

The Effect of Nutritional Additive of Glycyrrizic Acid, Glabridin and Resveratrol on SARS-coronavirus Replication

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Abstract: The outbreak of SARS requires the search for antiviral compounds to prevent and treat this disease. Currently, there is no specific treatment for coronavirus infection associated with SARS-COVID-19. Among researchers working in this area, there are various approaches to create an effective vaccine against this infection, there is no consensus on this problem. We will assess the antiviral potential of GA, GL, RT against two clinical isolates of coronavirus (M-1 and M-2) from SARS patients admitted to the Bukhara Multifunctional Clinical Center. The chemical structure of these substances interacts with the structures of the virus, changing the various phases of the viral cycle, which is accompanied by irreversible inactivation of viral particles (which are in a free state outside the cells), blocking the introduction of active viral particles through the cell membrane into the cell, as well as disrupting the ability of viruses to synthesize new structural components. Inhibits viruses at concentrations that are non-toxic to normally functioning cells. All these properties of these substances have attracted our attention, and some of them have confirmed their importance. Our results indicate that GA and RT should be used to treat coronavirus infection. RT, in turn, has antiviral, anticancer effects and will also be studied in detail.

Keywords: SARS Coronavirus, Glycyrrhizic Acid, Glabridin, Resveratrol, Replication, Enzymes

1. Introduction

There is an increasing demand for herbal medicines, nutritional supplements and cosmetics. A review of the chemical nature, namely the structural formula of the studied plant ingredients, shows both general similarities (the presence of phenolic groups) and some differences (not all have a heterocyclic ring, a carboxyl group). Nevertheless, they all have biological activity. For example, GA, GL, RT have antioxidant, antibacterial, antiviral and a number of other activities. These results indicate that research should be carried out more extensively in order to confirm the findings of other authors and to reveal other potential therapeutic effects of these compounds.

The new coronavirus COVID-19 has been identified in patients with severe respiratory syndrome (SARS). SARS is

an infectious disease with a high potential for transmission through close contacts. An outbreak of atypical pneumonia in several countries (pandemic) has led to the search for active antiviral compounds (vaccines) to treat this disease. In this work, we evaluated the antiviral activity of GA, GL and RT against two clinical isolates of coronavirus (M-1 and M-2) from SARS patients admitted to a multifunctional clinical center (Bukhara). GA and GL are active components of licorice roots [1, 2], and RT is isolated from mulberry leaves [3]. These compounds are used by patients because of their antiviral [4, 5], antitumor [6], immunosuppressive [7], etc. [8] activities.

2. Methods

On confluent layers of Vero cells, we evaluated the

cytopathogenicity of the virus evoked 4-5 days after infection. The ratio of the concentration of a compound that reduces cell viability by half (CC_{50}) to the concentration of a compound that inhibits cytotoxicity by the same value from the control (EC_{50}) gives us a selectivity index. The level of toxicity per cell was determined using a set of MMT-1 cells. (Eurodiagnostics, the Netherlands). In addition, inhibitors of the enzymes inosine monophosphate dehydrogenase ribavirin and mycophenolic acid were used in the study, which did not

affect the replication of SARS-associated coronaviruses (SARS-CV). Orotidine monophosphate decarboxylase inhibitors - 6-azauridine and pyrazofurin, suppressed the replication of SARS-CV at non-toxic doses with selectivity indices of 6 and 12, respectively. The selectivity index of GL and RT was 41 and 60, respectively. The most potent inhibitor of SARS-CV replication in Vero cells was GL, which had a selectivity index of 76 (Table 1).

Table 1. Activity of the compounds against SARS associated with coronavirus in Vero cell cultures.

No.	Compound	EC_{50} (mg / l) *	CC_{50} (mg / l) *	Selectivity index
1.	6-azauridine	18-8 (2-8)	107 (18)	6
2.	Pyrazofurin	4-2 (0-50)	50 (2-6)	14
3.	Mycophenolic acid	> 60	> 60	0
4.	Ribovirin	> 1100	> 1100	0
5.	Glycyrrhizic acid:			
	a) after adsorption of the virus	580 (70)	> 20,000 *	> 35
	b) during and after adsorption of the virus	280 (54)	> 20,000	> 68
	c) during the adsorption of the virus	2300 (410)	> 20,000	> 8-5
6.	Glabridin:			
	a) after adsorption of the virus	800 (82)	> 22000	> 76
	b) during and after adsorption of the virus	500 (66)	> 18000	> 70
	c) during the adsorption of the virus	2000 (380)	> 18000	> 12
7.	Resveratrol:			
	a) after adsorption of the virus	900 (91)	> 16000	> 41
	b) during and after adsorption of the virus	600 (56)	> 18000	> 64
	c) during the adsorption of the virus	1800 (380)	> 18000	> 24

EC_{50} is the effective concentration of the compound required to suppress the cytopathic effect up to 50% of the control value CC_{50} is the cytotoxic concentration of the compound that reduces cell viability to 50%, NC-not detected, * SD-average of 10 analyzes ** At the maximum concentration used (20 * 10^3 mg / l) there was a decrease in cell viability by 20-30%.

In addition to inhibiting viral replication, GA inhibits the adsorption and penetration of the virus into the host cell (Vero cells) - the first stages of the replicative cycle, GA was less effective when administered during the adsorption period than when added after virus adsorption (EC_{50} 580 mg / L versus 2300 mg / L, respectively) [9]. GA was most effective when added both during growth and after an adsorption period (EC_{50} 280 mg / L).

The effect of GA on SARS-CV replication in Vero cells has been shown. We have detected replication of SARS-CV with serum samples from SARS patients. The manifestation of antigens (viral) was much lower in the cultures treated with 1000 mg / l GA than in any other culture; high concentrations of GA (400 mg / l) completely suppressed viral replication. (Unpublished data from authors).

3. Results

When comparing the antiviral activity of 6-azauridine, ribovirin, GA against several pathogenic flavoviruses, it was found [6] that ribovirin and 6-azauridine were active, but not selective inhibitors when assessing them in relation to cell growth inhibition. GA had a low selectivity index, but was a significantly potent inhibitor of replication of all viruses tested. These authors report that the EC_{50} for GA was 316-625 mg / L (added twice during an incubation period of 7 days). Considering that compounds were added twice during the entire incubation period, the EC_{50} for GA that we found

(Table 1) indicates a higher sensitivity of SARS-CV to this drug than that found by Grance (6) and Toch (5) and Colleagues. The mechanism of GA activity against SARS is still unclear [10]. GA influences cellular signaling pathways such as protein kinase C, casein kinase II and transcription factors such as activator protein 1 and nuclear factor kB [5]. In addition, GA and its metabolite aglycone 18 β -glycyrrhetic acid enhance the expression of inducible nitrous oxide synthase (N_2O) and nitrous oxide production in macrophages. N_2O inhibits the replication of several viruses, such as Japanese encephalitis virus (a member of the Flaviviridae family), which can also inhibit GA.

Preliminary results of our experiments show that GA induces N_2O synthase in Vero cells and that viral replication is inhibited by the addition of a nitrous oxide donor (DETA NONO) to the culture medium.

GA has previously been used to treat patients with HIV-1 and chronic hepatitis C virus [14]. The resulting low P24 antigen concentrations in HIV-1 patients who were given this compound have been associated with upregulation of chemokines [11]. Rare side effects such as increased blood pressure and hypokalemia have been reported in some patients after several months of glycyrrhizin treatment [13]. SARS treatment is necessary only for a short time. Since the side effects of this compound are known and can be controlled, proper monitoring can lead to the effective use of GA as a drug for the treatment of SARS. It is reported (5, 6) that ribovirin has a number of toxic effects

when administered to patients with SARS, including hemolysis (76% of patients) and a sharp decrease in hemoglobin (49% of patients). However, although high doses of GA were used in clinical trials, this compound had few toxic effects compared to other treatment regimens, and the drug was reported to be clinically effective [15].

4. Conclusion

The studied compounds, in relation to the replication of the SARS-CV virus, require further analysis based on their structure, the reactivity of their functional groups in relation to oxidants, as well as their biochemical parameters (action, first of all, on the active centers of enzymes, then on the membranes (proteinaceous) [12].

The future search for compounds (preferably of natural origin) of therapeutic interest against SARS-CoV will be greatly facilitated by establishing the growth of SARS-CoV in human cells.

The study of the interrelation between the chemical nature and their biological, primarily antiviral activity is quite promising and serves as the goal of further research by the authors.

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