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Original Article

Influence of iodine-containing complex on the toxicity of drugs used for the treatment of acute respiratory viral diseases and influenza virus in combined application

[Influencia del complejo que contiene yodo en la toxicidad de los medicamentos utilizados para el tratamiento de enfermedades virales respiratorias agudas y del virus de la influenza en aplicación combinada]

Lyudmila Ivanova*, Ilya Korotetskiy, Bahkytzhan Kerimzhanova, Sergey Shilov, Natalya Zubenko, Tatyana Kuznetsova, Raikhan Parenova, Alexandr Ilin

JSC Scientific Center for Anti-Infectious Drugs, Almaty 050060, Kazakhstan.
*E-mail: lyudmila_69.69@mail.ru, l.ivanova@scaid.kz

Abstract

Context: One approach to assessing pharmacodynamic drug interactions is the practical application of the Chou-Talalay theory and its combination index theorem. The algorithm for studying and analysing the effects of combinations, according to Chou-Talalay, allows for the calculation of the direction and strength of pharmacodynamic interactions using the combination index (CI) values and to establish the dose reduction index (DRI) for each component of the combination. The algorithm allows for the analysis of combinations of different qualitative and quantitative compositions and calculates the result of interaction at any level of toxicological effect in laboratories engaged in drug development and research.

Aims: To evaluate the effect of FS-1 on the toxicity of rimantadine, oseltamivir, and acetylsalicylic acid (ASA) in combined *in vitro* experiments.

Methods: The combined cytotoxicity effect of drugs was evaluated using neutral red on MDCK cells. The type of drug interaction was determined using the principle of median-effect and CI by correlation of doses and effects, the ratio of the value of programmed cell death to the value of actual cell death using the Chou-Talalay method.

Results: It was found that the combination FS-1/rimantadine, FS-1/oseltamivir, and FS-1/ASA can significantly reduce their toxic properties. The CI of the combined use of FS-1/rimantadine was CI = 2.018; FS-1/oseltamivir CI = 13.726, and FS-1/ASA CI = 1.518.

Conclusions: The use of Chou-Talalay algorithm makes it possible to develop recommendations for the rational use and dosing of drug combinations in clinical practice, as well as to assess the prospects for the development of new combined therapeutic technologies.

Keywords: algorithm, drug toxicity; antagonism; Chou-Talalay modeling method; combined use; combination index.

Resumen

Contexto: Un enfoque para evaluar las interacciones farmacodinámicas de los fármacos es la aplicación práctica de la teoría de Chou-Talalay y su teorema del índice de combinación. El algoritmo para estudiar y analizar los efectos de las combinaciones según Chou-Talalay permite calcular la dirección y la intensidad de las interacciones farmacodinámicas mediante los valores del índice de combinación (IC) y establecer el índice de reducción de dosis (IRD) para cada componente de la combinación. El algoritmo permite analizar combinaciones de diferentes composiciones cualitativas y cuantitativas y calcula el resultado de la interacción a cualquier nivel de efecto toxicológico en laboratorios dedicados al desarrollo e investigación de fármacos.

Objetivos: Evaluar el efecto de FS-1 sobre la toxicidad de rimantadina, oseltamivir y ácido acetilsalicílico (AAS) en experimentos *in vitro* combinados.

Métodos: Se evaluó el efecto citotóxico combinado de los fármacos utilizando rojo neutro en células MDCK. El tipo de interacción farmacológica se determinó mediante el principio de mediana-efecto y el IC mediante la correlación de dosis y efectos, la razón entre el valor de la muerte celular programada y el valor de la muerte celular real mediante el método de Chou-Talalay.

Resultados: Se observó que la combinación de FS-1/rimantadina, FS-1/oseltamivir y FS-1/AAS puede reducir significativamente sus propiedades tóxicas. El IC del uso combinado de FS-1/rimantadina fue de 2,018; el de FS-1/oseltamivir fue de 13,726 y el de FS-1/AAS fue de 1,518.

Conclusiones: El uso del algoritmo de Chou-Talalay permite desarrollar recomendaciones para el uso racional y la dosificación de combinaciones de fármacos en la práctica clínica, así como evaluar las perspectivas de desarrollo de nuevas tecnologías terapéuticas combinadas.

Palabras Clave: algoritmo, toxicidad farmacológica; antagonismo; método de modelado de Chou-Talalay; uso combinado; índice de combinación.

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AUTHOR INFO
ORCID: 0000-0002-7194-0556 (LI) 0000-0001-6436-803X (NZ)
0000-0002-0397-7840 (IK) 0000-0003-4186-3948 (TK)
0000-0002-6860-3751 (BK) 0000-0002-8806-4214 (RP)
0000-0001-9490-9300 (SS) 0000-0001-9528-9721 (AI)

INTRODUCTION

Among viral infections, influenza is the most dangerous disease, one of the most frequent causes of death. According to WHO, diabetes and kidney disease rank eighth on the list of frequent causes of death (CDC, 2021; Ilyicheva et al., 2022; Thompson et al., 2009).

Antiviral drugs are an important means of mitigating the impact of annual influenza epidemics and potential pandemics (Burtseva et al., 2009; Jones et al., 2022).

Currently, three classes of antiviral drugs are approved for the prevention and treatment of influenza infection - M2 channel inhibitors (aminoadamantanes, amantadine, and rimantadine), neuraminidase inhibitors (NAI, oseltamivir and zanamivir) and polymerase acidic endonuclease inhibitor (baloxavir marboxil), which is new in terms of its mechanism of action (Batool et al., 2023; Duval et al., 2010; Swierczynska et al., 2022).

Oseltamivir, also known by the trade name Tamiflu, is an inhibitor of neuraminidase, an enzyme that plays a key role in the replication process of the influenza virus (Freund et al., 1999; Hayden et al., 1997; 2000; Savenkova et al., 2014). The drug is used to treat and prevent influenza types A and B, as well as to reduce the risk of complications from infection (De Clercq, 2019; Strachunsky et al., 2007). Oseltamivir has a broad spectrum of action and can be used in combination with other drugs to achieve the best effect (Batool et al., 2023; McKimm-Breschkin et al., 2003; Nguyen et al., 2010).

Rimantadine blocks the replication of the virus in the body's cells, thereby reducing its activity. Rimantadine is highly effective in the treatment of influenza and can be used as a stand-alone agent and in combination with other drugs (Cady et al., 2010; Hay, 1996; Mikhailov, 1983; Pettersen et al., 2006).

To reduce body temperature, as well as to reduce muscle, headache and joint pain, drugs with antipyretic and analgesic effects are recommended, among which are acetylsalicylic acid, paracetamol, and nonsteroidal anti-inflammatory drugs (Ghichloo and Gerriet, 2023; Saladino et al., 2010).

However, the prevalence of drug-resistant strains (Eccles, 2009; Ong and Hayden, 2007) may undermine their clinical benefit when used as monotherapy. Thus, in 2009, the Center for Disease Control and Prevention (CDC) reported that 100% tested isolate of seasonal H3N2 virus was resistant to adamantane in 99.6% of cases, and a tested H1N1 seasonal influenza virus carries an amantadine resistance associated S31N mutation in the M2 ion channel but remains sensitive to oseltamivir and zanamivir but remains sensitive to oseltamivir and zanamivir (Fischer and Hayden, 2008).

The recurrent emergence of influenza virus strains that are resistant to available antiviral drugs has become a global health concern, especially considering the possibility of a new influenza virus pandemic (Krammer et al., 2018; Kumar et al., 2018; Low et al., 2023). Currently, almost all circulating strains of influenza A virus are resistant to any of the two main classes of anti-influenza drugs (Loregian et al., 2014; Wathen et al., 2012).

The problem of the safety of combined pharmacotherapy is becoming more and more relevant today. Drug interaction is a qualitative change in the effects caused by drugs when two or more drugs are used simultaneously or sequentially (Han et al., 2022; Kovalskaya et al., 2018). So, to achieve the best therapeutic effect, patients are often prescribed combinations of several drugs aimed at treating various clinical manifestations of the existing disease (Matveyenko et al., 2013; Palleria et al., 2013; Shikh et al., 2017). However, little is known about the combined effect of the drugs used and how they interact with each other. Lack of knowledge in this area can lead to negative consequences, such as decreased efficacy, increased toxicity, and the occurrence of drug interactions that can cause adverse reactions in the patient's body (Nguyen et al., 2010; Smee et al., 2009). These circumstances emphasize the need to conduct studies of the combined effect of drugs to enhance or improve their therapeutic effect (Balanaser et al., 2023; Fischer and Hayden, 2008; Yusuf et al., 2014).

The combination of drugs is not just adding their individual effects (Kovalskaya et al., 2018). The basis for formulating a drug combination is to determine the direction and strength of the interaction of the components of the combination (Balanaser et al., 2023; Chou, 2006; 2010). This interaction may depend on various factors, such as the dose and method of administration, the time of administration, as well as the pharmacokinetics and pharmacodynamics of the drugs involved in the combination (Martsevich et al., 2021; Stielow et al., 2023). Studies may show that a certain combination of drugs is more effective in treating a certain condition than each drug individually (Sun et al., 2016). It is also possible to identify new therapeutic targets and develop new drugs that can be used in combination with existing treatment methods, which could lead to a significant improvement in the quality of patient care, treatment of various diseases, including those that are currently considered

incurable (Fang et al., 2015; Huang et al., 2017; Yusuf et al., 2014).

Examples of combined drug use include:

1. Antibiotics: in the treatment of infections, a combination of several antibiotics may be prescribed to provide broader coverage of pathogens and prevent the development of resistance (Sullivan et al., 2020).

2. Antitumor therapy: Cancer treatment often uses a combination of drugs that act on different stages of tumor development or mechanisms that promote its growth (Duarte and Vale, 2022).

3. Antihypertensive therapy: In treating high blood pressure, several drugs with different mechanisms of action may be prescribed to achieve a more effective reduction of blood pressure (Guerrero-Garcia and Rubio-Guerra, 2018).

4. Antidepressants: The combined use of various antidepressants may be prescribed when one drug is not effective enough or is not suitable for the patient (Fang et al., 2015; Henssler et al., 2022; Huang et al., 2017; Novikova et al., 2018; Yusuf et al., 2014).

Antiviral therapy can also serve as an example of the combined use of drugs. The simultaneous or combined use of rimantadine, oseltamivir, and aspirin in a clinical situation may be justified by several factors. The simultaneous or combined use of rimantadine, oseltamivir, and aspirin in a clinical situation may be justified by several factors (Batool et al., 2023; Galabov et al., 2006; Simeonova et al., 2012).

Firstly, each of these drugs has its own specific activity and mechanism of action, which allows it possible to achieve maximum effect in the fight against infection (Fang et al., 2015; Huang et al., 2017; Novikova et al., 2018).

Secondly, the combined use of drugs can enhance their effect and reduce the risk of the development of drug resistance to viruses (Novikova et al., 2018; Shyr et al., 2021; Siedentop et al., 2024).

Additionally, this combination may be helpful in relieving symptoms such as fever, pain, and inflammation. Thus, the simultaneous or combined use of rimantadine, oseltamivir, and acetylsalicylic acid may be an effective and reasonable approach in a clinical situation associated with influenza or other viral infections (Bijnsdorp, 2011; Chou, 2006; 2010; Chou et al., 1991).

Exposure-response relationships of combinatorial drug effects can be quantitatively described using pharmacodynamic interaction models that can be used to select optimal dose combinations (Bijnsdorp, 2011; Chou, 2006; 2010; Chou et al., 1991).

In Kazakhstan, an iodine-containing drug FS-1 has been developed, which is a chemically complex multicomponent compound containing molecular iodine, potassium and lithium halides, magnesium chloride, α-dextrins, and polypeptides (Yuldasheva et al., 2021). According to its physicochemical properties, FS-1 is an ionic nanostructured complex containing metal salts (LiCl(I), MgCl2), molecular iodine, and its ionized forms (Berdibay et al., 2017a; 2017b; 2017c; Turganbay et al., 2021; Yuldasheva et al., 2014). The structure of the active sites of the drug in the dextrin ring was calculated using the DFT/B3PW91 quantum chemical approach. Inside the dextrin helix, there are two active centers - a complex of molecular iodine with lithium halide (I), a binuclear complex of magnesium and lithium-containing molecular iodine, triiodide (II), and triiodide (Berdibay et al., 2017a; 2017c; Yuldasheva et al., 2021). This structure of the coordinated iodine-containing drug protects molecular iodine from interaction with bioorganic compounds after oral consumption and minimizes the toxic effects of iodine in the human body (Berdibay et al., 2017a; 2017b; 2017c; Yuldasheva et al., 2021). The main mechanism of action of FS-1 is membranolytic activity; the investigated drug also affects gene expression in bacteria (Ilyin and Kulmanov, 2014; Yuldasheva et al., 2021).

Our studies were aimed at studying the effect of the drug FS-1 on the toxicity of rimantadine and oseltamivir in combined use.

MATERIAL AND METHODS

Study drugs

Commercial drugs rimantadine (rimantadinum hydrochloridum J05A C02) manufactured by Merck (USA), oseltamivir (Tamiflu) manufactured by Hoffman-La-Roche (Switzerland), acetylsalicylic acid manufactured by JSC "Pharmstandard-Medicines" (Russia), and drug FS-1 manufactured by JSC "SCAID" (Kazakhstan) were purchased (Ilyin and Kulmanov, 2014).

Cell cultures

The studies were carried out on the transplantable cell culture MDCK. Madin-Darby canine kidney (MDCK) cell lines were obtained from RSE “Scientific Research Institute for Biological Safety Problems”. Cells were cultured using DMEM medium supplemented with 10% fetal bovine serum (FBS) (Applichem, Sigma, USA) and an antimycotic-antibiotic solution (100 U/mL penicillin, 0.1 mg/mL streptomy-

cin, and 0.25 µg/mL amphotericin B) at 37°C in an atmosphere of 5.0% CO_2 (Adams, 1980; Allen et al., 2005; Droganov, 2004; Freshney, 2005).

Determination of cytotoxicity of studied drugs *in vitro*

Determination of cytotoxicity concentration of the investigated drugs at individual and combined action *in vitro* was performed by using the neutral red test (Gomez-Gutierrez et al., 2021). The absorbance of each well was measured at the wavelength of the main filter of 540 nm and a reference wave of 620 nm in a microplate reader (Tecan Sunrise RC.4, Austria). The cytotoxicity concentration (CC_{50}) was calculated as the concentration of the plant extracts that reduced the viable cells to 50% of the untreated control.

Statistical analysis

The results are expressed as means ± SD (standard deviation of the mean). Statistical differences between groups were estimated using one-way analysis of variance (ANOVA) and were considered statistically significant at $p<0.05$. The analysis was done using GraphPad Prism version. 6.01.

Effect of the drug FS-1 on the toxicity of drugs rimantadine, oseltamivir, and acetylsalicylic acid with combined use

The study of the effect of the drug FS-1 on the toxicity of the drugs rimantadine, oseltamivir, and acetylsalicylic acid in combined use was carried out using the Chou-Talalay method, using CompuSyn 10.1 software (CompuSyn Inc., Paramus, NJ, USA). The algorithm for studying and analyzing combined effects according to Chou-Talalay allows for the quick calculation of the direction and strength of pharmacodynamic interactions by the value of the combination index (CI) for each component of the combination. It also allows analyzing combinations of various qualitative and quantitative compositions, calculating the result of interaction at any level of pharmacological (toxic) effect with any desired probability of an error-free prognosis (Bijnsdorp et al., 2011; Chou et al., 1991; Chou, 2006; 2010; Martsevich et al., 2021; Novikova et al., 2018). Interactions of biologically active substances "combinatorial index" (CI) calculated by formula [1].

$$CI = \frac{(D)_1}{(D_m)_1} + \frac{(D)_2}{(D_m)_2} \quad [1]$$

> 1 *antagonism*
$= 1$ *additivity*
< 1 *synergy*

Where: in the denominator (Dm)1 is the concentration (dose) of a biologically active substance that "changes the system" by x% (for example, causes cell death). (Dm)2 is the concentration (dose) of a physical factor that "changes the system" by x% (calculated by the median effect equation). In the numerator: (D)1 and (D)2 concentrations (doses) of a biologically active substance in combination that "change the system" by x%.

RESULTS AND DISCUSSION

Cytotoxicity of mono-action of drugs on MDCK cell culture

Cytotoxicity is the ability to cause changes in cellular behavior and essential processes that subsequently decrease the survival of a cell or trigger cell death. Cytotoxicity testing through various *in vitro* assays represents a crucial tool with applications in toxicological safety testing and drug discovery (Aleksandrova et al., 2018; Macarron et al., 2011; Szymański et al., 2012). Cells respond rapidly to toxic substances by changing their morphology, growth, behavior, and biochemical processes, controlling basic cell functions. *In vitro* cytotoxicity analysis, an alternative to animal testing, is an important tool for the specific screening of chemicals and the formation of comprehensive and reliable toxicological data useful for predicting toxic effects on humans (Mahto et al., 2010). *In vitro* cytotoxicity determination makes it possible to determine how toxic the drug is to cells. This is critical for assessing its potential harm and safety to living organisms. Safety at the cellular level is the first step in evaluating the risk to the patient (Danchenko, 2012; Wakuri et al., 1993). Determining the concentrations of the drug at which the toxic effect begins helps to establish safe dosages for clinical use. The results obtained in *in vitro* experiments serve as the basis for subsequent *in vivo* studies and clinical trials. They help to formulate hypotheses and develop protocols for further research (Eropkin and Eropkina, 2003).

The cytotoxicity of each drug was quantified separately *in vitro* on MDCK cell culture using a neutral red test (National Institute of Environmental Health Sciences, 2001). Registration of the results of cytotoxicity of mono-action of drugs on MDCK cell culture was carried out after 72 hours of incubation. Based on the data obtained, the values of CC_{50} were calculated (CC_{50} concentration of each drug, which inhibits cell growth by 50%). The results of CC_{50} are presented in Table 1 and Fig. 1.

The results of cytotoxicity of mono and combined action of drugs presented in Fig. 1 and Table 1 showed that rimantadine and oseltamivir have a pronounced cytotoxicity effect. Thus, the value of CC_{50} of the drug rimantadine is 0.049 mg/mL of the active substance; CC_{50} of oseltamivir is 0.149 mg/mL of the active substance. Moderate toxicity was found in the FS-1 preparation, the value of the CC_{50} of which was 0.430 mg/mL of the active substance. The least pronounced cytotoxicity effect, equal to 1.300 mg/mL, was shown by the drug acetylsalicylic acid.

Table 1. Evaluation of the cytotoxicity effect of anti-influenza and iodine-containing drugs on the MDCK cell culture model.

No.	Test system	Drug	[1]CC_{50} of active substance (mg/mL ± SD)[2]
1	MDCK	FS-1	0.430 ± 0.020
2		Rimantadine	0.049 ± 0.001
3		Oseltamivir	0.149 ± 0.005
4		ASA[3]	1.300 ± 0.090

[1]CC_{50} - is the concentration (dose) of each drug that inhibits cell growth by 50%. [2]Data are presented as means ± SD (n = 5). p<0.05 vs. control group. [3]ASA – acetylsalicylic acid.

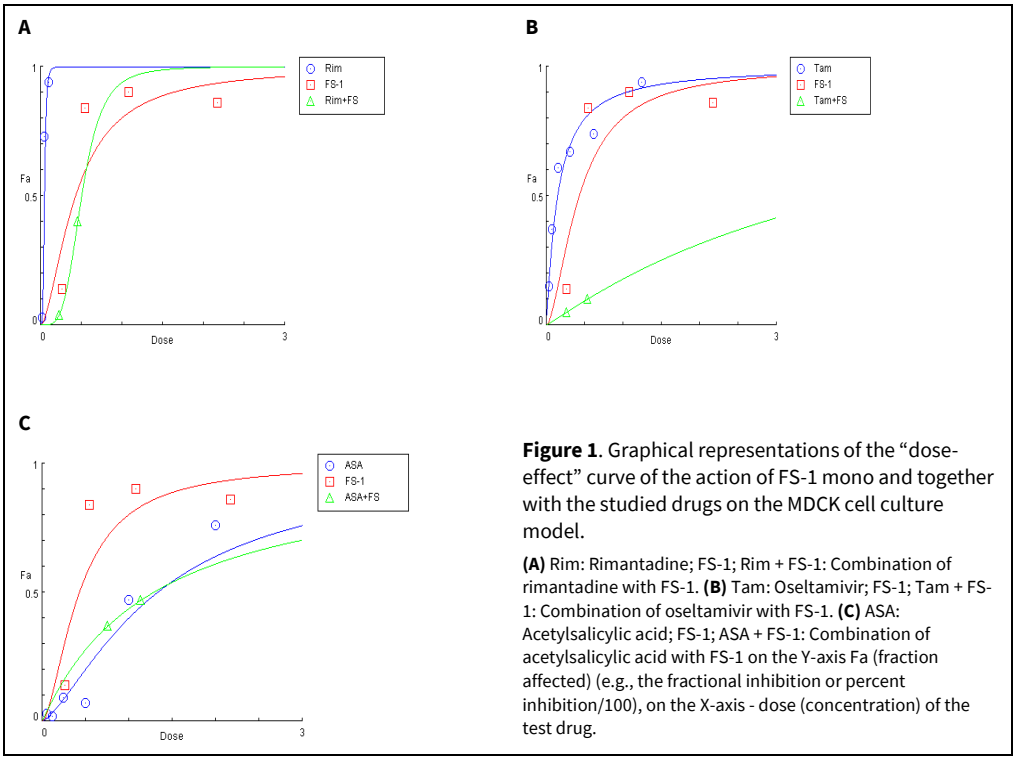

Figure 1. Graphical representations of the "dose-effect" curve of the action of FS-1 mono and together with the studied drugs on the MDCK cell culture model.

(A) Rim: Rimantadine; FS-1; Rim + FS-1: Combination of rimantadine with FS-1. **(B)** Tam: Oseltamivir; FS-1; Tam + FS-1: Combination of oseltamivir with FS-1. **(C)** ASA: Acetylsalicylic acid; FS-1; ASA + FS-1: Combination of acetylsalicylic acid with FS-1 on the Y-axis Fa (fraction affected) (e.g., the fractional inhibition or percent inhibition/100), on the X-axis - dose (concentration) of the test drug.

Combined interaction of the drugs rimantadine, oseltamivir, and acetylsalicylic acid under the action of FS-1

Studying the combined toxicity of drugs *in vitro* is important for understanding how drugs interact and affect their toxicity and effectiveness (Eropkin et al., 2012). Drug combination studies can determine whether synergistic effects occur, where two drugs work more effectively together than either drug alone, where a combination of drugs works better than either drug alone or where there is antagonism, where the effect of one drug reduces the toxicity of another. This may produce a more pronounced therapeutic effect at lower doses (Carrat et al., 2012).

Based on the theory of the median effect and the combination index by the ratio of doses (concentrations) and effects using the Chou-Talalay method, the combined effect of the drug FS-1 on the drugs rimantadine, oseltamivir, and acetylsalicylic acid was analyzed. The results of the analysis are presented in Table 2 and Figs. 2-4.

Thus, the concentration of the combined effect of using the drug FS-1 with rimantadine, inhibiting cell growth by 50%, was 0.507 mg/mL of the active substance. The result shows a decrease in the toxicity of one substance at the expense of another. According to the data obtained, the toxicity of the drug rimantadine decreased by 10 times and FS-1 by 1.2 times in relation to the mono effect of the drugs.

The concentration of the combined effect of oseltamivir in combination with FS-1 was 4.130 mg/mL of the active substance. A comparative study of oseltamivir and FS-1 preparations showed a significant decrease in toxicity with their combined use compared with a single use – a 27.7-fold decrease in oseltamivir toxicity and a 9.6-fold decrease in FS-1 toxicity.

Table 2. Comparative analysis of the combined interaction of the drugs rimantadine, oseltamivir, and acetylsalicylic acid under the action of FS-1.

No.	Name of the Drug A	Name of the Drug B	[1]D of active substance (mg/mL ± SD)[2]		[3]Dm of active substance (mg/mL ± SD)[2]	CI[4]
			Drug A	Drug B		
1	Rimantadine		0.049 ± 0.001		0.507 ± 0.03	2.018
2	Oseltamivir	FS-1	0.149 ± 0.005	0.430 ± 0.02	4.130 ± 0.50	13.726
3	ASA[5]		1.300 ± 0.090		1.300 ± 0.10	1.518

[1]D is the concentration (dose) of each drug that inhibits cell growth by 50%; [2]Data are presented as means ± SD (n = 5). p<0.05 vs. control group; [3]Dm is the concentration (dose) of the combined effect of the drug, which inhibits cell growth by 50%; [4]CI values of the combination index; [5]ASA: acetylsalicylic acid.

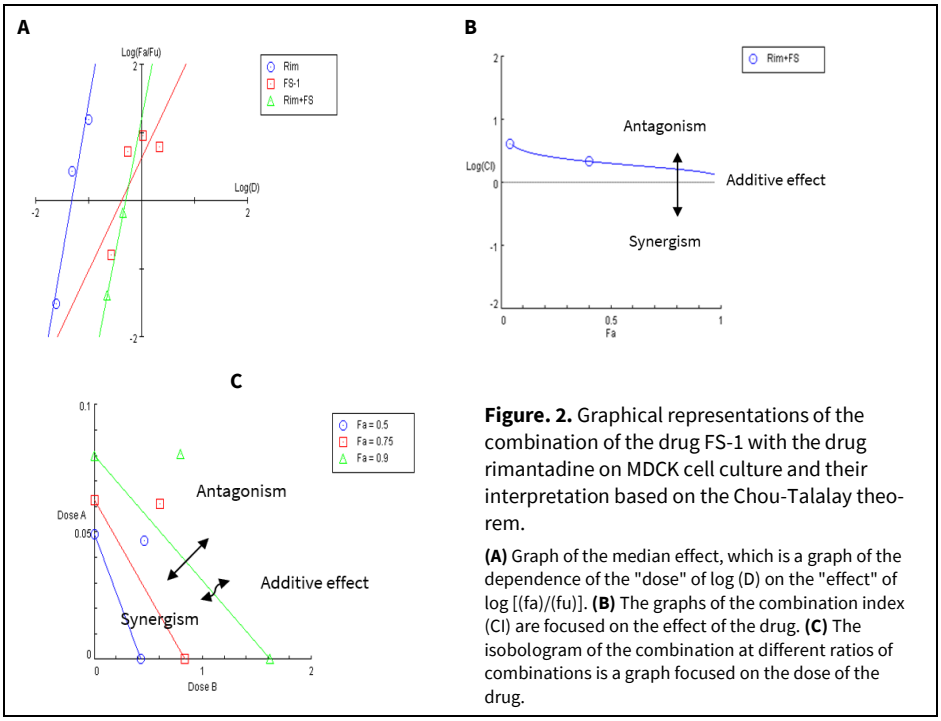

Figure. 2. Graphical representations of the combination of the drug FS-1 with the drug rimantadine on MDCK cell culture and their interpretation based on the Chou-Talalay theorem.

(A) Graph of the median effect, which is a graph of the dependence of the "dose" of log (D) on the "effect" of log [(fa)/(fu)]. **(B)** The graphs of the combination index (CI) are focused on the effect of the drug. **(C)** The isobologram of the combination at different ratios of combinations is a graph focused on the dose of the drug.

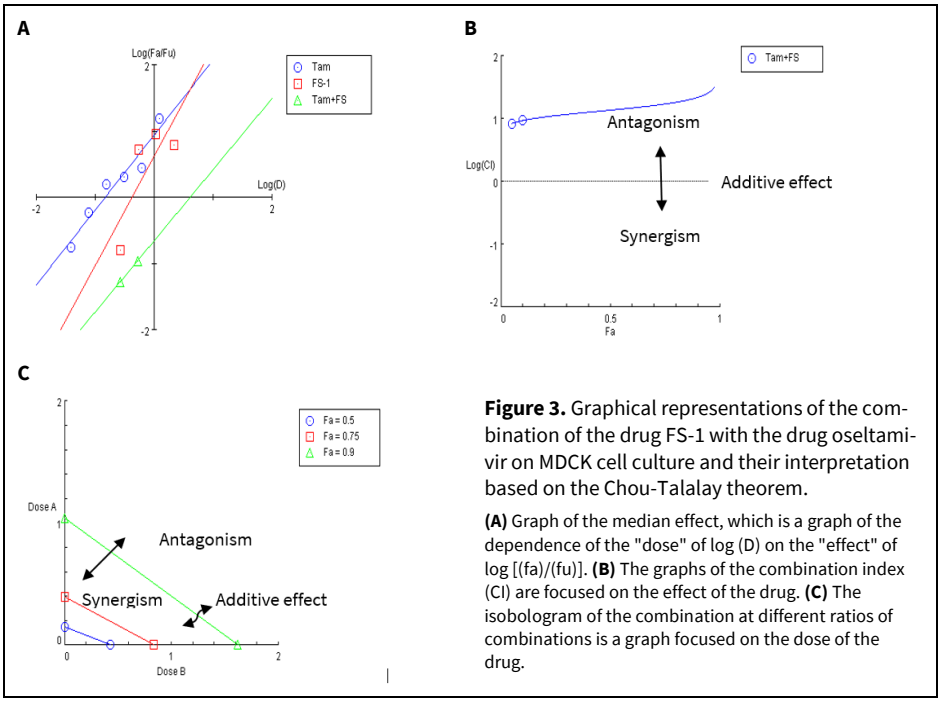

Figure 3. Graphical representations of the combination of the drug FS-1 with the drug oseltamivir on MDCK cell culture and their interpretation based on the Chou-Talalay theorem.

(A) Graph of the median effect, which is a graph of the dependence of the "dose" of log (D) on the "effect" of log [(fa)/(fu)]. **(B)** The graphs of the combination index (CI) are focused on the effect of the drug. **(C)** The isobologram of the combination at different ratios of combinations is a graph focused on the dose of the drug.

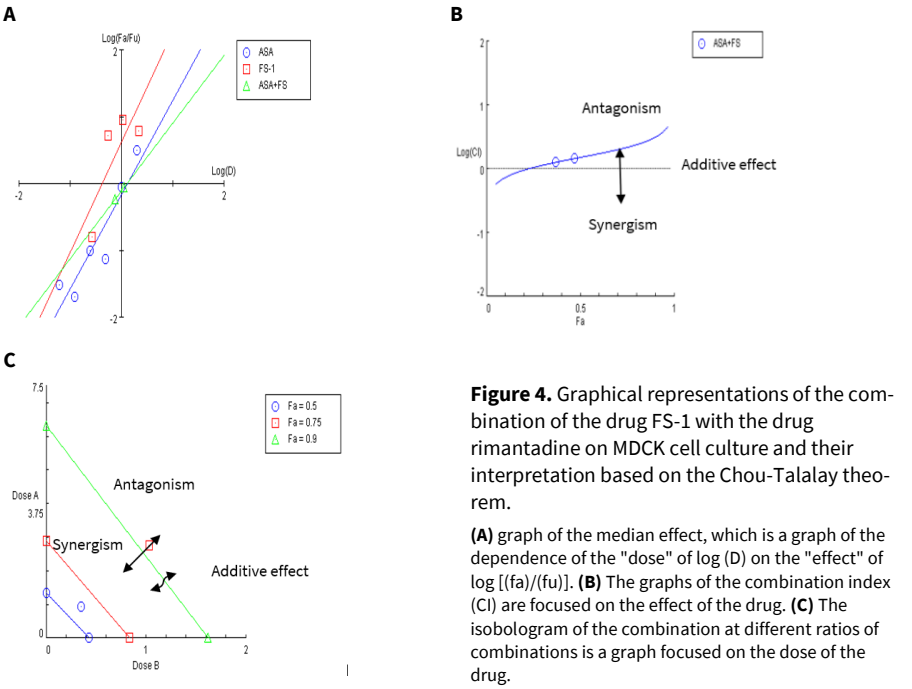

Figure 4. Graphical representations of the combination of the drug FS-1 with the drug rimantadine on MDCK cell culture and their interpretation based on the Chou-Talalay theorem.

(A) graph of the median effect, which is a graph of the dependence of the "dose" of log (D) on the "effect" of log [(fa)/(fu)]. **(B)** The graphs of the combination index (CI) are focused on the effect of the drug. **(C)** The isobologram of the combination at different ratios of combinations is a graph focused on the dose of the drug.

The concentration of the combined effect of the drug FS-1 with acetylsalicylic acid was 1.300 mg/mL of the active substance; no change in the toxicity of the combination was observed, but the toxicity of the drug FS-1 decreased by three times in relation to the mono action.

Thus, the analysis of the combined use of the anti-influenza drugs rimantadine, oseltamivir, and acetylsalicylic acid with FS-1 taken for the study showed that under the influence of the latter, there is a decrease in their toxic properties.

Graphs of the median effect equation, graphs of the combination index, and isobolograms of the drug effect showing the inhibition of the growth of toxicity of drug A, drug B, and the combination of drug A with drug B at a constant ratio (the dose of each drug inhibits cell growth by 50%), in Figs. 2-4.

Combination index (CI) graphs and isobolograms depict the quantitative values of the "dose-effect" for combinations of the studied drugs necessary for risk assessment. The use of the drug FS-1 together with the anti-influenza drugs rimantadine and oseltamivir in *in vitro* experiments showed that the combination index (CI) for all combinations is greater than one CI >1, for the combination of FS-1-rimantadine the index was CI = 2.018: for the combination of FS-1-oseltamivir CI = 13.726 and for the combination FS-1-ASA CI = 1.518. The values of the combination index (CI) of drugs were interpreted according to the following scale: CI <0.1 – synergism; CI = 0.90–1.10 – additive effect; CI >1 – antagonism.

The CI index >1 indicates the effect of antagonism, desirable in the study of toxicity, where one substance can eliminate and prevent the development of undesirable effects of another, preserving the main effect of the drug according to the pharmacodynamics interaction of drugs (Bijnsdorp et al., 2011).

Thus, the conducted studies show that the combined use of FS-1 with the anti-influenza drugs rimantadine, oseltamivir, and acetylsalicylic acid significantly reduces their individual toxic properties, which expands the range of applicable concentrations of these drugs for their joint use as antiviral agents.

CONCLUSION

In this study was investigated the influence of FS-1 on the toxicity of rimantadine, oseltamivir, and acetylsalicylic acid in combination *in vitro*. Employing the Chou-Talalay algorithm to analyze drug-drug interactions enabled us to quantify the impact of their combinations and identify the degree of interaction through the combination index (CI) value. The main hypothesis was that FS-1, as an ionic nanostructured complex, would reduce the toxicity of other drugs when administered together, while maintaining or improving their therapeutic efficacy. The result experiments support this hypothesis, as the combination of FS-1, rimantadine, and oseltamivir significantly reduced the toxicity of these drugs. This interaction appears to be favorable in terms of minimizing toxic effects while preserving the therapeutic action. Based on our findings, it is reasonable to conclude that FS-1

in combination with rimantadine, oseltamivir, and acetylsalicylic acid has the potential to decrease the toxicity of these drugs in clinical practice. This development may pave the way for the development of combination therapeutic approaches with improved safety profiles.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Ivanova L	Korotetskiy I	Kerimzhanova B	Shilov S	Zubenko N	Kuznetsova T	Parenova R	Ilin A
Concepts or ideas		x		x				x
Design		x		x	x			
Definition of intellectual content		x						x
Literature search	x	x		x	x	x		
Experimental studies	x			x	x			
Data acquisition	x			x	x			
Data analysis	x			x	x			
Statistical analysis	x	x		x	x	x	x	
Manuscript preparation	x	x	x			x		
Manuscript editing	x	x	x					x
Manuscript review	x	x	x	x	x	x	x	x

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