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Chloroquine defeats aging?

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Abstract

Autophagy, the turnover of cellular components including organelles, declines with age. Thus, enhancement of this characteristic process is hypothesized to improve health and extend lifespan. Two recent papers present data indicating that contrary to expectation, chloroquine (CQ), a nominal inhibitor of autophagy, extended the lifespan of middle-aged mice and rats by ~10%. Details of these studies provide a cautionary tale regarding traditional reagents or "tool compounds" of "established" mechanisms often used in cellular biological research. However, these and earlier studies support a deeper investigation of CQ or its more commonly used clinical analog, hydroxychloroquine (HCQ), as potential drugs to increase health span and slow the aging process.

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Introduction

1.1 Autophagy

Autophagy, literally “self-eating," was first described by Christian De Duve, who shared the 1974 Nobel prize for his discovery of lysosomes. Autophagy is an evolutionarily conserved catabolic process wherein lysosomes degrade various cellular components to maintain cytoplasmic quality control and thus cellular homeostasis. Several distinct forms of autophagy include macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). Dysregulation of this critical process has been associated with many pathological processes including infectious, metabolic, and neurodegenerative disorders, as well as cancer and autoimmunity. Reduced autophagy was identified by López-Otín et al. (2013) as a so-called "Hallmark of aging".

1.2 Chloroquine

Native peoples of South America have long used an extract of the bark of the Cinchona tree (*Cinchona officinalis*) as a remedy for fevers. By the 1600s, this herbal medicine was being used in Europe for treatment of malaria. Quinine was isolated from the bark in 1820. Seeking an alternative to quinine, Bayer chemists discovered chloroquine, a synthetic analog with a similar mechanism of action. A related analogue, 3-methyl-chloroquine, was utilized by the Germans in World War II. Material captured by the Americans in North Africa was analyzed by the United States government, resulting in corroborative studies and subsequent clinical approval in 1947 of chloroquine as a prophylactic treatment for malaria. While chloroquine and its derivative hydroxychloroquine are widely used in rheumatology (SLE, RA, etc.)[1], chloroquine-resistant malaria has mandated the development of newer antimalarials such as pyrimethamine, artemisinin and mefloquine[2].

Chloroquine inhibits hemozoin formation from the heme released by parasitic digestion of erythrocyte hemoglobin during Plasmodium infection. The free heme then lyses membranes and leads to parasite death[3, 4, 5]. Chloroquine passively diffuses through cell membranes and into

endosomes, lysosomes, and Golgi vesicles which become protonated, trapping the chloroquine in the organelles and raising the surrounding pH. The raised pH in the endosomal compartments (a so-called lysosomotropic effect), inhibits viral infectivity and various cellular processes such as autophagy.

Geroprotective activities of chloroquine

Li et al. reported the geroprotective effects of low-dose CQ on aged rats[6]. These studies were inspired by a perspective article published at the height of the COVID-19 pandemic by Sargiacomo et al. in 2020[7]. This British group sought to explain the considerably higher mortality rate in COVID-19 patients with advanced chronological age prior to the development of safe vaccines. They pointed out that the proposed SARS-Cov-2 viral receptors, CD26 and ACE2, were associated with senescence [8, 9]. Furthermore, at the time, azithromycin, quercetin, and HCQ were proposed as "off-the-shelf" treatments for SARS-CoV2 infection[10]. Quercetin has a senolytic activity[11] and HCQ is known to inhibit beta-galactosidase, a marker of senescence[12]. Although early and questionable trials demonstrated some benefit of HCQ[13] for SARS-CoV2, subsequent well-controlled trials demonstrated limited, if any, benefit[14, 15]. However, it is important to note that investigation of the role of the autophagy pathway in viral pathogenesis continues[16].

To investigate the geroprotective effects of HCQ, Li et al. treated 24-month-old Sprague Dawley male rats (n = 9) with CQ (0.1 mg/kg, in drinking water) twice weekly for 5 months[6]. Compared to controls (n = 13), the treated animals exhibited a 6% increase in medial survival and 13% increase in maximum lifespan ($p = 0.02$)[6].

Related to the work of Li et al., Doeppner et al. (2022) also reported a chloroquine-mediated increase in the lifespan of rodents, in this case mice[17]. Work by Fifan et al.[18] and others[19, 20] demonstrated that the polyamine spermidine increased the maximum life span of C. elegans and the median life span of mice (~10%). Because spermidine increases autophagy, they hypothesized that treatment with chloroquine, an inhibitor of autophagy, would shorten the lifespan of mice. Remarkably, addition of chloroquine (50 mg/kg) to the drinking water extended overall lifespan of middle-aged

male NMRI mice (n = 28; treatment initiated at age 500 days) by 11.4% (786 days) compared to control mice (n = 28; 689 days, p = 0.0002). Median life span of the middle-aged mice increased by 11.4%. Studies of chloroquine by these two groups and other data dating back a couple decades suggest the effect is real, although the precise mechanism is not clear. Mechanistic studies carried out by both groups suggest a variety of mechanisms, though this "ball of yarn" is far from being unraveled.

Mechanism studies

As noted above, CQ treatment was initiated at age 500 days when the mice weighed on average ~35 gm. Over the next 100 days, the control animals gained weight, reaching a maximum of 45 gm. In contrast, the CQ-treated animals did not gain significant weight over the duration of the experiment (almost 800 days). While the control animals consumed more liquid (p = 0.002), the food consumed by both groups was equal (~4 gm/day) ruling out the idea that CQ somehow reduced food intake through an artificial "calorie restriction" effect.

Previously, CQ (at 60 mg/kg) was reported to impair autophagosome-lysosome fusion rather than affecting the acidity and/or degradative activities of lysosomes[21]. In addition, HCQ treatment was associated with "autophagy-independent" disorganization of the Golgi and endo-lysosomal systems, with predominant Golgi disorganization seen in kidney and intestinal tissues. The microtubule-associated protein I light chain (LC3) family of proteins (LC3A, B, C)[22] is the major structural protein family of autophagosome membranes. LC3-II is generated by the conjugation of cytosolic LC3-I to phosphatidylethanolamine on the surface of nascent autophagosomes. Doeppner et al. showed that the CQ-treated mice exhibited a dose-dependent increase in LC3B-II as well as p62 in the liver and heart, as confirmed by transmission electron microscopy[17]. Curiously, the treated animals exhibited increased liver glycogen and reduced serum insulin growth factor binding protein 3 (IGFBP3), though no difference in IGF-1, IRS, or growth hormone levels[23, 24]. CQ treatment elicited a decrease in glycogenolysis in the liver.

IGFBP3 binds IGF1 and IGF2 to modulate their binding to the IGF-1 receptor. While IGFBP3 levels, like IGFs, are regulated by GH, expression in the liver is GH-independent[25]. IGFB3 mediates a plethora of other activities via its binding to a number of proteins and its interaction with various cell surface and nuclear signaling pathways. Several labs have demonstrated a beneficial role for reduced IGFB3 in senescence[26, 27, 28].

Li et al. found that low-dose CQ (0.1 mg/kg given twice a week) extended the lifespan of aged rats when given in late middle age[6]. This dose is ~100-fold lower than doses typically given as a putative autophagy inhibitor in cancer[29]. Qian et al. attributed the geroprotective effect of CQ to ATM activation leading to enhanced DNA damage repair within their C. elegans and progeria mouse strain models[30]. Others have shown that activation of ATM by CQ can slow atherosclerosis, improve insulin sensitivity, and rescue glucose intolerance in type 2 diabetes (T2D)[31, 32, 33].

Li et al. studied the transcriptomes from CQ-treated and control old rats to define CQ-induced, differentially expressed genes so-called “CQ DEGs” across multiple tissues. For example, 40% of kidney-specific aged genes, 30% of small intestine-specific aged genes, and 20% of liver-specific aged genes were "rescued" by CQ treatment. These changes were consistent with the reduced fibrosis and improved histology of the kidneys compared to other tissues. Contrary to expectation, CQ treatment augmented a number of genes associated with various cardiac diseases (e.g. heart failure, cardiac arrhythmias, ischemic cardiomyopathy (Caps2), hypertrophic cardiomyopathy, and cardiac arrhythmias (Myh7). Thus, CQ treatment may actually mediate pro-aging activity in certain tissues[6].

Summary

A number of labs have studied several animal species to generate data suggesting that CQ can mediate a pro-longevity effect. Mechanistic studies indicate that multiple mechanisms are at play, each addressing a hallmark of aging[34]. While CQ and HCQ are familiar to cell biologists as prototypic

lysosomotropic effect tool compounds, numerous diverse studies highlight the plethora of other activities mediated by CQ. Isolated papers over the past 20 years have identified non-autophagy-mediated effects of CQ. For example, CQ improves vasculogenesis[35], insulin metabolism[31], fibrosis[36], reduces oxidative stress[37], modulates autophagic flux[21], boosts DNA repair via STM[30], and attenuates atherogenesis[33] to name a few. When we began looking into this story, we sought a unifying mechanism, pathway, target, etc. to explain the increased lifespans. Unfortunately, at the present time, descriptive studies nominate many parallel mechanisms. The detailed transcriptome studies of Li et al. show quite variable tissue specific patterns, with some that are beneficial for health span and others that are consistent with cardiac diseases[6]. Of course, investigating the geroprotective effects of CQ using Horvath or other methylation clocks might lead to interesting findings.

Medical Implications

As noted above, CQ and HCQ are widely used drugs[38]. The studies of Li et al. and Doeppner et al. support further, possibly human studies of these drugs. On October 8–13, 2013, a workshop entitled 'Interventions to Slow Aging in Humans: Are We Ready?' was held in Erice, Italy[39]. At the time, a number of interventions were deemed worthy of human clinical trials. Since then, some of these ideas have been put to the test or are being tested . With regard to development of CQ/HCQ, suitable biomarkers will need to be validated. At low doses, CQ or HCQ seem to qualify for evaluation in humans. However, the safety profile of this class of drugs will need to be considered. For instance, periodic eye exams and cardiac monitoring are routine[40] at doses commonly used in rheumatology[38]. Clearly these anti-malarials have come a long way from the rainforests of South America!

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