan