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Lipids, statins and susceptibility to SARS-CoV-2 and influenza A viruses

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Abstract. The extensive and on-going epidemiology studies of the SARS-CoV-2 pandemic have raised interesting observations on statins reducing COVID-19 severity. In this review, literature is analysed to examine how statins affect COVID-19 and influenza A, another pandemic respiratory virus. This information could be useful to prevent or reduce disease severity caused by respiratory viruses.

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The respiratory viruses, influenza A virus (IAV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), have demonstrated their action to cause significant morbidity, mortality and socio-economic disruption. The 1918 influenza pandemic caused 20–100 million deaths, with one-third of the world's population being infected[1], while the current COVID-19 pandemic has resulted in 146 million confirmed cases and over 3 million deaths to date[2]. Our focus here is to review the potential for statins to affect patient outcomes for these viral infections.

Statins and cholesterol

Statins are among the most highly prescribed drugs used in the treatment of hypercholesterolemia, a major cause of cardiovascular disease. Diet has an effect on cholesterol levels, but our endogenous synthesis of cholesterol accounts for age-associated increases. To reduce plasma cholesterol to medically recommended levels of less than 4 mM, doctors prescribe statins to inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) (Figure 1). Cholesterol is essential so it is important that statins do not block cholesterol synthesis completely. As shown in Figure 1, inhibition of HMGCR also affects other L-mevalonate pathways including protein prenylation[3]. Interestingly, statins can target any HMGCR, including HMGCRs of pathogenic *Candida* species and *Aspergillus fumigatus*[4].

Statins were discovered in the soil fungus *Aspergillus terreus*, which is currently used to produce lovastatin, a precursor of simvastatin. Simvastatin and atorvastatin were the first blockbuster drugs, but many additional statins have since been produced. Statins are used to inhibit HMGCR in the liver, reducing plasma cholesterol levels. Cholesterol is also an essential component of cell membranes, which become integrated into viral envelopes, leading us to review what is known about the effect of statins on SARS-CoV-2 and the other respiratory virus associated with pandemics, influenza A virus (IAV). Our findings are summarised in Table 1.

Cholesterol levels

Cholesterol is a vital part of IAV and SARS-CoV-2. During viral budding, lipids and cholesterol from infected host cells become part of the viral envelope[19]. Dietary cholesterol levels were shown to affect influenza infection in a mouse study[7]. Compared to a controlled diet group, mice with a 2% cholesterol diet experienced increased morbidity over a 5-week period.

The underlying low-grade chronic inflammation due to the release of the pro-inflammatory mediators from adipocytes of obese individuals exacerbates the cytokine storm observed in COVID-19 disease[20]. Obesity is also associated with the upregulation of ACE2 expression. ACE2 is a receptor for SARS-CoV-2 spike proteins, so its upregulation could further enhance viral attachment and entry to the host tissue and increase severity[21]. The higher content of lipid rafts with high cholesterol levels in obese patients may also support SARS-CoV-2 attachment to host cells and its subsequent replication. Importantly, cholesterol-rich lipid rafts in the host cell membrane are favourable for enveloped viruses making cholesterol reduction a general strategy to thwart enveloped virus infection[22].

Effect of statins

Statins have been investigated to determine whether they affect outcomes of IAV and SARS-CoV-2 infections. While benefits of atorvastatin and rosuvastatin have been demonstrated in a model of IAV infection in cell culture[14,15], the benefits to statin users have varied. A study comparing 5181 statin users with 5181 non-users found small benefits that were not statistically significant[16]. On the

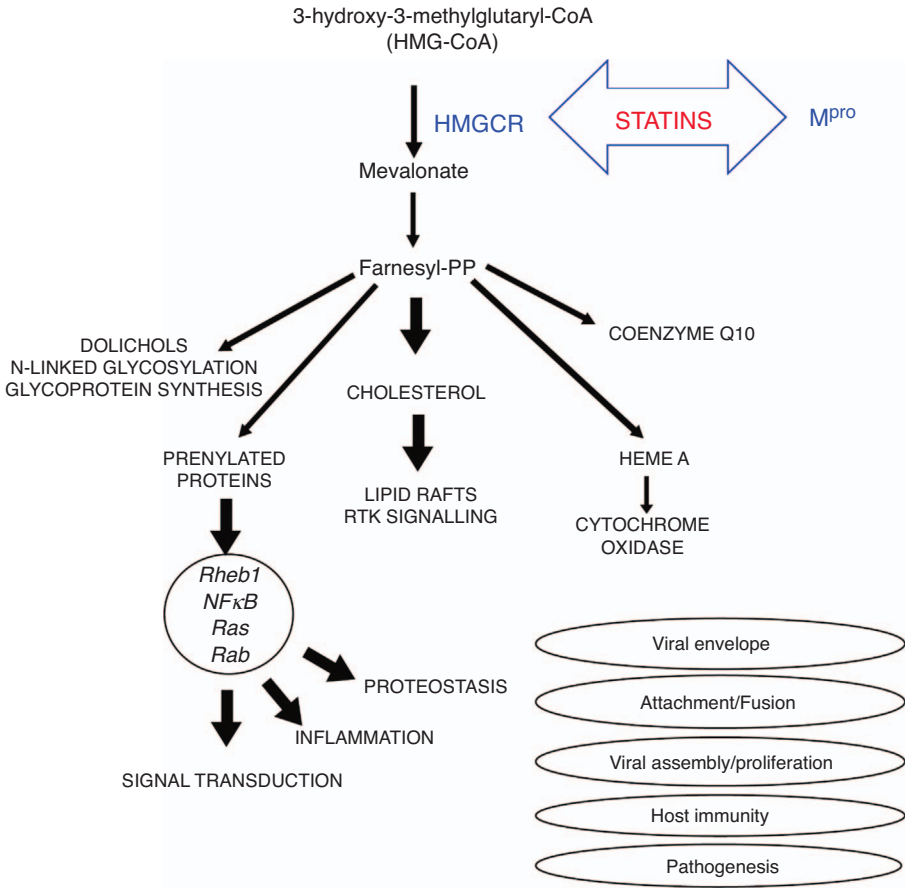

Figure 1. Molecular targets of statin treatment during SARS-CoV-2 infection showing inhibition of HMG-CoA reductase resulting in multiple effects.

Table 1. Effects of cholesterol and statins on SARS-CoV-2 and IAV infections.

	SARS-CoV-2	Influenza A virus
Effect of cholesterol/lipids	Host membrane cholesterol interacts with SARS-CoV-2 spike protein and facilitates viral entry to host cell[5] Dyslipidaemia is a common presentation in COVID-19 disease[5]	Envelope cholesterol is crucial for IAV entry and fusion to host cell membrane[6] Dietary cholesterol increased IAV infection in mice[7] Treatment with cholesterol lowering drugs significantly decreased IAV propagation in human airway epithelial cells[8]
Effect of statins	Statins reduced COVID-19 fatalities and severity by 30%[9] Use of statins reduced mortality due to COVID-19 in a retrospective observational study[10] Statin treatment in hospitalised COVID-19 patients reduced death rates and complications including acute kidney infection, sepsis and acute respiratory distress syndrome[11] Statin treatment reduced deaths due to COVID-19 in hospitalized patients[12] Potential binding with main protease (M^{pro}/NSP5), which is unique to and highly conserved in all coronaviruses[13]	Rosuvastatin and atorvastatin reduced IAV proliferation in kidney cells[14] Atorvastatin reduced IAV infection of MDK cells by >95%[15] A UK study showed a slight but not significant protection against hospitalisation and death in statin users[16] Statin usage in hospitalised patients with influenza was associated with reduced death rates[17] A moderate dose statin administration reduced the risk of death due to influenza and chronic obstructive pulmonary disease (COPD)[18]

other hand, in a large-scale matched cohort study (n = 76 232), moderate dose usage of statin was found beneficial by significantly reducing the risk of death due to COPD and influenza[18]. Similarly, in another multistate surveillance study, statin usage in patients hospitalised due to influenza was found associated with reduced mortality[17]. As influenza induces pro-inflammatory pathways by triggering the innate immune system, the anti-inflammatory pleotropic properties of statins have been studied to counteract it. Through *in vitro* tests, statins were able to inhibit IAV proliferation and possibly reduce inflammation by targeting Rho/Rho kinase pathways[14]. Several studies of patients with SARS-CoV-2 infection demonstrated the beneficial effects of statins, significantly reducing mortality rates and disease severity[9–12].

Mechanisms and thoughts on future therapeutics

It is now clear that statins have several additional effects apart from cholesterol synthesis inhibition which deserves further investigation.

SARS-CoV-2 main protease (M^{pro})

An *in silico* docking study demonstrated the potential of M^{pro}, the main protease of SARS-CoV-2, to bind a range of statins, possibly

explaining how statins can impede viral proliferation[13]. M^{pro} is essential for processing of the SARS-CoV-2 polyproteins[23]. Our BLASTP analyses show that sequences highly similar to SARS-CoV-2 M^{pro} are found in all other coronaviruses; however, they are absent in IAV (data available on request). The M^{pro} protein acts as dimer and its active site is composed of Cys-His dyad with the

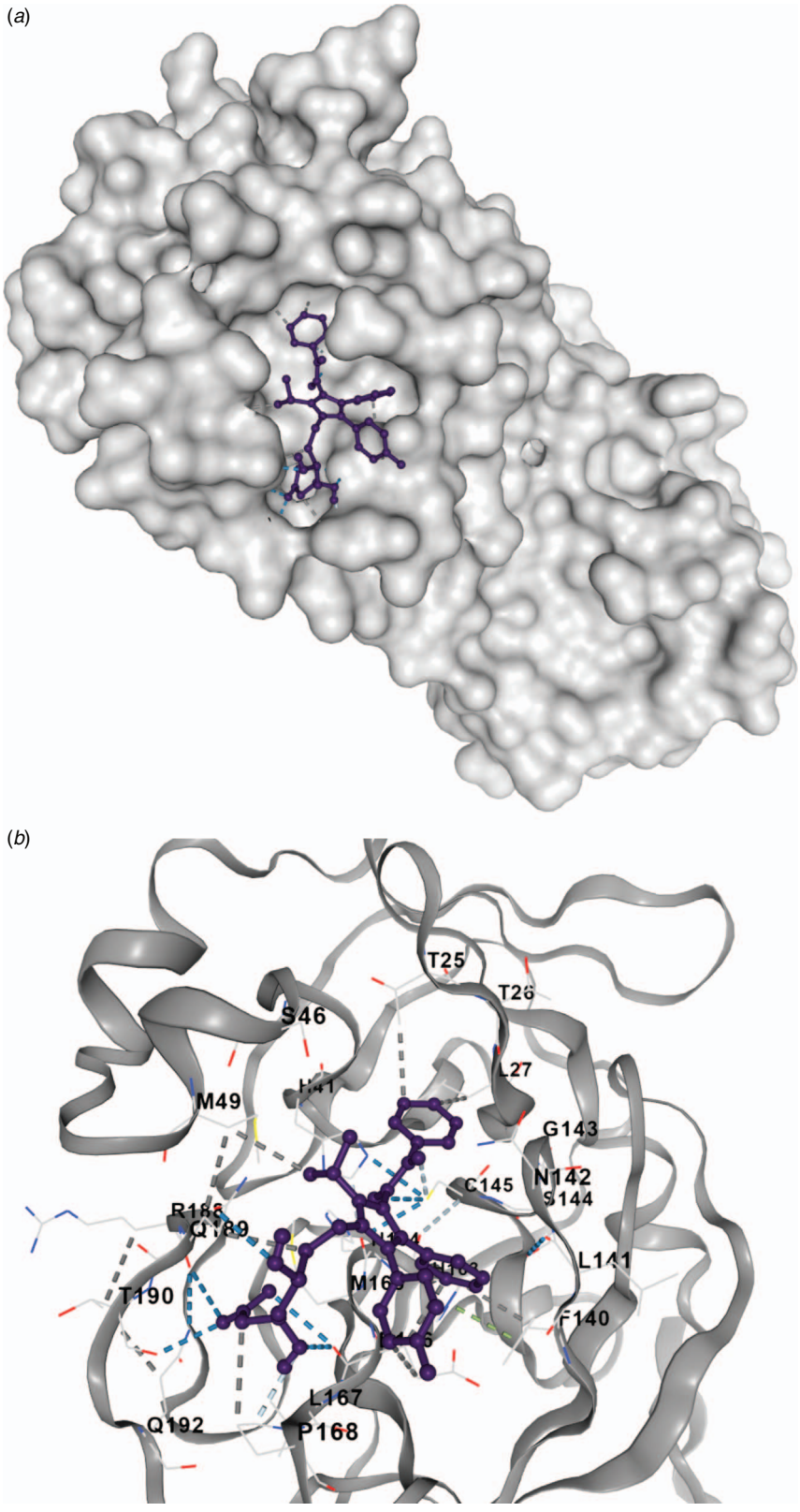

Figure 2. *In silico* docking analysis of SARS-CoV-2 M^{pro} structure 7JP1 (wild type structure retrieved from PDB database) with atorvastatin, performed using the CB-Dock online tool[30]. The binding of atorvastatin is shown at low (*a*) and high resolution (*b*). [Each of the statins, atorvastatin, fluvastatin, lovastatin, pitavastatin, rosuvastatin and simvastatin, bound M^{pro} at the catalytic Cys145 and His41 site with binding energies of –7.3, –7.1, –6.6, –6.9, –7.1 and –7.5 kcal/mol, respectively.]

Cys145 and His41 catalytic residues[24]. Our own *in silico* docking analysis of SARS-CoV-2 M^{pro} with statins demonstrates possible binding at the active site (including binding with Cys145 and His41) of the protease (Figure 2). This important knowledge may guide the design of better drugs to inhibit M^{pro} activity. The bodily distribution of statins is also important for drug targeting. To be effective, statins would need to reach the site of viral infection at levels sufficient for inhibition.

Proteostasis

Statins, like the lipophilic simvastatin, distribute widely in the body, and have additional effects like targeting protein turnover as well as providing an explanation of how simvastatin lowers the incidence of Alzheimer's disease[25,26]. One of the major effects of statin treatment is inhibition of protein prenylation by depleting farnesyl pyrophosphate and geranylgeranyl pyrophosphate. This reduction in protein prenylation also inhibits activation of proteins including Rheb1p, which in turn diminishes mammalian target of rapamycin (mTOR) mediated autophagy inhibition[27,28]. Such an effect of statin administration could enhance autophagy and associated lipolysis, which could further deplete intracellular lipids restricting the viral proliferation.

Inflammation

An additional effect of statin use is its ability to inhibit protein farnesylation, which causes adipogenesis arrest by lowering expression and activity of peroxisome proliferator activator gamma (PPARγ)[29]. Such interruption of adipocyte formation in statin users may lead to reduced release of pro-inflammatory markers, which has the potential to inhibit inflammation during COVID-19 infection[20]. Reduced protein prenylation due to statin treatment also produces an anti-inflammatory effect by inhibiting the activation of nuclear factor kappa B (NFκB)[27]. Another action of statins could include the effects on inflammation via the renin angiotensin system. The liver produces angiotensin that is converted to angiotensin I (inactive) by renin. The inactive angiotensin I is then converted to active angiotensin II, which plays a vital role in regulating inflammation, with the help of angiotensin converting enzyme 2 (ACE2). Angiotensin II, if acted on by ACE2, results in an anti-inflammatory effect. In contrast, angiotensin II interaction with the angiotensin II type 1 receptor (AT1R) proceeds towards release of pro-inflammatory mediators. However, an unhelpful effect of statins is the upregulation of ACE2 expression and the reduction of the pro-inflammatory pathway. On the contrary, overexpression of ACE2 due to statins may also potentially help SARS-CoV-2 viral entry to host[27].

Conclusion

Statins show promise in reducing severity of IAV and SARS-CoV-2, which could be attributed to inhibition HMGCR and a number of other targets. Specifically, the inhibition of protein prenylation has multiple effects including enhancing cytokine-induced inflammation, regulating proteostasis, and post-translational modifications of the intracellular proteins. These events are most likely to be involved in SARS-CoV-2 pathogenesis and viral proliferation as the virus utilises host machinery for survival and proliferation. Knowledge of the targeting of statins may improve the development of therapies for COVID-19 and IAV.

Conflicts of interest

Ian Macreadie is the Editor-in-Chief of *Microbiology Australia*, but was blinded from the peer-review process for this paper.

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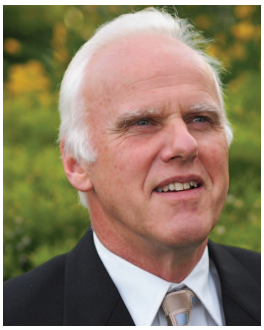

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