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QUERCETIN PHYTOSOME® AS A POTENTIAL DRUG FOR COVID-19

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Running head: Quercetin Phytosome® and COVID-19

Abstract

When looking for new antiviral compounds aimed to counteract the COVID-19, a disease caused by the recently identified novel coronavirus (SARS-CoV-2), the knowledge of the main viral proteins is fundamental. The major druggable targets of SARS-CoV-2 include 3-chymotrypsin-like protease

(3CLpro), papain-like protease (PLpro), RNA-dependent RNA polymerase, and spike (S) protein. Molecular docking studies have highlighted that quercetin, a natural polyphenol belonging to the flavonol class, inhibits 3CLpro, PLpro and S proteins. Biophysical technics have then very recently confirmed that quercetin is reasonably a potent inhibitor of 3CLpro. The likely antiviral properties of quercetin are anyway challenged by its very poor oral bioavailability profile and any attempt to overcome this limit should be welcome. A phospholipid complex of quercetin (Quercetin Phytosome®) has been recently tested in humans to evaluate a possible improvement in oral bioavailability. After hydrolysis of the conjugated form (mainly glucuronide) of quercetin found in human plasma, the pharmacokinetics results have demonstrated an increased bioavailability rate by about 20-fold for total quercetin. It has been also observed that the presence of specific glucuronidase could yield free systemic quercetin in human body. Taking also into considerations its anti-inflammatory and thrombin-inhibitory actions, a bioavailable form of quercetin, like Quercetin Phytosome®, should be considered a possible candidate to clinically face COVID-19.

Keywords: SARS-CoV-2; infectious diseases; pneumonia; nutraceuticals.

Introduction

Nevertheless the world has witnessed several viral epidemics over the past 20 years, including severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV-1) in 2003, the influenza disease called H1N1 in 2009, and the middle east respiratory syndrome (MERS) coronavirus (MERS-CoV) in 2012, viral diseases continue to pose a serious threat to public health. As globally known, between the end of 2019 and the beginning of 2020 an outbreak of pneumonia caused by a novel coronavirus, named initially 2019-nCoV, was detected in Wuhan City (China). Due to its genetic sequence and to the similarity of its associated symptoms to those induced by SARS-CoV-

1, the novel coronavirus was then named SARS-CoV-2. To date (September 2020) SARS-CoV-2 has provoked more than 26 million of infections and several hundreds of thousands deaths.[1]

Quercetin belongs to the flavonols class and cannot be produced by the human body. It is yellow colour and is poorly soluble in hot water but quite soluble in alcohol and lipids. It is one of the most abundant dietary flavonoids found in fruits (mainly citrus), green leafy vegetables as well as many seeds, buckwheat, nuts, flowers, barks, broccoli, olive oil, apples, onions, green tea, red grapes, red wine, dark cherries, and berries such as blueberries and cranberries. The highest concentrations of flavonols were found in vegetables such as capers, onions and broccoli, fruits such as apples, cherries, and berries, and drinks such as tea and red wine. Quercetin it widely used as a nutritional supplement and may be beneficial against a variety of diseases.[2] Some of the beneficial effects include cardiovascular protection, anticancer, antitumor, anti-ulcer, anti-allergy, anti-inflammatory activity, anti-diabetic, gastroprotective effects, antihypertensive, immunomodulatory, anti-infective, and thrombin-inhibitory actions. Noteworthy, quercetin has been widely described to exert a specific antiviral effect. Viruses which commonly respond to quercetin are adenovirus, herpes simplex virus, Japanese encephalitis virus, influenza virus and respiratory syncytial virus.[2]

Looking for new antiviral drugs by molecular docking

Despite hydroxychloroquine and chloroquine phosphate have shown apparently positive results[3,4] and new antiviral medications such as lopinavir, remdesivir and umifenovir,[5-7] also showing promising results, other potential molecules are currently under investigation, including lopinavir/ritonavir, nucleoside analogues, neuraminidase inhibitors, and the peptide EK1[8]. However, at present, no established antiviral therapies are available for beta-CoV.[9]

When looking for new antiviral compounds, knowledge of the main viral proteins is fundamental. The major druggable targets of SARS-CoV-2 include 3-chymotrypsin-like protease (3CLpro), papain-like protease (PLpro), RNA-dependent RNA polymerase, and spike (S) protein.[10] The S

protein interacts directly with the human ACE2 receptor, allowing the virus to enter the cells. Recently, in order to find new candidates expressing potential activity against these viral targets, a number of studies reporting the use of computer modelling for screening purposes have been published.[11,12] Typically, these models determine the free energy of binding between a ligand and a receptor.[13] A lower binding free energy indicates a stronger ligand–receptor interaction. Although obtaining comparable results via different modelling approaches can be a challenge, computer-based molecular docking allows visualisation of the relative binding affinity of thousands of molecules for the above-listed viral receptors.[14]

In addition to the speed and versatility of this method for rapidly finding a potent inhibitor of SARS-CoV-2, another advantage of molecular docking screening is the reduction of the high costs associated with physically screening the activity of large banks of natural substances.[15] Compounds that through this method demonstrate significant interaction with viral receptors can then be moved onto cell-based assays to verify effectiveness and toxicity. Confirmatory results can then accelerate testing in animals and clinical trials.

Recently, the virtual screening of 83 compounds commonly used in Chinese traditional medicine for activity against the RNA-dependent RNA polymerase of SARS-CoV-2 identified theaflavin, an antioxidant polyphenol, as a potential inhibitor.[11] Similarly, virtual screening of 115 compounds used within Chinese traditional medicine highlighted 13 for further studies.[12] Some of these were naturally occurring polyphenolic compounds such as quercetin and kaempferol, which have already received considerable attention for the treatment of other disease types.[16] In particular, the study showed a significant inhibition by quercetin of 3CLpro and PLpro with a docking binding energy corresponding to -6.25 and -4.62 kcal/mol, respectively.[12]

Moreover, Smith and Smith demonstrated for quercetin a theoretical, but significant, capability to interfere with SARS-CoV-2 replication, with the results showing this to be the fifth best compound out of 18 candidates.[17] In fact, a reasonable target for structure-based drug discovery was identified

to be the disruption of the viral S protein–ACE2 receptor interface. Once again, a computational docking model was used to identify small molecules that were able to bind to either the isolated viral S protein at its host receptor binding region or to the S protein–human ACE2 receptor interface, to potentially limit viral recognition of host cells and/or to disrupt host–virus interactions. Among the natural compounds tested, quercetin was identified as being between the top scoring ligands for the S protein:ACE2 receptor interface, further confirming its role as a promising antiviral agent that should be further investigated. Other Authors have demonstrated similar results for quercetin and have revealed that this molecule binds with the ACE2-S complex with low binding free energy, likely through Arg403 and to Ser494 of the S protein and to His34 of ACE2 protein.[18,19] An in vitro molecular docking study was also performed to analyse the probability of molecular docking between quercetin and viral protease. Proteases play essential roles in viral replication, and specifically, 6LU7 was determined to be the main protease (Mpro) found in SARS-CoV-2. Quercetin formed H-bonds with the 6LU7 amino acids His164, Glu166, Asp187, Gln192, and Thr190, with all of the H-bonds interacting with amino acids in the virus Mpro active site.[20]

More recently, by screening of a chemical library consisting of about 150 compounds, quercetin was identified as reasonably potent inhibitor of SARS-CoV-2 3CLpro with a Ki of about 7μM.[21] Nevertheless, three years have passed since the potential role for quercetin were obtained and no cell-based assay of antiviral activity has been performed.

Quercetin Phytosome®

As the low solubility (0.01 mg/mL)[22] and reduced absorption of quercetin limits its practical use[23] considerable effort has been directed towards overcoming those limitations. Different delivery systems to enhance the water solubility of quercetin and its bioavailability have been developed, including systems based on liposomes, nanoparticles, nano-emulsions, and micelles. The encouraging results of those studies, mainly performed in vitro and evaluated in vivo in rodent

species, support the hypothesis that increasing the water solubility of quercetin could enhance its oral bioavailability. Recently, quercetin formulated with sunflower lecithin in a 1:1 weight ratio with a HPLC-measured total quercetin content of approximately 40% (Quercetin Phytosome®) was tested in humans to evaluate a possible improvement in bioavailability. The results clearly showed the attainment of very high plasma levels of quercetin, up to 20 times greater than those usually obtained following a dose of quercetin, without any notable side effects.[24] Quercetin, when administered orally, undergoes a series of metabolic degradation steps[25] at different levels of the gastrointestinal system: (a) interactions with salivary gland proteins to form aggregates in the mouth;[26] (b) degradation to phenolic acids (for example, protocatechuic acid) with limited absorption in the stomach at low pH, as well as extensive glucuronidation, sulfation, and methylation at the intestinal and hepatic levels;[27] and (c) reconversion of the resulting gut glucuronide derivatives to quercetin by enzymes present in the microbiota.[28] Regardless of the microbiota action, quercetin-3-O-β-D-glucuronide is the major metabolite of quercetin circulating in the bloodstream.[24]

Deconjugation of quercetin glucuronide

Quercetin is known to exert an antihypertensive role in rats and this effect was prevented by β-glucuronidase inhibitors.[29] These data strongly suggest that the sequence of liver–intestine conjugation and vascular deconjugation processes is required for this effect of quercetin. Indeed, in rats, quercetin glucuronide rapidly disappears from the plasma and its fast decay is not compatible with renal excretion, suggesting that quercetin glucuronide is first metabolised and then accumulates in tissues. Moreover, deconjugation is proposed to occur intracellularly as these enzymes are located in the lysosomes. Therefore, the aglycone is formed within the vessel and probably in the cytosol of endothelial cells where it is believed to interact with its possible targets. In this regard, deconjugation of the glucuronide metabolites of the flavonoids by increased β-

glucuronidase activity at the site of inflammation has been suggested as a plausible mechanism for the protective effects of flavonoids in vivo.[30] Thus, glucuronidation protects quercetin from metabolism and helps to carry the flavonoid to the tissues where the free aglycone is released.[31] Of course, polymorphisms of UDP-glucuronosyltransferases (encoded by the UGT1 and UGT2 loci), which are common in humans,[32] and changes in the β-glucuronidase activity, could result in a variable response to quercetin. It is possible to speculate that the same cytolytic effect exerted by the virus on endothelial cells[33] should paradoxically also free quercetin, allowing it to interact with viral protein targets (Figure 1).

Multifaceted quercetin and its clinical relevance

On the basis of the results obtained by computational methods on molecular docking and by biophysical characterization of the structural stability and the catalytic activity of 3CLpro from SARS-CoV-2, it is anticipated that quercetin could have an effect on SARS-CoV-2 by interacting with 3CLpro, PLpro, and/or S protein. In addition to these targets, other findings could prompt us to consider quercetin as being endowed with a general, that is, not specific only for CoV, antiviral role.[34] In any case, based on the strong inflammatory cascade and the blood clotting phenomena triggered during SARS-CoV-2 infection, the multifaceted aspect of quercetin, which has been well described as exerting both anti-inflammatory (quercetin dose-dependently decreases the mRNA and protein levels of ICAM-1, IL-6, IL-8, and MCP-1) and thrombin-inhibitory actions, should be taken into consideration.[35,36]

To date, a considerable amount of data has been accumulated describing the potential antiviral role (among others) of quercetin. Indeed, several studies, using computational models and in vitro and in vivo assays, would seem to confirm this. At the present time, however, the critical lack of high-quality clinical data must be highlighted, although some empirical and/or case–control clinical evaluations would appear encouraging. A randomised study performed a decade ago enrolled 1002

adult subjects affected by viral infections of the upper respiratory tract; this showed that quercetin administered at very high dosages for 12 weeks reduced the days of illness in middle-aged and elderly subjects.[37] More recently, an empirical study conducted at a Wuhan hospital showed that an approach where, in addition to conventional therapies, patients were treated with traditional Chinese medicine remedies, including herbs with a high quercetin content, was medically safe, free from particular side effects additional to those obtained with the conventional approach alone, and was able to improve the symptoms of patients with COVID-19.[38]

Finally, a recent our review shows that quercetin may have a well-defined role in the treatment of COVID-19, because shows pharmacological activity such as antiviral, anti-atopic, pro-metabolic, and anti-inflammatory effects.[39]

Conclusion

In the recent past, there have been several pandemics. Within the context of globalisation, some of these pandemics have truly raised the global risk to humankind. The COVID-19 pandemic is the latest of these and is, so far, completely unresolved. In the meantime, as we await the development of an effective vaccine, researchers worldwide must focus all their efforts on selecting possible effective treatments, bearing in mind that the plant kingdom supplies chemical skeletons that, since ancient times, have provided humans with “drugs”.

A number of data seems to suggest quercetin as a potential molecule candidate for an anti-COVID-19 and recommends the execution of in vitro tests able to reveals if the molecule is endowed with a real and SARS2 viral effect and a case–control clinical study with the aim of realising its possible efficacy within the context of this disease. On the basis of its poor pharmacokinetics profile, any galenic formulation aimed to improve its rate of absorption should be considered important. At our knowledge, the Phytosome form of quercetin could be a possible candidate. Our group is indeed currently investigating both the *in vitro* and the clinical effect in COVID-19 patients of Quercetin

Phytosome® at University of Oxford (UK) and Liaquat University of Medical and Health Sciences (LUMHS), Jamshoro (Pakistan), respectively. The clinical trial is currently under application on www.clinicaltrial.gov.

Conflict of interest

The authors have no relevant conflict of interests.

Contributions section

All authors equally contributed in writing the manuscript. All authors read and approved the final version of the manuscript.

References

1) Kannan S, Shaik Syed Ali P, Sheeza A, Hemalatha K. COVID-19 (Novel Coronavirus 2019) - recent trends. Eur Rev Med Pharmacol Sci. 2020;24(4):2006-2011.
2) Anand David AV, Arulmoli R, Parasuraman S. Overviews of Biological Importance of Quercetin: A Bioactive Flavonoid. Pharmacogn Rev. 2016;10(20):84-89.
3) Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. J Crit Care. 2020;57:279-283.
4) Gao J, Hu S. Update on use of chloroquine/hydroxychloroquine to treat coronavirus disease 2019 (COVID-19). Biosci Trends. 2020;14(2):156-158.
5) Yao TT, Qian JD, Zhu WY, Wang Y, Wang GQ. A systematic review of lopinavir therapy for SARS coronavirus and MERS coronavirus-A possible reference for coronavirus disease-19 treatment option. J Med Virol. 2020;92(6):556-563.

6) Yin W, Mao C, Luan X, et al. Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. Science. 2020;368(6498):1499-1504.

7) Khamitov RA, Loginova SIa, Shchukina VN, Borisevich SV, Maksimov VA, Shuster AM. Antiviral activity of arbidol and its derivatives against the pathogen of severe acute respiratory syndrome in the cell cultures. Vopr Virusol. 2008;53(4):9-13.

8) Lu, H., 2020. Drug treatment options for the 2019-new coronavirus (2019-nCoV). Biosci Trends 14, 69-71.

9) Sohrabi C, Alsafi Z, O'Neill N, et al. Corrigendum to "World Health Organization declares Global Emergency: A review of the 2019 Novel Coronavirus (COVID-19)" [Int. J. Surg. 76 (2020) 71-76]. Int J Surg. 2020;77:217.

10) Wu C, Liu Y, Yang Y, et al. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharm Sin B. 2020;10(5):766-788.

11) Lung J, Lin YS, Yang YH, et al. The potential chemical structure of anti-SARS-CoV-2 RNA-dependent RNA polymerase. J Med Virol. 2020;92(6):693-697.

12) Zhang DH, Wu KL, Zhang X, Deng SQ, Peng B. In silico screening of Chinese herbal medicines with the potential to directly inhibit 2019 novel coronavirus. J Integr Med. 2020;18(2):152-158.

13) Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ. Computational protein-ligand docking and virtual drug screening with the AutoDock suite. Nat Protoc. 2016;11(5):905-919.

14) Aldeghi M, Heifetz A, Bodkin MJ, Knapp S, Biggin PC. Accurate calculation of the absolute free energy of binding for drug molecules. Chem Sci. 2016;7(1):207-218.

15) Chen Y, de Bruyn Kops C, Kirchmair J. Data Resources for the Computer-Guided Discovery of Bioactive Natural Products. J Chem Inf Model. 2017;57(9):2099-2111.

16) Cassidy L, Fernandez F, Johnson JB, Naiker M, Owoola AG, Broszczak DA. Oxidative stress in alzheimer's disease: A review on emergent natural polyphenolic therapeutics. Complement Ther Med. 2020;49:102294.

17) Smith, M., Smith, J.C., 2020. Repurposing therapeutics for COVID-19: supercomputer-based docking to the SARS-CoV-2 viral spike protein and viral spike protein-human ACE2 interface. ChemRxiv https://doi.org/10.26434/chemrxiv.11871402.v3.

18) Williamson G, Kerimi A. Testing of natural products in clinical trials targeting the SARS-CoV-2 (Covid-19) viral spike protein-angiotensin converting enzyme-2 (ACE2) interaction. Biochem Pharmacol. 2020;178:114123.

19) Pandey P, Rane JS, Chatterjee A, et al. Targeting SARS-CoV-2 spike protein of COVID-19 with naturally occurring phytochemicals: an in silico study for drug development [published online ahead of print, 2020 Jul 22]. J Biomol Struct Dyn. 2020;1-11.

20) Khaerunnisa, S, Kurniawan, H, Awaluddin, R, Suhartati, S, Soetjipto, S, 2020. Potential inhibitor of COVID-19 main protease (Mpro) from several medicinal plant compounds by molecular docking study. Preprints 2020030226.

21) Abian O, Ortega-Alarcon D, Jimenez-Alesanco A, et al. Structural stability of SARS-CoV-2 3CLpro and identification of quercetin as an inhibitor by experimental screening. Int J Biol Macromol. 2020;164:1693-1703.

22) Gao L, Liu G, Wang X, Liu F, Xu Y, Ma J. Preparation of a chemically stable quercetin formulation using nanosuspension technology. Int J Pharm. 2011;404(1-2):231-237.

23) Wang W, Sun C, Mao L et al. The biological activities, chemical stability, metabolism and delivery systems of quercetin: a review. Trends Food Sci Technol. 2016; 56, 21-38.

24) Riva A, Ronchi M, Petrangolini G, Bosisio S, Allegrini P. Improved Oral Absorption of Quercetin from Quercetin Phytosome®, a New Delivery System Based on Food Grade Lecithin. Eur J Drug Metab Pharmacokinet. 2019;44(2):169-177.

25) Mullen W, Edwards CA, Crozier A. Absorption, excretion and metabolite profiling of methyl-, glucuronyl-, glucosyl- and sulpho-conjugates of quercetin in human plasma and urine after ingestion of onions. Br J Nutr. 2006;96(1):107-116.

26) Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004;79(5):727-747.

27) Rich GT, Buchweitz M, Winterbone MS, Kroon PA, Wilde PJ. Towards an Understanding of the Low Bioavailability of Quercetin: A Study of Its Interaction with Intestinal Lipids. Nutrients. 2017;9(2):111.

28) Németh K, Plumb GW, Berrin JG, et al. Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. Eur J Nutr. 2003;42(1):29-42.

29) Galindo P, Rodriguez-Gómez I, González-Manzano S, et al. Glucuronidated quercetin lowers blood pressure in spontaneously hypertensive rats via deconjugation. PLoS One. 2012;7(3):e32673.

30) Shimoi K, Saka N, Nozawa R, et al. Deglucuronidation of a flavonoid, luteolin monoglucuronide, during inflammation. Drug Metab Dispos. 2001;29(12):1521-1524.

31) Schewe T, Steffen Y, Sies H. How do dietary flavanols improve vascular function? A position paper. Arch Biochem Biophys. 2008;476(2):102-106.

32) Crettol S, Petrovic N, Murray M. Pharmacogenetics of phase I and phase II drug metabolism. Curr Pharm Des. 2010;16(2):204-219.

33) Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet. 2020;395(10234):1417-1418.

34) Fanunza E, Iampietro M, Distinto S, et al. Quercetin Blocks Ebola Virus Infection by Counteracting the VP24 Interferon-Inhibitory Function. Antimicrob Agents Chemother. 2020;64(7):e00530-20.

35) Cheng SC, Huang WC, S Pang JH, Wu YH, Cheng CY. Quercetin Inhibits the Production of IL-1β-Induced Inflammatory Cytokines and Chemokines in ARPE-19 Cells via the MAPK and NF-κB Signaling Pathways. Int J Mol Sci. 2019;20(12):2957.

36) Liu L, Ma H, Yang N, et al. A series of natural flavonoids as thrombin inhibitors: structure-activity relationships. Thromb Res. 2010;126(5):e365-e378.

37) Heinz SA, Henson DA, Austin MD, Jin F, Nieman DC. Quercetin supplementation and upper respiratory tract infection: A randomized community clinical trial. Pharmacol Res. 2010;62(3):237-242.

38) Luo E, Zhang D, Luo H, et al. Treatment efficacy analysis of traditional Chinese medicine for novel coronavirus pneumonia (COVID-19): an empirical study from Wuhan, Hubei Province, China. Chin Med. 2020;15, 34.

39) Derosa G, Maffioli P, D'Angelo A, Di Pierro F. A role for quercetin in Coronavirus disease 2019 (Covid-19). Phytother Res. Accepted.

Figure 1 Hypothetical pathways of the quercetin glucuronide and free-quercetin inside a coronavirus-infected host

Thanks to ACE2 protein coronavirus enters inside the cell where it replicates until provoking cell lysis and death (A). Glucuronidases of the host free quercetin from quercetin glucuronide allowing it to inhibit viral proteases inside host's cells (B). Cell lysis provoked by coronavirus frees quercetin outside host's cells allowing it to limit the contact between S-protein and ACE2 and therefore inhibiting the new entry of the virus (C).