

### **Mode of action of chloroquine and hydroxychloroquine as anti-viral agents**

Antimalarials anti-viral activity was identified since the late 1960s and was extensively reviewed by Rolain et al in 2007 [S1]. CQ and HCQ exert their antiviral activity mainly by increasing pH within acidic organelles, including endosomes, lysosomes and Golgi vesicles; in particular, these drugs could inhibit the viruses requiring a pH-dependent step for entry into their host cells. In fact, some viruses, in a low-pH milieu, could change their structure facilitating fusion, penetration and/or uncoating. CQ might prevent the uncoating of influenza B virus by increasing the lysosomal pH above the critical value required for inducing fusion between virus envelope and lysosomal membrane; similarly, CQ seems to be able to inhibit uncoating of the hepatitis A virus [S1]. Moreover, antimalarial drugs seem to inhibit post-translational modifications of glycoproteins of virus envelope acting on proteases and glycosyltransferases, enzymes needing an acid pH milieu: by increasing pH, CQ/HCQ might impair the envelope maturation [S1].

The specific effect of CQ against corona viruses was studied soon after the first SARS epidemic.

In 2004 Keyaerts et al showed that after one day of incubation of Vero E6 cells with 4  $\mu$ M CQ, no significant replication was observed, and 16  $\mu$ M was required to inhibit by 99% the viral replication; CQ was equally effective when added during or 1 hour after the infection [S2].

In 2005 Vincent et al confirmed the ability of CQ to increase endosomal pH required for virus/cell fusion, as well as in interfering with the glycosylation of cellular receptors of SARS-CoV [S2]. In particular, antimalarial drugs could reduce the glycosylation of ACE2, which has been identified as a functional cellular receptor of SARS-CoV spike protein [S2].

Few years later, Keyaerts et al confirmed the data obtained *in vitro* in a murine model, showing a long-lasting protective effect of CQ against lethal coronavirus OC43 infection in new-born mice treated via maternal milk [S4].

#### Supplementary references

- S1. Rolain JM, Colson P, Raoult D. Recycling of Chloroquine and Its Hydroxyl Analogue to Face Bacterial, Fungal and Viral Infections in the 21st Century. *Int J Antimicrob Agents* 2007; 30 (4), 297-308.
- S2. Keyaerts E, Vijgen L, Maes P et al, In Vitro Inhibition of Severe Acute Respiratory Syndrome Coronavirus by Chloroquine. *Biochem Biophys Res Commun* 2004; 323 (1), 264-8.
- S3. Vincent MJ, Bergeron E, Benjannet S et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virology* 2005; 2:69.
- S4. Keyaerts E, Li S, Vijgen L, et al. Antiviral activity of chloroquine against human coronavirus OC43 infection in newborn mice. *Antimicrob Agents Chemother* 2009; 53: 3416–3421.