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Brief report on the HMGB1-antagonism exerted by glycyrrhizin could be fruitful against COVID-19

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Abstract. The COVID-19 pandemic era is causing a relevant issue for the health. There is no specific drug able to antagonize the SARS-CoV-2 infection. As a consequence, there is growing interest about potential molecules able to contrast infection. In this regard, HMGB, an alarmin, may play a relevant role in pathogenic mechanisms induced by SARS-CoV-2. As HMGB1 is antagonized by glycyrrhizin, this substance could be potentially useful as ancillary treatment in COVID-19. (www.actabiomedica.it)

Keywords: COVID-19, SARS CoV-2, HMGB1, Glycyrrhizic acid, Prophylaxis.

The pandemic COVID-19 has so far affected around 220 million confirmed patients, and nearly 4.5 million people have died worldwide as at 30 August 2021 (1). SARS-CoV-2, a single-strand-RNA coronavirus, is the pathogen for COVID-19. SARS-CoV-2 uses the ACE2 receptor as a gateway to enter the human cell, as its protein S can bind this cellular receptor (2). Once inside the cell, SARS - CoV-2 begins the replication phase, usually in the respiratory system, but also in digestive and nervous system, inducing a defensive host's immune response. However, some patients (about 5-10%) break out in severe disease, they frequently have associated comorbidities, including obesity, hypertension, diabetes mellitus, and old age. These conditions are characterized by a low-grade chronic inflammation that may amplify the immune response against the virus, such as the cytokine storm syndrome, characterized by an overproduction of pro-inflammatory cytokines, including tumor necrosis factors, interleukins, and chemokines (2). The overproduction of these factors leads to severe tissue damage, progressing rapidly towards acute respiratory distress (ARDS), sepsis, multi-organ failure, and disseminated intravascular coagulation.

At present, a mass vaccination program is ongoing (August 2021) as there is no specific drug to treat COVID-19. In this regard, there is growing interest in plants with activity against COVID-19, including *Achillea millefolium, Allium sativum, Aloe vera, Bidens Pilosa, Curcuma longa, foeniculum vulgare, Glycine max, Momordica charantia, Morus indica, Phyllanthus amarus, Phyllanthus niruri, Punica granatum,* and *Zingiber officinalis* (3). Evidence is accumulating on phytochemicals' potential effectiveness in contrasting COVID-19, mainly concerning immunomodulation (4-7).

Virally infected cells release substances (damage-associated molecular pattern molecules) that alarm the immune system to adequately contrast infection. For this reason, these molecules take the name alarmin. High mobility group box-1 (HMGB1) is a prototypical alarmin and participates in nuclear and extracellular pathways (8). Intranuclear activity concerns transcriptional phases, as HMGB1 binds to chromatin, histones, DNA, and RNA. Otherwise, HMGB1 is released from dead or damaged cells. Extracellular HMGB-1 interacts with two primary receptors, such as toll-like receptors (TLR) and receptor advanced glycosylation end-product (RAGE) (9). An axis HMGB1/RAGE/

TLR is deeply involved in many respiratory infections, including influenza, bronchiolitis caused by the human respiratory syncytial virus, bacterial pneumonia, and acute lung injury (10). In particular, RAGE, a member of the immunoglobulin superfamily, may be involved in acute lung and systemic inflammatory events in severe COVID-19 patients (11). Also, severe COVID-19 patients express high HMGB1 levels (9). Noticeably, HMGB1 may promote a cytokine storm via TLR interaction and increase ACE2 expression through RAGE stimulation in COVID-19 patients (12).

Based on this background, HMGB1 could be an attractive target in treating COVID-19 (13). In this regard, an innovative approach glimpses the use of a saponin phytochemical: glycyrrhizin (14). The term saponin derives from the plant *Saponaria officinalis* that was used as a natural soap to wash the wool.

Glycyrrhizin is a triterpene saponin extracted from the root of *the Glycyrrhiza glabra* plant, typically cultivated in Europe. The term glycyrrhiza derives from the old Greek as means γλυκύς (sweet) and ῥίζα (root). The main constituent of roots is glycyrrhizin, a triterpenoid saponin that is almost 50 times sweeter than sucrose, being the primary active ingredient (15). Glycyrrhizin represents about 10% of the liquorice root dry weight, being a mixture of potassium, calcium, and magnesium salts of glycyrrhizic acid that varies between 2% and 25% (16). After oral administration, glycyrrhizin is metabolized to 18-glycyrrhetic acid 3-omonoglucuronide and glycyrrhetic acid by intestinal bacteria (15). Licorice is typically the main source of intake; however, also white walnut and cicely are foods rich in glycyrrhizin.

Glycyrrhizin is a component of traditional Chinese medicine and boasts a millennial tradition in Western popular medicine (17). The perennial appeal relies on the broad mechanisms of action, including anti-inflammatory, antioxidant, corticosteroidal, antiallergic, antiviral, antimalarial, antihyperglycemic, antitussive, immunostimulating, and anti-HIV activity (18). For these reasons, glycyrrhizin has been popularly used to treat many diseases, such as stomach ulcers, heartburn, acidity, flatulence, constipation, fever, cough, respiratory infections, asthma, and jaundice (19). The recommended daily dosage is about 5-10 grams of pulverized root (corresponding to about 200-400 mg of glycyrrhizin) *per os*. However, there is accumulating evidence that glycyrrhizin has been used as a true drug for treating liver diseases, mainly concerning chronic hepatitis, for more than 40 years, at the same posology. The rationale for this use depended on the documented activities, such as the inhibitory effect on pro-inflammatory cytokines, activation of $CD8^+$ T cells, and proliferation of T regulatory cells (20).

Concerning COVID-19, as specific treatments are still lacking, glycyrrhizin could potentially prevent and/or contrast SARS-CoV-2 infection. A recent paper summarized the multiple mechanisms through which glycyrrhizin could be beneficial for COVID-19. Glycyrrhizin binds to ACE2, so diminishing the viral docking, downregulates pro-inflammatory cytokines release, and upregulates expression of anti-inflammatory mediators acting as a corticosteroid (21). Namely, the glycyrrhizin/RAGE axis promotes or inhibits the nuclear factors' activity so suppressing or enhance the immune response. Glycyrrhizin actually has a steroid-like molecular structure and also exerts mild mineral active activity. However, glycyrrhizin does not provide the classical adverse events associated with corticosteroid use. Therefore, glycyrrhizin could be administered for longer periods than corticosteroids. Also, glycyrrhizin inhibits the formation of reactive oxygen species consequent to viral infection so exerting antioxidant activity. Glycyrrhizin is a selective inhibitor of thrombin and consequently could block the activation of the coagulation and complement cascade. Glycyrrhizin also inhibits the MUC5AC gene transcription involved in mucus production, so that reduces mucus hypersecretion. Glycyrrhizin fosters the production of interferon so increasing type 1 immune response against viral infection. In addition to these mechanisms of action, glycyrrhizin has a specific activity on HMGB1. Glycyrrhizin physically binds to HMGB1 (trapping) to inhibit the interaction between HMGB1 and its receptors RAGE and TLR. Glycyrrhizin *de facto* sequesters extracellular HMGB1 inhibiting its intranuclear translocation. Consequently, glycyrrhizin induces a relevant anti-inflammatory activity, so damping inflammatory phenomena dependent on innate immunity stimulation by viral infection (13). As HMGB1 is an alarmin, its effects are systemic, such as targeted towards many organs. HMGB1 inhibition

could be, therefore, an intriguing strategy to contrast viral infection. Thanks to the optimal safety profile, glycyrrhizin could be envisaged as a multi-target compound able to fight viral disease.

From a practical point of view, glycyrrhizin is available in different oral formulations, including tablets, elixir, syrups, or for topical administration (dipotassium glycyrrhizinate), such as nasal spray, eye drops, and mouthwash, combined with other natural substances, for example, zinc, vitamin D, hyaluronate, and lactoferrin. These substances can have synergic antiviral activities.

In conclusion, glycyrrhizin is an interesting phytochemical provided by multifaceted antiviral and anti-inflammatory activities and good safety and tolerability. In particular, there is growing interest about this molecule as documented by the several reviews, and *in silico* and *in vitro* evidence recently published (22-28). It has also been hypothesized the use of saponins as adjuvants for the COVID-19 vaccines (29). Nevertheless, there is the need to further studies that document the real beneficial effect of glycyrrhizin in the treatment (or even prevention) of COVID-19. In this regard, a retrospective observational investigated the use of giammonium glycyrrhizinate and vitamin C in patients with COVID-19. This multicomponent compound reduced the incidence of acute respiratory distress syndrome (30). However, we are still waiting for a randomized controlled study that provide adocumented evidence.

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