Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Commentary

Testing of natural products in clinical trials targeting the SARS-CoV-2 (Covid-19) viral spike protein-angiotensin converting enzyme-2 (ACE2) interaction

Gary Williamson*, Asimina Kerimi

Department of Nutrition, Dietetics and Food, School of Clinical Sciences at Monash Health, Faculty of Medicine, Nursing and Health Sciences, Monash University, BASE Facility, 264 Ferntree Gully Road, Notting Hill, VIC 3168, Australia

A B S T R A C T

Commonly used drugs for treating many conditions are either natural products or derivatives. In silico modelling has identified several natural products including quercetin as potential highly effective disruptors of the initial infection process involving binding to the interface between the SARS-CoV-2 (Covid-19) Viral Spike Protein and the epithelial cell Angiotensin Converting Enzyme-2 (ACE2) protein. Here we argue that the oral route of administration of quercetin is unlikely to be effective in clinical trials owing to biotransformation during digestion, absorption and metabolism, but suggest that agents could be administered directly by alternative routes such as a nasal or throat spray.

1. Introduction

The first disease-initiating interaction of the SARS-CoV-2 virus with the human organism at the molecular level is through binding of the Viral Spike Protein with ACE2 (angiotensin converting enzyme-2). ACE2 is an enzyme attached by a single tail to the outer cell membrane and is particularly highly expressed in the goblet and ciliated cells of the nasal cavity [1]. *In silico* modelling of the interaction between the SARS-CoV-2 Viral Spike Protein and ACE2 identified quercetin from a database of 8,000 small molecule candidates of known drugs, metabolites, and natural products as one of the top 5 most potent compounds for binding to the interface site and potentially disrupting the initiating infection process [2] (Fig. 1). In support of this hypothesis, quercetin was active against infection in a model of virus cell entry and also inhibited the 3C-like protease of SARS-CoV *in vitro* [3,4]. Based on these findings, it has been suggested that quercetin be incorporated into trials against Covid-19 [5].

2. Bioavailability

A key aspect of the therapeutically successful action of any drug is delivery to the appropriate site of action. When targeting the interaction of the Viral Spike Protein with ACE2 on the surface of the epithelial cells, the proposed bioactive molecule must reach that site at a concentration sufficient to interfere with the interaction. Based on *in silico* modelling [2] where the energy of the interaction (Vina score) was ~ -30,500 J/mol, quercetin would theoretically give 50% inhibition (based on $\Delta G = -RT \ln K_a$, where ΔG is the change in Gibbs free energy, R is the gas constant, T is the temperature, K_a is the association constant; assumption here is that Vina score *in silico* $\approx \Delta G$) at a concentration of very approximately 7 μM. This calculation serves to help estimate the dose that might be needed at the site of action. Quercetin is a plant-based naturally occurring flavonoid. Plants store quercetin attached to sugars which substantially interfere with protein interactions [6] but are released in the human digestive tract [7]. The oral bioavailability of quercetin is very well understood [8]. Even after administration of a high oral dose of quercetin, the maximum concentration of the free aglycone in plasma is only in the low nM range [9], several orders of magnitude lower than required based on the above calculation. Canonical drug-metabolising pathways [10] convert most of the quercetin to sulfate, glucuronide and methyl conjugates. The concentration at the necessary site of action would therefore not match or even approach that required for an effect even with very large doses. However, although oral administration of quercetin is unlikely to be effective in disrupting SARS-CoV-2 Viral Spike Protein/ACE2 interactions (Fig. 2), other routes of delivery could be effective. A nasal spray containing

Abbreviations: SARS, severe acute respiratory syndrome; ACE2, angiotensin converting enzyme-2; ADAM-17, ADAM metallopeptidase domain 17; TMPRSS2, transmembrane protease, serine 2

* Corresponding author.
E-mail address: gary.williamson1@monash.edu (G. Williamson).

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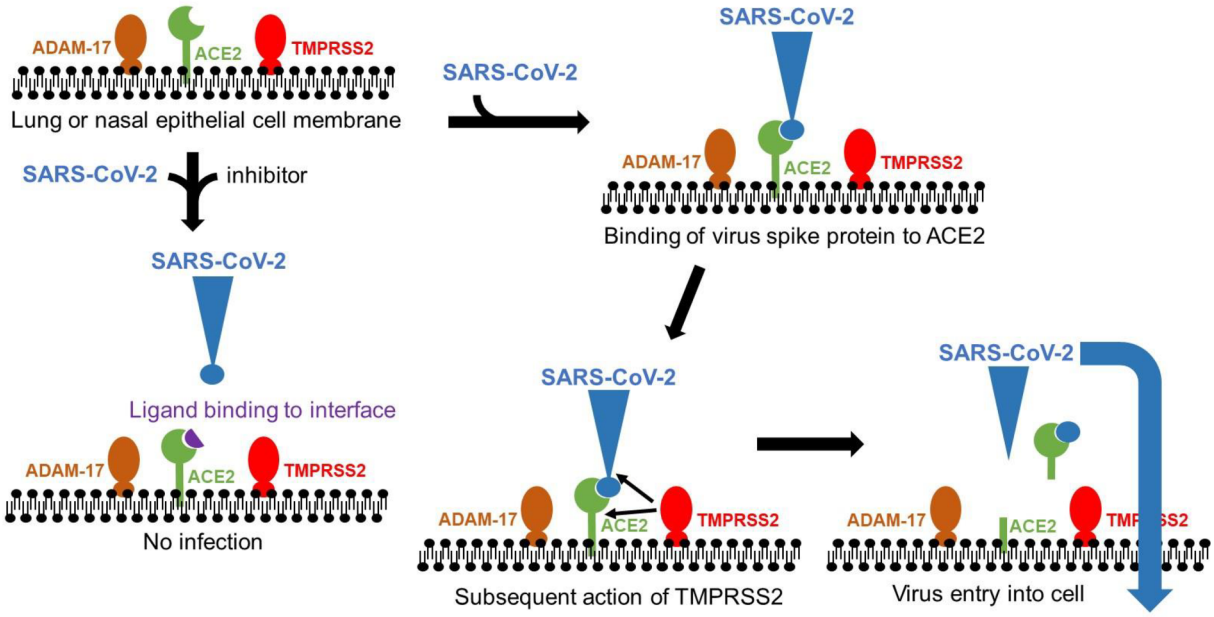

Fig. 1. Interaction between ACE2 and the viral spike protein leading to infection. Representation of the interaction of an inhibitory ligand on the interface between ACE2 and the viral spike protein. The epithelial cells at the infection site express the enzymes ACE2, ADAM-17 and TMPRSS2 on the outer surface. The SARS-CoV-2 virus spike protein binds to the ACE2 enzyme as the very first step of a potential invasion. If this occurs, then the proteases ADAM-17 or TMPRSS2 can cleave ACE2, and in the case of the latter, also cleave the spike protein leading to infection of the epithelial cells and beginning of the invasion [18]. A ligand that binds to the interface of the ACE2 protein and the viral spike protein can inhibit the interaction, reducing the chance of infection. Note that the ligands that bind to the interface are not necessarily inhibitors of ACE2 enzyme activity. ADAM-17, ADAM metallopeptidase domain 17; TMPRSS2, Transmembrane protease, serine 2.

quercetin in a suitable form could deliver the appropriate concentration directly in the active molecular form, i.e. free unconjugated quercetin. Any formulation could include agents to improve stability and solubility [11].

3. Doses required

For a solution of quercetin to be active, a target of at least three times the inhibition constant is reasonable at the site of interaction, i.e. ≥ 25 μM (≥ 7.6 μg/ml). A single 10 ml nasal dose would therefore need to contain ~ 76 μg of quercetin. Although the required effective dose of quercetin is contained, for example, in only ~ 0.06 g of fresh red onion (www.phenol-explorer.eu), eating even large amounts of onions would not be effective owing to the bioavailability issues raised above. These points highlight that if any clinical studies were to be conducted to harness the *in silico* and *in vitro* data, the delivery method of the bioactive molecule is of critical importance. The United States Food and Drug Administration has already approved oral doses of quercetin as safe for human consumption. Quercetin given nasally was effective in a rat model of allergic rhinitis [12], and the safety of quercetin has been favourably assessed [13]. Quercetin at high doses, like any other bioactive compound, could have potentially off-target effects. Following local application by a nasal spray the possibility exists that quercetin could be transported or diffuse to other tissues such as the lungs and blood. Previously found beneficial effects on cardiovascular health biomarkers after regular consumption of quercetin [14] could deliver an additional positive outcome. Patients with pre-existing cardiometabolic syndromes such as hypertension are at increased risk during Covid-19 infection, and the protective effects of quercetin increase the justification for trials. However, quercetin treatment should be cautioned in the case of pre-existing lung cancer. Although many of the effects of quercetin were beneficial in that setting, the fact that quercetin can reprogram cellular energy metabolism should be taken into account [15,16]. We have recently published a review which traces the history of research on quercetin and other flavonoids [17]. One study on Covid-19 and quercetin has so far been entered into www.clinicaltrials.gov (20th June 2020), and the ready availability of quercetin and its low toxicity [13] supports the potential beneficial outcomes of clinical trials involving quercetin [5].

4. Recommendations

We encourage researchers to consider bioavailability issues before embarking on expensive clinical trials. Based on the evidence presented here, it is possible that a nasal spray of dilute quercetin in a suitable vehicle, administered regularly at low doses during the early stages of infection, could attenuate entry of the virus into cells and so halt progress of the infection, possibly leading to a reduced need for hospitalisation. We suggest that clinical trials should be conducted in a timely fashion to test this. However, we emphasize that oral administration of even high doses of quercetin, either as a drug in pure form or in food, is unlikely to be successful owing to the known metabolism of quercetin, involving extensive conjugation and leading to low plasma concentrations of free quercetin.

5. Authors' contributions

Both authors contributed equally to writing the article.

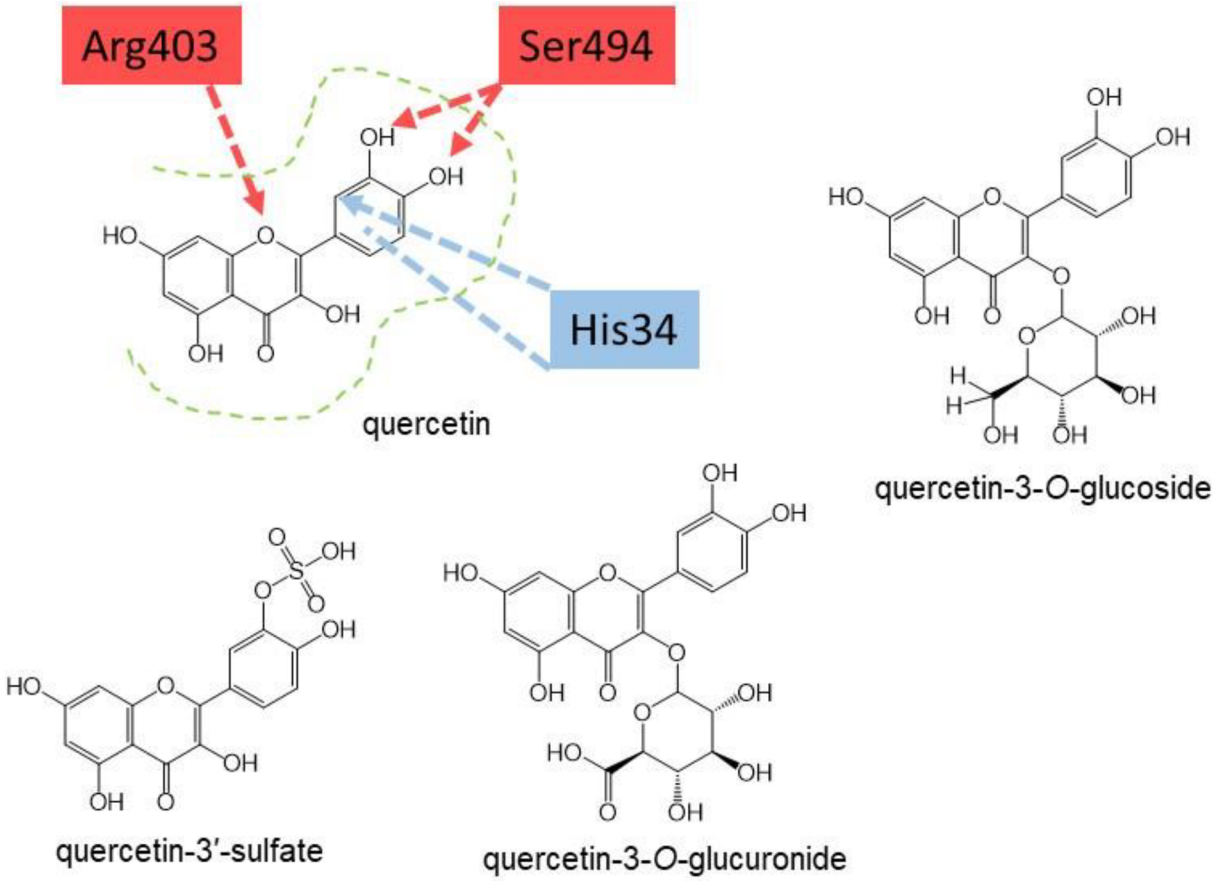

Fig. 2. Putative interactions between quercetin and the ACE2-spike protein interface. The residues shown are redrawn for quercetin based on the interaction reported for the related flavonoid eriodictyol [2]. Amino acids in red belong to the spike protein and those in blue to ACE2. The structures of the two most abundant quercetin conjugates found in the blood after oral consumption of quercetin [6,7,9] are shown to illustrate that the bulky attached moieties would preclude binding to the same site as quercetin. Quercetin-3-*O*-glucoside is also shown as the major form of quercetin in onions [19] before digestion, which has a bulky glucose moiety attached. The crystal structure between ACE2 and the viral spike protein has recently been refined [20]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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