Mexican Journal of Technology and Engineering *Volumen 1, Número 2*

Artículo de revisión https://doi.org/10.61767/mjte.001.2.0921

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Recibido:11-08-2022 *Revisado: 15-09-2022* *Aceptado: 04-10-2022* *Publicado: 11-10-2022*

GLYCYRRHIZIN AND GLYCYRRHETINIC ACID: PHARMACOLOGICAL POTENTIAL FOR THE TREATMENT OF VIRAL RESPIRATORY INFECTIONS GLICIRRIZINA Y ÁCIDO GLICIRRETÍNICO: POTENCIAL FARMACOLÓGICO PARA EL TRATAMIENTO DE INFECCIONES RESPIRATORIAS VIRALES

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Abstract

Glycyrrhizinic acid (also known as glycyrrhizin) (GA) and its derivative 18-β-Glycyrrhetinic acid (18b-GA), which are isolated from the plant *Glycyrryza glabra,* show several therapeutic properties, including antioxidant, anti-inflammatory, and antiviral activity. These are therefore being evaluated for several medical proposes, among them the treatment of respiratory infections induced by viruses. The current review aimed to highlight the potential of GA and 18b-GA as efficient drugs. Both molecules have demonstrated antiviral activity against SARS-CoV-2, due to different mechanisms; for instance, blocking key enzymes for virus entry to the cell or by limiting virus replication. Those properties are well known from in vitro and animal assay research, however, reports of their effects on humans are scarce. One of the main challenges of using GA and 18b-GA as a drug is improving their solubility and permeability, as well as using therapeutic doses without toxic effects. Due to the biological properties of GA and 18b-GA, the dose and administration type are crucial to achieving a high local concentration and therefore a therapeutic effect.

Keywords: Glycyrrhizin, 18-β-Glycyrrhetinic acid, respiratory infections, antiviral activity, antioxidant activity, anti-inflammatory activity.

Resumen

El ácido glicirricínico (también conocido como glicirricina) (GA) y su derivado, el ácido 18-β-glicirretínico (18b-GA), aislados de la planta *Glycyrryza glabra*, muestran diversas propiedades terapéuticas, que incluyen actividad antioxidante, antiinflamatoria y antiviral. Por lo tanto, estos

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fármacos están siendo evaluados con diferentes propósitos médicos, entre ellos el tratamiento de infecciones respiratorias inducidas por virus. El objetivo de la presente revisión fue resaltar el potencial de GA y 18b-GA como fármacos eficaces. Ambas moléculas han demostrado actividad antiviral frente a SARS-CoV-2, a través de diferentes mecanismos; por ejemplo, bloqueando enzimas clave para la entrada del virus a la célula o limitando la replicación del virus. Estas propiedades se conocen a partir de la investigación en ensayos in vitro y en animales, sin embargo, los reportes de sus efectos en humanos son escasos. Uno de los principales retos del uso de GA y 18b-GA como fármaco es mejorar su solubilidad y permeabilidad, así como utilizar dosis terapéuticas sin efectos tóxicos. Debido a las propiedades biológicas de GA y 18b-Ga, las dosis y el tipo de administración son cruciales para lograr una alta concentración local y, por lo tanto, un efecto terapéutico.

Palabras clave: Glicirricina, ácido 18-β-glicirretínico, infecciones respiratorias, actividad antiviral, actividad antioxidante, actividad antiinflamatoria.

1. Introduction

Glycyrrhizinic acid or glycyrrhizin (GA) is a triterpene saponin mainly found in the root of the Leguminosae *Glycyrrhiza glabra* Linn. This molecule has also been found in other *Glycyrrhiza* species such as *G. triphylla*, *G. uralensis Fisch, G. inflata Bat*, and even in an edible marine alga *Hizikia fusiformis* (Harvey) Okamura, a brown seaweed [1]. The GA-producing *G. glabra* is commonly known as "licorice" and has long been used in traditional Chinese, Indian and Tibetan medicines due to its broad range of biological properties, including antibacterial, antiviral, anti-inflammatory, antioxidant, and antidiabetic activities. Owing to these properties, *G. glabra* extracts are currently used in the pharmaceutical, cosmetic, and food industries. It has been used widely in the manufacture of food supplements and natural sweeteners; in fact, licorice is well known as a natural sweetener and flavoring additive, as GA is reported to be 30-50 times sweeter than sucrose and is recognized as generally safe by the U.S. Flavor and Extract Manufacturers Association [2, 3]. In addition, *G. glabra* is used in the production of food additives, with the therapeutic properties of its compounds being a promising field, particularly for the treatment of cancer and respiratory infections. Among the main compounds of *G. glabra*, GA has two residues of glucuronic acid, whose glycosidic bond can be hydrolyzed. This chemical modification produces the glycoside of GA, 18-β-Glycyrrhetinic acid (18b-GA), also known as "enoxolone", and thus 18b-GA can be released by the activity of bacterial gastrointestinal enzymes when it is consumed by animals [2]. GA and its derivative 18b-GA have shown antioxidative, anti-inflammatory, anti-asthmatic, anti-allergenic, antiviral, antiparasitic, hepatoprotective, anti-ulcerative, antimicrobial, anticarcinogenic, antimutagenic, anticoagulant and neuroprotective activity. Despite these properties, reports of their effects on humans are scarce and more studies about GA and 18b-GA pharmaceutical effects are needed. Thus, this work aimed to provide an updated and critical overview of the current knowledge of GA and 18b-GA pharmacological properties and their application in potential drugs for the treatment of respiratory infections.

2. Glycyrrhizin and Glycyrrhetinic acid have several therapeutic activities

Anti-inflammatory, antioxidant, and anti-viral properties of both GA and 18b-GA have been broadly studied. The well-known GA and 18b-GA antioxidant activity, by inhibition of free-radical generation and lipid peroxidation, may contribute

to neuroprotective, hepatoprotective, and anticarcinogenic effects.

For instance, GA extract inhibits cytotoxicity produced by reactive oxygen species (ROS) generation, as well as glutathione (GSH) down-regulation, the main cause of increased oxidative stress in dementia [4]. In addition, 18b-GA can up-regulate the activities of antioxidant enzymes Superoxide Dismutase and Glutathione Peroxidase in skin under UV-induced stress [4].
Anti-inflammatory properties that inhibit factors responsible for inflammation could mainly confer anticarcinogenic effects and recovery of renal and liver complications effects [1, 2, 5]. In fact, cytokine inhibition and modulation by GA and 18b-GA may be the main benefit in multiple pathologies such as depression, Parkinson's disease, gastritis infection; different cancers such as leukemia, breast and ovarian cancer [3,6]; and prevention of contact dermatitis induced by contact allergens [7]. Moreover, the anti-inflammatory effects of GA were described as similar to those of glucocorticoids and mineralocorticoids, which are widely used while treating cancer to combat chemotherapy's side effects [3].

The antiviral activity of both compounds contributes to alleviating viral infections. GA has been investigated for use against the multiplication of various viruses, including herpes simplex, Epstein–Barr, human cytomegalovirus, hepatitis A, B, and C, influenza, HIV, varicella zoster, and severe acute respiratory syndrome (SARS) coronavirus. GA is also active against flaviviruses and its derivatives were observed as Dengue virus inhibitors [8,9]. Both GA and its metabolite 18b-GA have demonstrated several antiviral mechanisms. They are effective in preventing the early stage of virus infection by affecting viral attachment and penetration; GA also inhibits adsorption and penetration of SARS coronavirus during and after the viral adsorption period [10]. Furthermore, several viral proteins can be inhibited by both compounds, thus avoiding viral entry to cells or viral replication. For instance, GA strongly binds to the S subunit of the spike protein [11], while molecular docking shows both compounds have a strong binding affinity for the main protease (MPro) of SARS-CoV-2 virus [12].

Besides targeting proteins, inhibition of virus-induced intracellular ROS accumulation by GA can also reduce the activation of nuclear factor kappa beta (NF-κB), c-Jun N-terminal kinase (JNK), p38, and redox-sensitive signaling events, thereby suppressing the virus replication process, as observed for influenza A virus [10,13].

3. Therapeutic properties for human respiratory infections

GA and 18b-GA have broad-spectrum antiviral activity against respiratory disease viruses, DNA and RNA viruses such as SARS coronavirus, influenza, and respiratory syncytial virus [10, 13]. For instance, GA has demonstrated activity against SARS-associated coronavirus (SARS-CoV), MERS-CoV, and the influenza virus [8, 14]. GA inhibited SARS-CoV replication in Vero and Vero-E6 cell lines but was ineffective in fRHK-4 cells [15]. Inhibition of viral replication by GA was later confirmed by Sharma et al [16] in a comparison study where GA demonstrated more effectiveness in inhibiting replication of the SARS-associated virus than four other antiviral compounds: ribavirin, 6-azauridine, pyrazofurin, and mycophenolic acid. In this same study, GA was the most potent inhibitor of SARS-CoV replication in Vero cells, with a selectivity index of 67 [16]. Another study demonstrated that GA inhibits influenza A/H1N1 infection by preventing virus uptake into the cell [8] and inhibits virus replication and pro-inflammatory gene expression in H5N1 influenza virus-infected cells [13]. Despite 18b-GA being a GA derivative, the two molecules show different effects; for instance, in a study with human respiratory tract cells, 18b-GA, but not GA, showed an effect on the infection rate with the human respiratory syncytial virus [17].

Inhibition of virus replication by GA or 18b-GA may be due to several mechanisms, including by reducing ROS production, which in turn reduces

activation of signaling events, or by inhibiting the activity of key enzymes for virus replication [12, 13]. For infection by SARS-CoV-2 virus which causes coronavirus disease (COVID-19), one of those enzymes is the Mpro, a broadly studied coronaviral target since it participates in a critical step during viral replication. Molecular docking studies demonstrated that GA binds strongly with some of the amino acid residues in the active site of Mpro [12] and in vitro assays have demonstrated inhibition of the Mpro activity by GA [18]. Another target enzyme for therapeutic activities that treat respiratory infections could be the type II transmembrane serine protease (TMPRSS2), which is one of the main enzymes involved in virus entry, involved in both corona and influenza virus infection [19]. Recently, Murck et al., [20] proposed that GA and its metabolites have two main mechanisms in combating COVID-19: direct inhibition of TMPRSS2 expression and providing an Angiotensin Converting Enzyme-2 (ACE2) independent anti-inflammatory mechanism.

During SARS-CoV-2 infection, virus binding to ACE2 dysregulates the renin-angiotensin system (RAS) balance, which results in ROS production by ACE activity (Figure 1). This in turn induces the release of pro-inflammatory cytokines, exacerbating inflammation and acute respiratory distress syndrome. GA inhibits cytokine levels by decreasing the inflammatory mediator toll-like receptor (TLR) activity, through reduction of the ROS production (antioxidant activity) and by avoiding virus binding to ACE2 receptor [1, 10, 21]. Inhibition of the high mobility group box 1 (HMGB1) protein is another anti-inflammatory GA-mediated pathway in infections by SARS-CoV-2 virus. While extracellular HMGB1 promotes release of proinflammatory cytokines, nuclear HMGB1 regulates ACE2 expression (Figure 1). GA can physically bind to HMGB1, thus its binding to DNA or other proteins can be reduced and several HMGB1-mediated pathological conditions are affected [22].

Therapeutic activities of licorice to treat bronchial cough, catarrh, and sore throat may be attributed to the existence of GA and its derivatives within licorice, which also help to relieve congestion in the upper respiratory tract by accelerating the secretion of the bronchial mucosa [4, 16,23]. In fact, GA and enoxolone have demonstrated prophylactic and therapeutic effects in different stages of respiratory infections caused by viruses. In the severe stage of SARS-CoV-2, GA may reduce the severity of infection with COVID-19 by blocking the number of entry points (Spike protein and ACE2 Receptor) and providing an ACE2 independent anti-inflammatory mechanism [10]. Because elevation in serum HMGB1 levels has been reported in severe COVID-19 patients, GA could also ameliorate the inflammatory symptoms through inhibition of the HMGB1 protein [1, 22]. In this regard, alleviating lung inflammation, which is the main cause of life-threatening respiratory disorders at the severe stage of SARS-CoV-2, could result in decreasing the risk of coagulation and multiple organ failure due to a cytokine storm [10]. Because GA can inhibit GSH down-regulation, it could also restore endogenous GSH levels associated with severe symptoms of COVID-19 [4, 21]. Additionally, at the early stage of infection by SARS-CoV-2, the anti-inflammatory and antioxidant properties may prevent the progression of inflammation and thereby avert the induction of a state of hyper inflammation or cytokine storm syndrome [8].

A pharmacological approach for treating respiratory infections would suggest the use of validated drugs for either blocking viral replication or for inhibiting inflammation. Unfortunately, for some diseases like COVID-19, inflammatory inhibitors have several side effects. Considering the biological properties of GA and 18b-GA, they could be used for both purposes, i.e., as antiviral and anti-inflammatory drugs. Besides therapeutic properties, either GA and 18B are safe, non-cytotoxic and non-carcinogenic compounds, thus both molecules can be applied as drugs for treating COVID-19 as they would not lead to cancer if the patient is treated for prolonged periods [11, 12]. GA also has demonstrated good ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties [12]. For

example, GA reports a low log P value (0.99) [12], which indicates that it can be absorbed easily by the cell since values higher than 5 predicts poor absorption or permeation [24]. Therefore, some of the pharmacological features of GA and 18b-GA are described next.

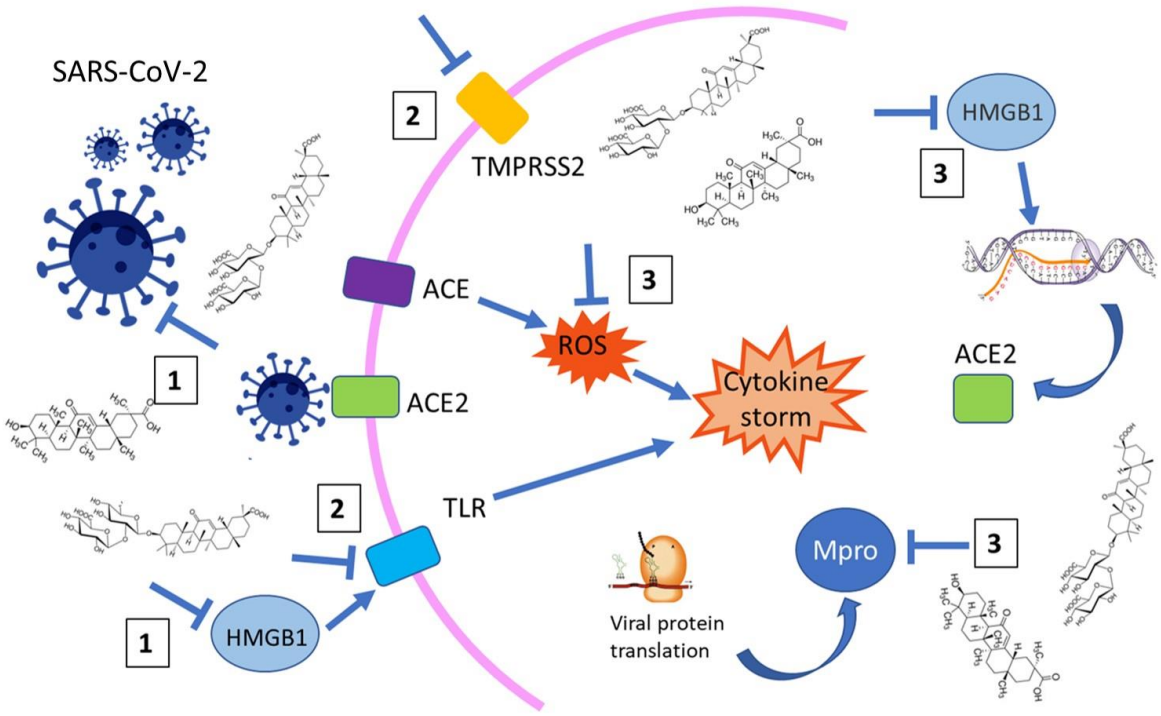

Figure 1. Scheme of the effect of GA against SARS-CoV-2 infection. 1): Effect at extra cellular level; 2): Effect at membrane receptor level; 3); Effect at intracellular level. ACE: *Angiotensin-converting enzyme;* ACE2: *Angiotensin Converting Enzyme-2;* TLR: toll-like receptor; TMPRSS2: type II transmembrane serine protease; HMGB1: high mobility group box 1; Mpro: main protease. Flat lines symbolize inhibition; arrows symbolize activation.

4. Pharmacological features of Glycyrrhizin and Glycyrrhetinic acid

Because the pharmacological effects of GA can be attributed to GA itself or its aglycon 18b-GA, which is produced when GA is metabolized, the pharmacological properties of both compounds have been extensively studied. Both compounds show very diverse pharmacological properties and have been employed in the past few decades as starting materials for the chemical synthesis of new derivatives or new drugs (Table 1). In this sense, GA and 18b-GA have emerged as promising drugs for the treatment of respiratory diseases, including COVID-19. However, to use them as effective and secure drugs, there are some crucial points that should be studied and considered: 1) Administration and optimal dose; 2) Side effects; 3) Biological effects in humans [10].

Table 1. Examples of 18b-GA and GA derivatives reported with effective antiviral activities.

Modified compound	Derivative	Target virus	Reference
18b-GA	3-O-acetyl-30-aminopyridine	Zika virus	[25]
	Amino and Thiol derivatives	Influenza virus	[26]
	Modified derivatives on rings A, C and E.	Hepatitis B virus	[27]
	Soloxolone methyl Modification of the A and C rings	H1N1 influenza A virus	[28]
GA	Glycyrrhizic methyl ester conjugates	Influenza virus	[29]
	Glycyrrhizic conjugates with free 30-COOH	Influenza virus	[30]
	Carboxy-substitued conjugate	Human immunodeficiency virus)	[31]
	Dipeptide GA derivative	Human immunodeficiency virus	[32]
	Diammonium glycyrrhizinate	Coronavirus	[33]
	Glycyrrizic conjugate; 2-acetamido- β-D-glucopyranosylamine	SARS-CoV virus	[34]
	Glycyrrhizic acid conjugate with isoleucine and 11-ami-noundecanoic acid	Dengue virus	[9]
	Derivatives with amino acid residues intro the GA carbohydrate part	Epstein-Bar virus	[35]

4.1. Administration and optimal dose

GA or 18b-GA can be consumed as licorice or as a GA or 18b-GA drug. However, their bioavailability is reduced when they are consumed as licorice because it contains at least more than one GA-related saponins, besides other different compounds (vitamins, tannins, pectins flavonoids etc) [1]. Around 10% of licorice (root dry weight) correspond to GA thus a Licorice extract dose administration decreases GA and 18B therapeutic effects [3]. For that reason several trials have focused on establishing effective dose-effect levels in animals and humans, high enough to achieve their therapeutic effects without having toxic effects [11]**.** Murck et al. [20] mention that, for 18b-GA induced action, the oral administration of GA is crucial as it is not metabolized to 18b-GA systemically. Conversely, an intravenous or inhaled administration may be required for a localized effect of GA. However, in this case it should also be combined with oral administration. Both oral and intravenous formulations of GA are currently used in humans. GA oral administration shows an impaired bioavailability due to its metabolism. Although GA is metabolized into 18b-GA by intestinal bacteria and absorbed via the intestine, GA is detected in human plasma at very low concentrations after oral administration of a clinical dose of 100–1600 mg/kg [36]. After being hydrolyzed to 18b-GA, it is rapidly absorbed and transferred by carrier molecules to the liver. There it is metabolized by lysosomal enzymes to sulfate

conjugates and 3-mono-glucuronyl 18 β-glycyrrhetinic acid (3MGA), which subsequently degrade back to 18b-GA and is reabsorbed. Studies on rodents and humans demonstrated that GA is poorly absorbed by the gastrointestinal tract but extensively metabolized by the intestinal microflora to 18b-GA and 3MGA, which are both readily absorbed. Thus, an enterohepatic circulation of 18b-GA can occur, requiring several days for complete body elimination and leading to a significant delay in terminal plasma clearance [3, 4]. The plasma clearance of GA and 18b-GA is only dose-dependent at high doses that exceed the serum protein binding saturation, while at low doses (below 120 mg) it is not dose-dependent in healthy people **[**4]**.** For example, a study of GA pharmacological properties demonstrated that after its oral administration in healthy persons, the serum level after 100 mg of GA was not detectable. Even with a 200 mg dose of intravenous administration, the peak serum level was only 80 µg/ml which is still below the half maximal effective concentration (EC50) of GA [37].

For SARS treatment, a dose has been recommended for oral administration of up to 300 mg or a dose of approximately 240 mg for intravenous administration [37]. In fact, for clinical trials carried out in China, a dose of 300 mg GA orally/day was used [23]. Although doses ranging from 10-400 mg/day could produce unwanted effects, a dose of GA up to 100 mg/day used long-term could be safe [20]. Moreover, based on in vivo and clinical evidence, Isbrucker and Burdock [38] propose an acceptable daily intake of 0.015–0.229 mg/kg(bodyweight)/day of GA. Licorice extract containing GA could also have prophylactic and therapeutic effects against COVID-19 depending on the GA concentration; small doses (10-50 mg) are proposed to be a daily prophylactic dose and large doses (50-100 mg) a therapeutic dose to prevent the progression of the disease and eradicate the virus [8]. Optimal doses of GA or 18b-GA for treating respiratory viral infections need to be studied, as well as other administration routes, for example, nebulization or inhalation.

Administration through inhalation is a promising alternative to improve the GA and 18b-GA bioavailability and achieve a high local concentration of those active ingredients in the respiratory tract, nasal passages, pharynx, larynx, trachea, bronchi, or lungs, the last of which are especially vulnerable to infection by viral agents. For the specific case of inhaled drug delivery, it does not necessarily require absorption, since the largest proportion of the drug would be in contact with the site of action. However, increasing the absorption of the drug at the site of action enhances its therapeutic effect [39]. An inhaled administration was recently tested in a clinical study; thirty-three people were treated with a vaporizer solution containing licorice, 18b-GA, Resveratrol, and GA. The treatment consisted of three vaporizer inhalations administered three times a day over a minimum of 5 days, with a dose per inhalation containing 0.83 µg of 18b-GA and 4.166 µg of GA. This had a preventive effect on COVID-19 positive subjects who were otherwise healthy, as well as a curative effect on symptomatic COVID-19 subjects [40].

Recently, the company SPV TIMSER, S.A.P.I. de C.V. (TIMSER) observed that inhaled administration of a GA and 18b-GA-based drug in mice provided an initial surface effect in the upper airways, exerting their antimicrobial and antiviral effect to subsequently produce a lengthened systemic effect, greater than 24 hours, with reduced biotransformation into 18b-GA. Additionally, in this same study, a synergistic effect was obtained when GA and 18b-GA were administered as a mixture; changes in their proportions modulated their systemic effect, independently of the concentration used in the formulation [39]. Another interesting drug delivery route has been reported by Tong et al., [41], whereby the study revealed that a polymeric form of GA in monodisperse carbon dots (spherical carbon particles) leads to remarkable antiviral effects, with stimulation of antiviral innate immune responses, inhibition of Porcine reproductive and respiratory syndrome virus (PRRSV) replication and PRRSV virus-induced ROS accumulation. Moreover, this GA-based product

was also effective against the related porcine epidemic diarrhea virus **(**PEDV).

4.2. Side effects

As GA has a rapid metabolism in humans when it is administered orally or through injection, the method of dose administration could be crucial to achieving the local concentrations required for therapeutic effect. However, the necessary concentrations to generate antiviral effects are extremely high in comparison to what could be considered completely safe. High doses could produce irritability and toxicity because of the similarity in structure of 18b-GA to the structure of hormones secreted by the adrenal cortex, which can produce mineralocorticoid and glucocorticoid activity. Thus, exposure to high levels of GA can produce hypermineralocorticoid-like effects [42]. GA and licorice saponins can inhibit 11-β-hydroxysteroid dehydrogenase enzymes, leading to a cortisol-induced mineralocorticoid effect and a consequent tendency for the elevation of sodium and reduction of potassium levels [3, 38]. Furthermore, the toxic effects of GA consist of depressing the renin-angiotensin-aldosterone system, which would lead to headaches and increased arterial blood pressure, meaning that administration of GA in elderly patients with heart disease and hypertension should be performed with caution [20]. As GA-induced pseudohyperaldosteronism syndrome has been reported when doses ranging from 100 to 400 mg/day are administered, side effects could be doses dependent. For example, Chen et al. [37] indicate that a long-term dose of up to 100 mg/day of GA is safe and does not lead to side effects that have been observed with long-term use in higher doses. On the other hand, people with kidney impairment, hypertension, and heart failure are more sensitive to the side effects of licorice and GA; hence administration of oral contraceptives, hydrocortisone, and prednisolone are contraindicated in patients who uses large doses of GA [8]. Therefore, more studies about dose-dependent side effects are needed.

4.3. Biological effects in humans

The biological effect of GA has been widely demonstrated by in vitro and animal experiments but this may not correspond with clinical efficacy in humans. In case of antiviral activity against SARS-CoV-2, it could be due to in vivo experiments are performed in genetically modified animals expressing ACE2 receptor, hence, triggered physiologic response may not include proteins and ACE2-associated pathways which naturally play a role in humans. As a result, dose toxic effects dependence is different between animals and humans. For instance, GA doses ranging 30-229 mg/day in male mice and up to 407 mg/day in female mice have not shown chronic toxicity or tumorigenicity [38], however, in humans GA doses of 10-100mg/day are considered as safe doses, while above 200 mg/day several side effects have been reported [20, 43]. Since safe doses range is different between animals and humans, dose-dependent therapeutic activity and pharmacokinetic needs to be defined in humans as well.

To date, few GA-based compounds have been used in humans for treating different diseases. In Japan, GA has been used for more than 60 years as a coadjutant for the treatment of chronic hepatitis C. In two clinical trials, a GA preparation, Stronger Neo-Minophagen C, caused a remarkable decrease in alanine transaminase (ALT), gamma-glutamyl transferase (GGT), and aspartate transaminase (AST) levels, with increasing histological evidence of reduced necrosis and inflammatory lesions in the liver [4]. In addition**,** injection to treat hepatitis produced few adverse reactions, while significant inhibition in the progression of cirrhosis and hepatocarcinoma was observed [3,44]. Treatment for acquired immunodeficiency syndrome patients based on a GA drug proved a marked inhibition of HIV-1 replication, while doses of 400–1600 mg/day induced no detection of viral antigen at the end of the treatment [1]. In fact, this drug inhibits both virus-cell binding and replication of HIV-1 virus in human cells and is also active against some animal viruses such as duck hepatitis virus (DHV) and avian infectious bronchitis virus [1].

Although thrombin inhibition by GA or 18b-GA has not been demonstrated in humans, an in vivo assay performed in rats demonstrated that the intravenous administration of GA causes a dose-dependent reduction in thrombus size on a venous thrombosis model, combining stasis and hypercoagulability [45]. This property could make GA a potential candidate for counteracting the effect of snake venom; in a rat model, Assafim et al. [46] demonstrated that GA prevents venom-induced changes in hemostasis, inhibiting thrombus formation, thus suggesting potential antiophidic activity.

Another GA and 18b-GA effect is the inhibition of damage by drugs. For example, according to Rizzato et al. [47], both prevent drug-induced liver injury and ensure the disruption of bile acid metabolism in humans [3].

Effects on humans during treatment for respiratory illness have been scarcely explored. In China, a suspected COVID-19 patient took an alternative medicine based on oral administration of 150 mg of Diammonium Glycyrrhizinate (the diammonium salt of GA) three times per day, combined with a corticosteroid. After 12 hours of treatment, severe symptoms including fever, shortness of breath, and coughing were relieved. This improvement could be attributed to the GA antiviral and anti-inflammatory effects on respiratory and neurological systems [33]. In addition, a clinical trial reports a preventive and curative effect of a treatment consisting of a vaporizer solution containing 18b-GA and GA. In the trial, all 8 COVID-19 positive subjects, who had severe symptoms, were symptom-free after 48 hours of treatment [40].

5. Glycyrrhizinic acid and Glycyrrhetinic acid-based drugs

Due to the diverse biological properties of GA and 18b-GA, drugs based on GA and 18b-GA have been used for different purposes; for example, for treating chronic hepatitis, different forms of cutaneous inflammation, and respiratory tract infections.

Combined with methotrexate, GA has been used successfully to treat erythrodermic psoriasis [1]. Grippaudo and Di Russo [48] described the effects of the topical application of GA, combined with a fractional carbon dioxide laser, for 4 weeks, for the benign treatment of hand hyperpigmentation.

In Japan and other countries, a preparation of GA combined with l-cysteine and glycine, called Stronger Neo-Minophagen C (SNMC), is administered intravenously and has been used with apparent success for the treatment of chronic viral hepatitis [49] and upper respiratory tract infections [15]. In the treatment of chronic hepatitis C, SNMC was effective in preventing liver carcinogenesis [49].

Although numerous clinical trials are underway worldwide to find effective drugs for COVID-19 treatment, no drug has been announced to be effective so far. Drugs similar to corticosteroids have been used to suppress lung inflammation in patients; however, multiple side effects have been observed. Since both GA and 18b-GA have antiviral, antioxidant, and anti-inflammatory properties, they could provide an effective and suitable drug for treating COVID-19 or other severe respiratory diseases. In fact, GA has proved to attenuate COVID-19 symptoms in non-hospitalized patients. In China, Diammonium Glycyrrhizinate enteric-coated capsules were combined with corticosteroids in the treatment for a non-hospitalized COVID-19 patient. Upon implementation of this alternative medicine, the symptoms of the patient were significantly relieved, and it eventually recovered [33].

To date, information is scarce related to administration of GA, 18b-GA, or drugs based on both compounds, in humans. One of the main challenges in using them as a drug is improving their solubility and permeability. Due to the amphiphilic and anisotropic structure of GA, it is soluble in water and can form microstructures like micelles and fibrils, which have been used in different drug delivery systems [1, 50]. However, in contrast to other saponins like digitonin, GA exhibits reduced permeability and hence its

absorption is a function of the excipients and manufacturing processes.

GA has a similar issue in terms of solubility. It shows low polarity, high hydrophobicity, and moderate permeability. Hence, the limiting step for absorption into the human bloodstream is the dissolution of the drug, which is then absorbed and distributed to different organs or to the target site [1]. The selection of excipients and pharmaceutical forms therefore define the absorption of the drug and thus the potentiation of the desired therapeutic effect. Due to this, new and improved pharmaceutical compositions are developing. Unlike for oral administration, when GA is absorbed without passing through the gastrointestinal tract its metabolism into 18b-GA is reduced and only the fraction that passes through this tract is then metabolized [39]. In order to keep 18b-GA properties after administration through inhalation, both compounds should be included in the drug formula for administration to the lungs or the epithelial airways. As a result, the company TIMSER developed pharmaceutical compositions that include GA and 18b-GA, with synergistic compositions whose GA and 18b-GA proportions are designed to reduce toxicological effects, irritability, and provide effective pharmacokinetics, compared to the application of the same components individually [39]. In addition, it should be noted that GA could also induce immune stimulation when combined with a COVID-19 vaccine, as has been demonstrated previously with duck hepatitis virus (DHV) vaccine. Soufy et al. [51] found that GA has excellent immunostimulant properties and induces a synergistic effect with the DHV vaccine by activating T lymphocyte proliferation.

6. Conclusion

GA and 18b-GA show diverse therapeutic properties, including antiviral, anti-inflammatory, and antioxidant activity which have mostly been demonstrated through in vitro tissue culture. However, their effects on humans are insufficiently investigated and more studies about the biological effects, optimal doses, and methods of administration are needed for different medical applications. In the case of viral respiratory infections like COVID-19, both compounds show antiviral activity against SARS-CoV-2, through different mechanisms, that prevent virus entry to the cell or replication. On the other hand, their low solubility and permeability make their application as an efficient drug difficult. Recent studies have demonstrated a range of dose-dependent response and toxicity effects, signifying that doses high enough to show therapeutic effect could cause toxicity. Administration type is a promising research field to improve GA and 18b-GA bioavailability, potentially enabling a high local concentration and therefore a therapeutic effect for treating viral respiratory infections.

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