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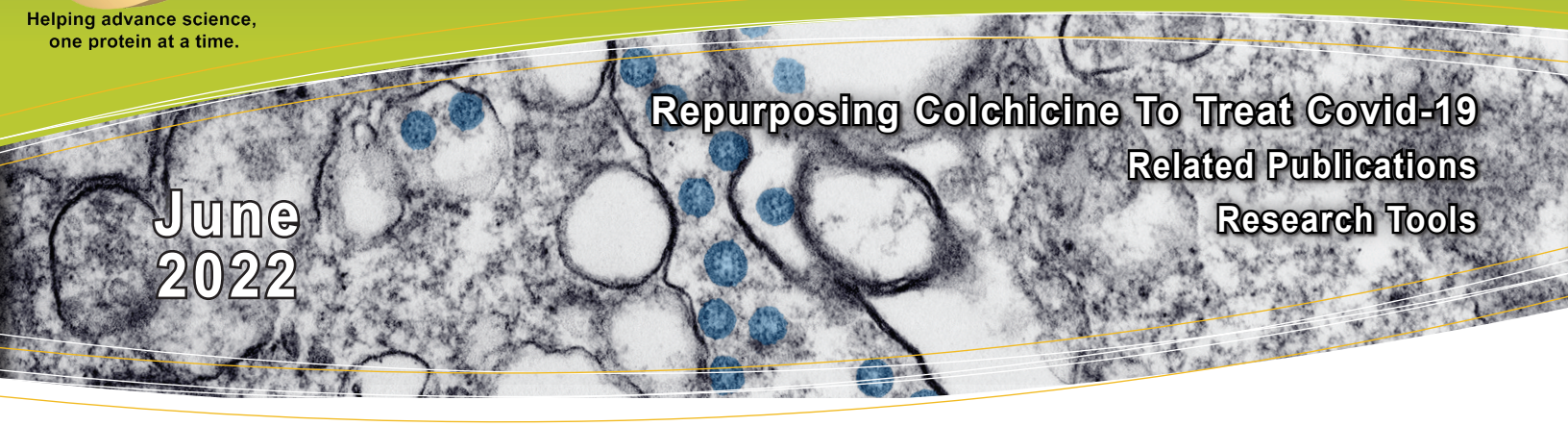

Repurposing Colchicine To Treat Covid-19

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Repurposing Colchicine To Treat Covid-19

Introduction

SARS-Cov2 is a virus which evolved in 2019 and spread rapidly through the world using inter-personal viroid containing aerosols as a vector. The disease, Covid-19, causes traumatic respiratory problems in patients, and has devastated old, diabetic and immune-compromised people with 5-50% death rates. Recent variants are less severe but spread rapidly all the same. Epidemiologists predict that new variants will continue to move through the world population for many years[1]. Vaccinations have been and will continue to be the major line of defense against infection and serious illness, however their efficacy depends on which variant is infecting, therefore a new therapeutic paradigm with small molecule inhibitors is emerging as a broad-spectrum approach. This brief review will focus on tubulin and colchicine as one possible target although there are many others[2].

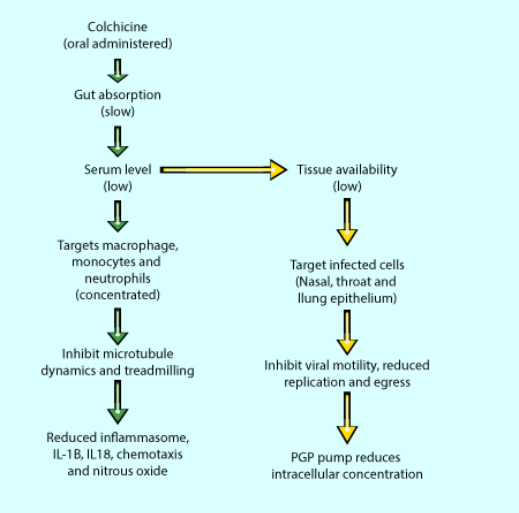
Colchicine Historical Perspective and Cellular Targets

Colchicine (CLN) and its herbal extract has been used to reduce inflammation in illnesses such as gout since 1550 BC[3] and, more recently, iatrogenic allogenosis (a type of implant rejection). The connection to inflammation induced by SARS-CoV-2 in Covid-19 illness was quickly established in 2020[4,5]. In particular, the innate immune system's neutrophil cells were known to migrate to areas of tissue and cell damage and activate inflammation. The terms neutrophil swarm and cytokine storm are descriptive terms that highlight the dramatic consequence of an uncontrolled neutrophil response. Due to the similarities between other inflammatory illnesses and Covid-19, CLN clinical trials were allowed to proceed by many government bodies, utilizing multi-national institutes[6].

Considering CLN as an anti-inflammatory, the mechanism of action is thought to be multi-factorial acting through monocytes, macrophages and neutrophils. Documented effects include; reducing neutrophil chemotaxis[7,8], inhibition of nitrous oxide production[9], inhibiting the NACHT-LRRPYD containing protein 3 (NCPA3) inflammasome and downstream interleukin secretion[10]. In neutrophils, cell motility and chemotaxis are strongly inhibited because CLN is concentrated 13 to 68-fold inside these cells compared to other cell types[11,21]. This phenotype is thought to be a consequence of a lack of PGP-efflux pumps to remove the compound from the cytoplasm.

Colchicine Molecular Mechanisms

What are the molecular mechanisms that underlie CLN's effects on migration, IL secretion, inflammasome and mitosis? In short, CLN has two sites of action, the most well-known being the CLN binding site on ß-tubulin[12,13], and the second is a site on the mitochondrial membrane which inhibits ATP from entering mitochondria via the voltage dependent channel[14]. Clearly the direct effect on tubulin and microtubules can to a large degree explain inhibition of motility, secretion, and mitosis due to microtubule's critical role in those functions. Whereas the mitochondrial site may affect the amount of energy available for a neutrophil's innate response, or the amount of acetyl-CoA for phospho-lipid synthesis in infected cells[15] (see later).



Above: Therapeutic pathways of colchicine through the body.

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CLN's therapeutic serum level is about 3 nM but varies widely between patients[21]. *In vitro*, CLN inhibits microtubule polymerization with an IC50 of 1-3 μM[22], whereas its Kd (ratio of off rate versus on rate) is about 10^{-7}M[23], which is almost impossible to measure due to its nearly 1-week dissociation half-life rate. The actual mechanism of inhibition *in vitro* was binding of a tubulin/colchicine complex to the ends of microtubules which inhibited the rates of polymerization and depolymerization by 50% at 0.16 μM[24,25]. The IC50 for dynamic instability of microtubules is closer to 100 nM[26], which is closer to the 3 nM serum level when combined with the 13 to 67-fold increased concentration in neutrophils[11,21]. Therefore, the primary target of CLN in neutrophils could be microtubule dynamic instability and treadmilling.

Another interesting angle is the mitochondrial target of CLN[14]. Clearly, affecting mitochondrial function will reduce energy availability in the cell and decrease the rate of energy dependent processes such as polymer construction (RNA, fatty acids and protein synthesis). A connection between mitochondrial function and lipid metabolism is the acetyl-CoA shuttle[27]. Therefore, limiting mitochondrial function will reduce acetyl-CoA synthesis and result in less phospho-lipid production and could therefore reduce virion production[28].

Colchicine Targets the Viral "Life-Cycle"

From another perspective, the cytoskeleton is a key component of the viral "life-cycle" which includes infection, intra-cellular transport to a peri-nuclear position, replication, transport towards the plasma-membrane, and egress functions[16,17,18,19]. Within this scope, tubulin and microtubules are involved mostly in viral transport and replication, and Oliva et al. directly measured SARS-CoV-2 infection *in vitro* in the presence of CLN, among other cytoskeleton inhibitors, and found a 30% reduction ($p<0.05$ confidence with n=9 replicates) in viral spread[20]. A CLN binding site compound, podophyllotoxin, had even greater inhibition of viral infection and spread, a 60% reduction ($p<0.0001$), indicating that CLN site binding compounds can inhibit viral spread via interference with microtubule architecture in the absence of tissue and organism level inflammation. Other CLN site compounds such as mebendazole and febendazole also show efficacy, and have less side effects, which opens a path for therapy with less side effects than steroids or CLN. However, much of the early work in 2020 utilized typical virus culturing cell types such as Vero E6[29], Caco-2[30] and A549[31], which did not effectively replicate the characteristics of covid infected cells. Therefore, a pluripotent stem cell-derived AT2 cell line was developed by Hekman et al. 2020[32], which had improved alignment with alveoli AT2 cell type characteristics, and it resulted in a different pharmacological profile than the previous mentioned viral cell models.

Colchicine Analogs: Potential Therapeutic for Covid-19

Early and late stages Covid-19 therapy have quite different requirements, whereas early disease stage would require direct viral binding, replication and virion assembly inhibitors, the later stages require neutrophil and inflammation reduction. *In vitro*, CLN and its analogs appear to treat both aspects, however viral spread as judged by disease progression did not decrease significantly in clinical studies[28], possibly due to CLN's poor

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uptake rate and serum availability making for a poor effect[11] (Fig.1). Other cytoskeletal components may be more efficacious targets e.g. F-actin and vimentin[18] in the early stages of Covid-19.

It is critical to note that late stage Covid-19 clinical trials of CLN were halted because it was no more efficacious over steroid use[28] e.g. dexamethasone, which was being administered at the same time to the same patients receiving CLN. However, a mid-sized community trial (COLCORONA) administered to a pool of early PCR-positive infected people[28] reported a 50% reduction in hospitalizations and deaths i.e, late-stage disease, but these were not significant in the small numbers collected. The early stage Covid-19 score was not affected by CLN.

Neither pre-Covid 19 infection or late-stage CLN-alone trials have been attempted because steroids are quite effective at the late stage and CLN has a narrow therapeutic window in humans (0.5-2mg/day) to be administered with tolerable side effects. To improve the outcomes, second generation CLN-analogs and CLN binding site compounds are currently being assessed in the clinic. A recent example was a study with Sabizabulin from Veru Inc.[33] The Phase II trial reported 82% reduction in mortality when 9 mg doses were given to moderate and high symptom patients. As in the COLCORONA trial, there were low numbers of mortalities which made the data less substantial but still very encouraging, and even led to emergency use authorization for hospitalized moderate to severe COVID-19 patients at high risk for acute respiratory distress syndrome. Even so, based on the current information, it's possible that different compounds will be needed for treating early and late Covid-19 cases as they manifest in different molecular models.

Header Image Credit: CDC/Hannah A Bullock; Azaibi Tamin

Tubulin Live Cell Imaging Products

Product	Ex / Em	Amount	Cat #
SiR-Tubulin™ Kit Includes SiR-Tubulin, and Verapamil	630 / 680 nm	50 nmol	CY-SC002
Cytoskeleton Kit Includes SiR-Actin, SiR-Tubulin and Verapamil	630 / 680 nm	50 nmol each	CY-SC006
SiR700-Tubulin Kit 35 nmol SiR700-Tubulin and 1 μmol verapamil	680 / 720 nm	50 nmol	CY-SC014
SPY555-Tubulin Kit Includes SPY555-Tubulin and Verapamil	555 / 580 nm	100 stains	CY-SC203
SPY650-Tubulin Includes SPY650-Tubulin and Verapamil	652 / 674 nm	100 stains	CY-SC503

Tubulin Kits

Product	Assays	Cat #
Tubulin polymerization HTS assay using >97% pure tubulin OD based - Porcine	24	BK004P
Tubulin polymerization assay using >99% pure tubulin OD based - Porcine	24-30	BK006P
Tubulin polymerization assay using >99% pure tubulin Fluorescence based	96	BK011P
Microtubule Binding Protein Spin-Down Assay Biochem Kit	50-100	BK029
Microtubule/Tubulin In Vivo Assay Biochem Kit	30-100	BK038

References

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Purified Tubulins and MAPs

Product	Amount	Cat #
Microtubules (Taxol Stabilized and Lyophilized)	4 x 500 µg 1 x 10 mg	MT002-A MT002-XL
Microtubule associated protein rich fraction Source : porcine brain	1 x 100 µg 5 x 100 µg	MAPF-A MAPF-C
Tau protein Source : bovine brain	1 x 50 µg 3 x 50 µg	TA01-A TA01-B
Tubulin protein (>99% pure) Source : porcine brain	1 x 1 mg 5 x 1 mg	T240-A T240-B

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