Promising Therapeutic Approach for SARS-CoV-2 Infections by Using a Rutin-Based Combination Therapy

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The development of new therapeutic approaches for SARS-CoV-2 infections is of particular current importance. The combination therapy proposed here is based on already proven, safe and inexpensive compounds. The natural compound rutin, one of the six therapy components, has the potential to inhibit both viral and host cell targets. In addition, this therapy involves the use of acetylsalicylic acid, vitamin C and vitamin D_3, which should be administered together with calcium and magnesium. The importance of each component is briefly described in this article. Due to multiple anti-infective properties of rutin, it provides a basis for combating a SARS-CoV-2 infection as well as various viral and bacterial co-infections. There are strong indications for a good effect of this simple combination therapy, especially in the early stages of infection. It has the potential to be of interest both prophylactically and therapeutically, and offers the possibility of protection against severe disease progression.

The search for new therapeutic approaches against the COVID-19 disease caused by SARS-CoV-2 viruses is of highest current importance. The worldwide enormous activities are manifold and the summary of the already published studies would exceed the scope of this article. The activities focus, on the one hand, on the identification and testing of known antiviral agents[1,2a] and, on the other hand, on the design of new compounds.[2a–c] When testing existing antiviral drugs, both monotherapies and combination therapies[1,3] (e.g. lopinavir/ritonavir, sofosbuvir/velpatasvir or chloroquine/hydroxychloroquine combinations) were taken into account. Although promising results could be obtained, there is still no proven antiviral therapy against SARS-CoV-2 infections.

SARS-CoV-2 is an enveloped, positive sense, single-stranded RNA virus (β-coronavirus, such as SARS-CoV) and its entry into host cells is mediated by human angiotensin-converting enzyme 2 (ACE2), which binds to spike surface glycoproteins of SARS-CoV-2.[4] Thus, the use of ACE2 inhibitors preventing the entry of the SARS-CoV-2 virus into the cell represents one of the possible strategies to combat infection.

Furthermore, the inhibition of the catalytic activity of 3-chymotrypsin-like protease ($3CL^{pro}$),[2a,5] the main protease of SARS-CoV-2 and other coronaviruses, is considered as one of the best strategies to block the viral replication in affected patients. Because of high similarity of the active site of $3CL^{pro}$ among different variants of SARS-CoV-2 as well as other coronaviruses, inhibitors of this viral protease have the potential to act as broad-spectrum agents. Given the importance of inhibiting ACE2 and $3CL^{pro}$, the identification of compounds possessing the ability to bind to one or both of the mentioned targets is of high significance.

It is particularly desirable to develop effective therapies based on already proven, safe and inexpensive compounds. The combination therapy proposed here meets these requirements and is based on six components. Of particular note, the natural compound rutin (Figure 1) has the potential to inhibit both human ACE2 and viral $3CL^{pro}$ as well as other viral and host cell targets (see below). In addition, this therapy involves the use of acetylsalicylic acid, vitamin C and vitamin D_3, which should be administered together with calcium and magnesium. Some observations and experiences indicate a good effect of this combination therapy,[6] especially in the early stages of infection (there are strong indications for prevention of severe disease progression). The importance of each component is briefly described below.

Rutin belongs to the class of flavonoids and has been identified as a substance with a very broad pharmacological spectrum of activity.[7a–c] The numerous beneficial activities reported so far include, among others, antibacterial, antimicrobial, anti-inflammatory, antiallergic, analgesic, antioxidant, and

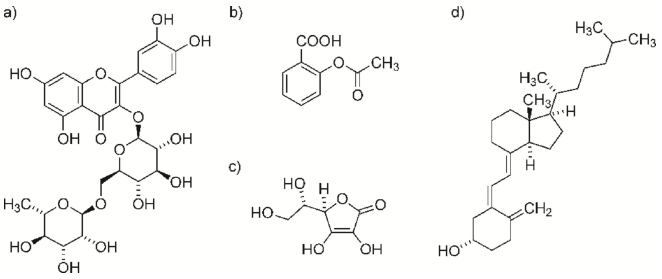

Figure 1. Structures of (a) rutin (quercetin-3-*O*-rutinoside), (b) acetylsalicylic acid (ASA, aspirin), (c) vitamin C (L-ascorbic acid) and (d) vitamin D_3 (cholecalciferol).

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antidiabetic properties.[5,7a,b] It should be especially emphasized that rutin has also a broad antiviral activity against a diverse group of viruses, including the influenza virus and the hepatitis C virus.[5,7c]

With regard to SARS-CoV-2, various computational studies, such as docking and molecular dynamics simulations, have identified rutin as a potential inhibitor of SARS-CoV-2 main protease.[5,8,9] Experimental studies confirmed this ability and provided information about inhibition constants and thermodynamic parameters of the binding process (for description of inhibition assays and microcalorimetric investigations, see ref.[8]). According to the molecular docking studies, rutin is able to associate in the active site of $3CL^{pro}$ and interact with the catalytic dyad (His 41/Cys 145). The intermolecular interactions formed between rutin and $3CL^{pro}$ include conventional hydrogen bonds and CH-π contacts as well as cation-π and sulfur-π interactions (for examples, see Figure 2).[9a] The published comprehensive computational studies on rutin also contain a detailed description of the intermolecular interactions that are supposed to be responsible for effective binding of rutin to human ACE2.[9b,c] Furthermore, rutin has also been recognized as a potential inhibitor of SARS-CoV-2 papain-like protease (PL^{pro}),[5,9b] RNA-dependent RNA polymerase (RdRp)[9d] and helicase.[9d] In addition, rutin was also reported to have the ability to interact with the host toll-like receptors (TLRs),[9e] which play an important role in the host-based anti-CoV activity [for a review on virus-based and host-based therapeutic options for coronaviruses (CoVs), especially for SARS-CoV and MERS-CoV, see ref.[10]]. Besides the inhibition of SARS-CoV-2 $3CL^{pro}$ and the human ACE2, the blocking of the last-mentioned targets also plays an important role in combating SARS-CoV-2 infection.

The positive synergism of flavonoids and ascorbic acid indicates simultaneous administration of rutin and vitamin C, which is known to have wide ranging effects on the immune system during infections.[11a–d] Some studies supported the beneficial role of vitamin C in reducing the incidence of severity of bacterial and viral infections.[11d] Its potential role in the fight against COVID-19 was also evaluated.[11a] The effect of various vitamin C applications as both prophylaxis and therapy of the COVID-19 disease has been investigated, but the results are controversially discussed. However, all literature data to date show that vitamin C acts on various pathophysiological processes of SARS-CoV-2 infections without significant side effects. Given the potential favorable role of vitamin C in treatment of sepsis and ARDS (acute respiratory distress syndrome),[11d] there is a great interest in investigating whether supplementation of this vitamin could be beneficial in the case of COVID-19. Since a viral infection leads to a strongly increased vitamin C requirement,[11a–c] its intake is recommendable. It should also be mentioned that drug forms containing both rutin and vitamin C have long been available on the market and have been suggested, among others, for use in viral infections, including influenza.[11e]

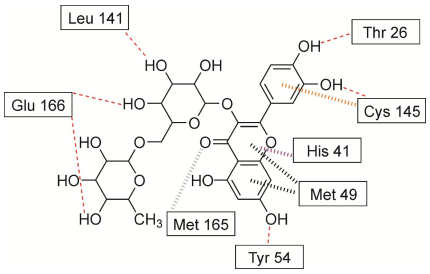

Figure 2. Examples of intermolecular interactions (dashed lines) between rutin and $3CL^{pro}$ of SARS-CoV-2 indicated by molecular docking experiments:[9a] hydrogen bonds (red), CH-π (black), cation-π (magenta), sulfur-π (orange) and van der Waals interactions (grey).

Acetylsalicylic acid (ASA, aspirin) has multiple effects on different components of innate and adaptive immunity and may therefore influence susceptibility to viral infection.[12a] Although the mode of action of ASA has been studied very intensively, information about its efficacy against viral pathogens is less frequently published. Studies have been reported, for example, on the antiviral activity of ASA against RNA viruses of the respiratory tract, such as influenza A H1N1 virus and human rhinoviruses (HRV).[12b] In the case of SARS-CoV-2 some studies suggest that ASA use may have beneficial effects in patients with COVID-19.[13] For example, it has been reported that patients who were treated with acetylsalicylic acid at a low-dose for cardiometabolic disease prior to their infection with SARS-CoV-2 had a significantly milder course of their COVID-19 disease in an observational study.[13a,b] The effect of low doses of ASA on mortality and viral duration of the hospitalized adults with COVID-19 was also examined.[13c] In this study, low-dose aspirin medication (100 mg/day) among patients with COVID-19 was associated with a lower risk of mortality compared with those who did not take aspirin. It should be noted that considering the mode of action of ASA, the observed positive effect appears quite comprehensible.

With regard to the problem of co-infections among patients with COVID-19, the need for combination therapy with non-anti-SARS-CoV-2 agents has been addressed in the literature [such co-infections have also been observed in patients with severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS)].[14] Various bacteria as well as viruses,[14] such as influenza, rhinovirus, parainfluenza and metapneumovirus have been identified as co-pathogens (influenza A was one of the most common co-infective viruses). It has been pointed out that co-infection with other respiratory pathogens cannot be ruled out when diagnosing a SARS-CoV-2 infection, nor can COVID-19 be ruled out by detecting non-SARS-CoV-2 respiratory pathogens.[14] Taking the co-infections into account, the use of aspirin in low doses (e.g. 100 mg/day) gains additional importance. However, the remarkable broad biological activity of rutin plays a particularly important role in this context.

There is evidence in the literature that insufficient serum vitamin D levels are associated with an increased risk of acute respiratory infections, including the COVID-19 disease.[15a–d] Several studies suggested that taking vitamin D_3 (see Figure 1)[15e] may have a positive effect on the course of SARS-CoV-2 infections and provided strong evidence of a causal relationship between low vitamin D status and an increased risk of

severe COVID-19 disease.[15a–c] Due to a lot of encouraging data, the use of vitamin D to reduce the severity of SARS-CoV-2 infections is receiving considerable attention.

The synergistic action of vitamin D, magnesium and calcium[15f] indicates the necessity of the consideration of the two minerals. In addition, some studies revealed that COVID-19 severity was also associated with lower serum concentration of magnesium and calcium. Low blood calcium levels (hypocalcaemia) are very common in COVID-19 patients. In studies, up to 82% of all patients had low blood calcium levels on hospital admission[16a–e] and these patients were found to have a significantly worse course of disease. It is controversial whether the low calcium status is caused by the infection or whether calcium deficiency negatively affects the immune defense.[16f] However, not replacing the calcium loss at all would lead to hypocalcaemic disorders and calcium mobilization from the bones, so that moderate supplementation combined with magnesium and vitamin D_3 is recommended in COVID-19.[16e]

Compared to the numerous discussions on the role of calcium, studies that have assessed the relationship between magnesium status and COVID-19 infection have been published less frequently. Based on the results showing the possible supportive effects of magnesium on disease outcomes, monitoring of magnesium status and treatment of magnesium deficiency in COVID-19 patients have been suggested.[16g]

At this point, it should be emphasized that the significance of the individual components of the proposed combination therapy can only be briefly outlined, since a detailed consideration of their mode of action and the description of the extensive literature references would exceed the scope of this concept article.

The therapeutic approach for SARS-CoV-2 infections by using a rutin-based combination therapy has numerous potential advantages. The benefits include, among others, broad pharmacological spectrum of activity, good safety profile, availability and low cost.

According to the computational and experimental studies, rutin can potentially act as both a virus-based and host-based anti-CoV therapeutic by inhibiting SARS-CoV-2 and host targets. Of particular advantage is that rutin has the potential to inhibit various stages of the virus life cycle. Due to its known multiple anti-infective properties, rutin provides a basis for combating SARS-CoV-2 infection as well as various viral and bacterial co-infections, which have been observed in patients. In this context, the use of the other therapy components also plays a very important role.

The remarkable efficacy of rutin against a whole range of bacterial and viral pathogens, as well as its proven use in vitamin C preparations and in traditional medicine (recently also against COVID-19, as reported for traditional Chinese medicine[17]), numerous literature references to the positive effects of the other components on the course of SARS-CoV-2 infections, and, last but not least, our own very positive experiences[6,18] make this simple combination therapy a promising and attractive therapeutic approach for SARS-CoV-2 infections. It has the potential to be of interest both prophylactically and therapeutically, and offers the possibility of protection against severe disease progression. Therefore, detailed validation of this approach in well-designed clinical studies is highly desirable.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Natural products · antiviral agents · viruses · vitamins · flavonoids · antioxidants · molecular recognition · noncovalent interactions

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[18] a) Exemplary medication is given in the following (for details, see ref. [6]). Morning: 50 mg rutin trihydrate and 200 mg vitamin C (corresponds to two tablets of Rutinoscorbin), 100 mg ASA (taking into account the time interval); lunch: 5 μg vitamin D_3, 600 mg calcium and 300 mg magnesium; evening: 50 mg rutin trihydrate and 200 mg vitamin C (two tablets of Rutinoscorbin); b) The aspect of possible enhancement of the effect of ASA on the stomach by rutin should be considered; c) It is necessary to clarify whether the possible formation of complexes between rutin and magnesium or calcium ions can strengthen or weaken the positive effect of rutin (currently it is recommended to consider time intervals when taking these components).

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In Reference [18a], the correct lunch dosage of vitamin D_3 is 5 μg (micrograms) and not 5 mg as mentioned in the first version of record published on 24 May 2022.