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Izatizon Efficacy against Coronavirus Infection in Model of Transmissive Gastroenteritis Virus of Pigs

S. Rybalko[1], D. Starosyla[1], L. Zaika[2], O. Bolsunova[2], A. Potopalskyi[3], B. Zadorozhniy[2], M. Arkhipova[1], O. Deriabin[1], M. Zavelevich[4]

[1]*SI "L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases, the NAMS of Ukraine", Kyiv, Ukraine*
[2]*Institute of Molecular Biology and Genetics of the NAS of Ukraine, Kyiv, Ukraine*
[3]*Institute of Health Promotion and Rebirth of People of Ukraine, Kyiv, Ukraine*
[4]*RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the NAS of Ukraine, Kyiv, Ukraine*

Abstract:
Background*: The search for the substances being effective against coronavirus infection is in the limelight of pharmacology and virology taking account of the current COVID-19 pandemic. This study was aimed at assaying in vitro Izatizon activities against transmissible gastroenteritis virus (TGEV) of pigs representing highly contagious coronavirus.*
Materials and Methods*: Izatizon activities were assayed in SPEV cells infected with TGEV and pretreated with various concentrations of Izatizon. CC_{50} and EC_{50} were calculated based on the visual analysis of cythopathogenic effects as well as results of MTT test.*
Results*: Izatizon inhibit effectively TGEV reproduction decreasing the infectious titer of virus by 4 $lgID_{50}$ even in the lowest of studied concentrations 0.6 µg/ml. CC50 of Izatizon in SPEV cells is 20 µg/ml. Therefore, selectivity index of Izatizon in this system is more than 34.*
Conclusion*: Izatizon is highly efficient substance in inhibition of TGEV reproduction in vitro suggesting its advantageous effects as antiviral agent that requires further study.*
Key Words*: Izatizon, coronavirus, transmissive gastroenteritis of pigs, antiviral effect, selectivity index.*

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I. Introduction

The vast spread of both existing and emergent infectious agents threatening the humanity necessitates the urgent need in developing novel agents possessing antiviral and antibacrerial activities with parallel immunomodulating effect. Designing the targeting drugs is an integral part of the search for novel medicinal products based on the analysis of the molecular recognition between three-dimensional targets[1].

The problem of coronavirus infections of humans became highly topical since the appearance of the dangerous emergent infection caused by SARS-Cov-2 that has resulted in the pandemic. As of October 2021, more than 238 million cases and 4.87 million deaths due to COVID-19 have been confirmed worldwide.

SARS-Cov-2 is a single-strand beta-coronavirus. The genome of SARS-Cov-2 codes for non-structural proteins such as PL protease, helicase, RNA-dependent RNA-polymerase, structural proteins such as spike glycoprotein, nucleocaspsid protein, membrane protein, and accessory proteins.

Late in 20th century, the antiviral activity of several thiosemicarbasons was demonstrated and its relation to the molecular structure of these substances was proven[2]. Izatizon represents the novel form of Methisazone, namely pegylated Methisazone – izatin-beta-thiosemicarbazone[3]. The broad spectrum of Izatizon activities is based on the conformationally labile structure of Methisazone molecule representing the major active component of the composition depending on the properties of the solvent and microenvironment[4].

Earlier, the effects of Izatizon were confirmed in several DNA and RNA viruses[5]. Moreover, Izatizon possesses pronounced immunomodulating properties[6].

The coroniviruses affecting livestock animals and poultry that are of economic importance for Ukraine have been extensively studied. Among these viruses is transmissible gastroenteritis virus (TGEV) of pigs[7] that belongs to alpha-coronaviruses. The analysis of the substances that are effective against coronaviruses of different subgroups could be helpful in elucidating the mechanisms of such anti-coronavirus effects and searching for the effective drugs against SARS-Cov-2.

The current study is aimed at assaying *in vitro* Izatizon activities against transmissible gastroenteritis virus (TGEV) of pigs representing highly contagious causative agent of gastrointestinal disease.

II. Materials and Methods

Viruses

TGEV – etiologic agent of trasnsmissive gastroenteritis of pigs. The strain used: $D_{52\text{-}5}$ (BRE_{79}) – highly pathogenic virus for pigs of all age groups was obtained following five passages in the continuous cell line of swine fetal testes (ST cells). The virus is tropic to the cells of gastrointestinal and respiratory tract. The virus strain was kindly provided by Dr. Hubert Laude from the Molecular Virology and Immunology Lab in Biotechnological Center INRA in Jouy-en-Josas.

Infectious titer

Virus-containing material was titered in cell lines by two techniques. In the end-dilution method, the cytopathogenic effect was recorded and the infectious titer was estimated by Kerber-Ashmarin technique and expressed as tissue cytopathogenic doses (TCD_{50}) per ml. In the negative agar colonies technique, the infectious titer was estimated as BFU/ml. The results were recorded in 120 h following infection.

Cell cultures

SPEV – permanent cell line of the swine embryonic kidney
ST – permanent cell line of pig testicles

Izatizon

Izatizon was produced in the laboratory of the Institute of Molecular Biology and Genetics of the NAS of Ukraine and the Institute of Health Promotion and Rebirth of People of Ukraine according to the Patent of Ukraine No. 1780, 1994. The purity of the final product was confirmed by analytical assays.

Cytotoxicity assay

The cytotoxicity of Izatizon was assessed by MTT test in SPEV cells. The cells were seeded in 96-well plates and incubated for 5 days at 37 °C in a 5% CO_2-humidified incubator with various concentrations of the substance under study. The various concentrations of the assayed substance were added in triplicate. CPE (cell rounding, degeneration, cell detachment from the surface of the wells) was assessed by scoring the cell layer by 5-point scale such as follows:

"–" – degeneration is absent
"+" – damage of cell layer by not more than 25%
"++" – damage of cell layer by not more than 50%
"+++" – damage of cell layer by not more than 75%
"++++" – complete degeneration of cell layer

50% cytotoxic concentration (CC_{50}) was then calculated as the compound concentration (μg/mL) required for the reduction of cell viability by 50% in accordance with the regulatory guidelines on in vitro study of antiviral substances.

MTT assay

Cells were cultured in 96-well plate with or without different concentrations of Izatizon as indicated above. Then MTT (5 mg/ml in PBS) was added to each well. After incubation for 4 h, MTT solution was removed and DMSO was added as the solvent. The plate was incubated with shaking for 10 min at 37 °C and read on a Bio-Tek Elx 800 ELISA reader at 570 nm.

Assay for effective concentration (EC_{50})

For assaying antiviral activity of Izatizon, virus was added to cells grown in 96-well plate in a dose of 100 TCD_{50}/0.1 mL. Upon absorption of virus (60 min, 37 °C), the cells were washed and the maintenance medium (RPMI-1640 with 2% of fetal calf serum) was added. Then Izatizon was added in different concentrations. Upon incubation, the cell viability was assessed by MTT assay and half-maximal effective concentration EC_{50} was then calculated as the concentration of the compound, which inhibits virus replication by 50%.

The selectivity index (SI) was then calculated as CC_{50} to EC_{50} ratio.

Statistical processing

The significance of the differences was analyzed by Student *t*-test by Microsoft Excel and Microcal Origin software. Curve fitting was performed using linear regression model in GraphPad Prism. The difference were considered as significant if $p < 0.05$.

III. Result

The cytotoxicity of Izatizon in SPEV cells was assayed within the range of 5-100 µg/ml. The results were assessed by MTT assay following 120 h of incubation. The results of MTT assay (Figure) show that the treatment of SPEV cells with Izatizon causes dose-dependent cytotoxicity with CC_{50} being of 20 µg/ml.

The antiviral effect of Izatizon was studied in SPEV cells infected with TGEV. The TGEV titer was 8.5 $lgID_{50}$. The cells were preincubated with different concentrations of Izatizon for 1 h and then the cells were infected with 100 TCD_{50} of TGEV. The cells were incubated for 3 days. The development of cytopathogenic effect was followed by the gross appearance of cells under inverted microscope. Cytopathogenic effect morphologically was manifested as small-cell degeneration. Following the incubation, the infectious titer of TGEV was assessed. The results are presented in the Table.

Table: Infectious titer of TEGV in cells pretreated with Izatizon

Treatment	Infectious titer, $lgID_{50}$
Control of virus	8.9 ± 0.2
Izatizon 5.0 µg/ml	4.2 ± 0.1
Izatizon 2.5 µg/ml	4.0 ± 0.3
Izatizon 1.25 µg/ml	5.2 ± 0.1
Izatizon 0.6 µg/ml	5.1 ± 0.1

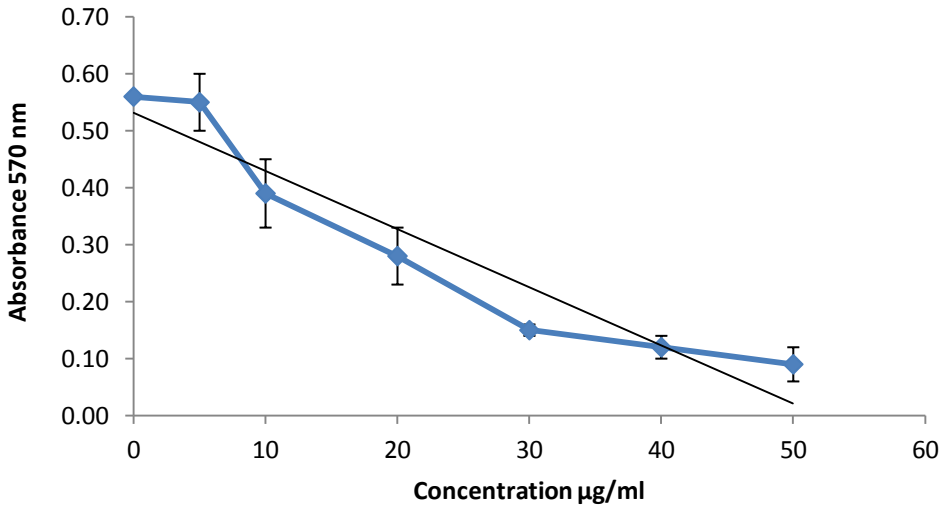

Figure: Cytotoxicity of Izatizon assayed in SPEV cells. Cells were incubated with various concentrations of Izatizon for 5 days. Percent of viable cells was assayed in MTT test

The data in the Table show that Izatizon in the concentration ranged from 5.0 to 0.6 µg/ml inhibits effectively TEGV reproduction decreasing the infectious titer by about 4 $lgID_{50}$ even in the lowest of studied concentrations 0.6 µg/ml.

The criterion for the assessment of the inhibitory activity of antiviral drugs in *in vitro* systems is represented by the decrease in the infectious titer of virus at least by 1.5-2.0 $lgID_{50}$. In the range of the dose under study, the decrease of TEGV infectious titer caused by Izatizon surpassed this value significantly. The selectivity index (SI) calculated as CC_{50} to EC_{50} ratio is therefore much more than 34 since EC_{50} value was not reached with the lowest of Izatizon concentrations being assayed.

SI is a critical measure to determine whether an antiviral compound is potent and selective for further drug development.

Further studies of Izatizon in different models could be relevant since values of SI exceeding 8-10 are recommended as an acceptance criterion for the substances deserving further preclinical studies[8,9].

IV. Conclusion

The advantageous antiviral effects of Izatizon against TGEV are achieved with low toxicity providing for high values of selectivity index that deserves further studies.

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