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Innovative therapeutic approach to chemical burns produced by vesicants; an experimental study

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ABSTRACT

Vesicants are compounds that cause severe toxic effects on various tissues. Such chemical action causes tissue necrosis, with clinical expression of skin lesions with a burning character and characteristic blisters. Clinical toxic effects of cutaneous vesicles are correlated with the absorbed dose and exposure time. The goals of the study are to evaluate in vitro the skin toxicity produced by the vesicant chemical compound 2-chloroethyl-ethyl sulfide (CEES), to develop a complex antidote formula, and to optimize the therapeutic efficacy by inclusion in controlled release systems. The experimental protocol aims at the in vitro evaluation of the cytotoxicity of the vesicant compound CEES and of the optimized complex antidote, using the MTT cell viability test. Optimization of the complex antidote formula was achieved by developing and in vitro and in vivo testing of a fixed combination of active substances with anti-inflammatory and antioxidant effects, formulated as a solution with cutaneous administration. In vitro cytotoxicity tests on fibroblast cultures revealed the protective effect of the newly developed antidote solution, specifically a dose-related effect in the case of vesicant exposure.

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Introduction

Vesicants are compounds that cause serious toxic effects to the eyes, lungs and skin, either by direct contact or through inhalation of vapors. This class of substances includes mustard gas, mustard nitrogen, halogenated oximes and arsenic agents [1,2]. Halogenated vesicants are a family of compounds based on sulfur, nitrogen and oxygen, with similar chemical and biological effects.

There are two types of mustard-based vesicants: nitrogen mustards, known for their use as chemotherapy drugs, and sulfur mustards, that are known only for their applications as warfare agents [3]. When dermally absorbed, molecules of sulfur-based vesicular compounds cyclize forming a highly reactive intermediate that binds to tissue proteins [4]. This chemical action causes tissue necrosis, clinically manifested as burn-like skin lesions and characteristic phlyctenules (blisters). Basal epidermal keratinocytes are the first target. After this, vesicant compounds cause cell death (apoptosis), followed by digestion of the anchoring filaments' protease at the level of dermal-epidermal junctions, which leads to separation and the appearance of vesicles [5-7].

The present study performs an in vitro evaluation of skin toxicity produced by the vesicant chemical compound, 2-chloroethyl-ethyl sulfide (CEES). The objectives of this study are represented by the development of a complex antidote formulation, which pharmacodynamically should be able to antagonize the toxicity of vesicants. As a result of this action, a possible remission of severe lesions is expected, in order to delay the surgical procedure and thus to optimize the therapeutic effectiveness by including it in the systems with a possible controlled release (in the form of microemulsions).

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Materials and Methods

Materials and reagents

The following materials and reagents were used to perform the experimental study:

- MTT cell viability determination kit – SIGMA ALDRICH Germany product;
- 2-chloroethyl-ethyl sulfur (CEES) 97% purity – Sigma Aldrich Germany product;
- dexamethasone - CAS number 50-02-2 99% purity, produced by Sigma-Aldrich Chemie GmbH, Germany;
- acetylcysteine - CAS number 616-91-1 99% purity, product of MERCK, Germany;
- nicotinamide mononucleotide CAS number 1094-61-7 95% purity produced by Sigma Aldrich, Germany;
- hydrolyzed hyaluronic acid - CAS number 9004-61-9. Purity ≥98%, produced by PARCHEM, USA;
- recombinant human epidermal growth factor, expressed in E. coli, ≥98% packed as 0,1 mg, produced by Alomone Laboratories, Israel;
- ketoprofen CAS number 22071-15-4 98% purity produced by Sigma-Aldrich Chemie GmbH, Germany.

Laboratory equipment

EnSight™ Multimode Microplate Reader multimodal reader, PERKIN ELMER, used for optical density reading in the MTT method for determining cell viability;

- NANO ZETASIZER MALVERN PANALITICAL standard cell analysis system, used for dynamic light diffusion measurements in the study of microemulsions;
- HPLC LC 4500 JASCO INC system, used to determine the analytical method for dosing antidote components in solutions and microemulsions;
- ChromNAV 2.0 HPLC software.

The experimental design went through the following stages:

Pharmaceutical formulation of the complex antidote

A complex formulation was prepared in the form of a percutaneous solution whose active substances are capable to pharmacodynamically antagonizing the toxicity of the studied vesicant compound. The following composition is studied: 10% acetylcysteine (antioxidant); 5% ketoprofen (non-steroidal antiinflammatory compound); 4% dexamethasone (steroidal antiinflammatory compound); 10% nicotinamide nucleotide (PARP inhibitor); epidermal growth factor (EGF) 0,1 µg/mL (the stock solution dosage form, 10 µg/ml in concentration, was prepared in 0.9% w/v NaCl); PBS excipients ad 100 ml buffer containing disodium phosphate, sodium chloride, potassium phosphate and potassium chloride, achieving a pH of 7.8, necessary for faster transdermal absorption.

Experimental protocol

In vitro evaluation of 2-chlorodiethyl-ethyl sulfide (CEES) vesicant's cytotoxicity and of the optimized complex antidote, using the MTT cell viability test.

An experimental model for in vitro toxicity assessment was performed by incubating a culture of fibroblasts (106 cells/ml) with doses of CEES 1: 7.78 × 10-2 M and CEES 2: 7.78 × 10-3M. The cell viability testing was performed using the MTT kit and 3T3 fibroblast cell line.

The 3T3 fibroblast cell line was cultured in 25 cm^2 cell culture dishes using Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 50 µg/ml gentamicin.

Cells in a 25 cm2 dish were trypsinized with 0,025% trypsin-EDTA and then centrifuged, while the pellet was resuspended in 5 ml of complete DMEM medium. Initial cell count was performed by Tripan Blue 1:1 staining (50 µl cell suspension + 50 µl Tripan Blue). The cell suspension has a concentration of 1.07 x 106 cells/ml.

To perform the cytotoxicity test, the cell suspension is cultured in a 96-well flat-bottomed microplate of 200 µl/well (2.14 x 105 cells/well) and incubated in a CO2 incubator (5%) at 370 C, for 24 hours.

The next day, the medium is changed as follows:

- remove the medium from the control cell wells and add 200 µl of completely fresh DMEM medium;
- in the wells with CEES toxic control, remove the medium and add 100 µl of fresh DMEM medium + 100 µl of CEES;
- the medium is removed from the wells containing solutions to be tested and 100 µl of CEES + 100 µl of test treatment solution in different concentrations (dilutions) are added;
- only 200 µl of complete DMEM medium is added to the blank wells; incubate the microplate in the CO2 incubator (5%) at 370 C, for 24 hours.

After 24 hours, the 3-(4,5-)dimethyl-2-thiazolyl-2,5-diphenyl-2H-tetrazolium bromide (MTT) test is performed to measure the conversion of MTT to a stained product in living cells. For this test, we used the MTT-based cell growth assay kit (Sigma) containing the MTT solution (5 mg/ml MTT in RPMI-1640 without phenol red) and the MTT solvent (0.1 N HCl in anhydrous isopropanol).

Remove the microplate from the incubator, add 20 µl MTT/well (10% of the medium's volume) and proceed with dark incubation for 4 hours, at 370C, in CO2. After 4 hours, remove the microplate from the incubator, remove the medium and add the MTT solvent, 200 µl/well. Read the optical density at a wavelength of 570 nm within one hour of adding the solvent using the PerkinElmer multimodal reader (EnSight™ Multimode Microplate Reader).

Obtaining and characterizing a delivery and controlled release system for the formulation of a chemical burn antidote

Obtaining microemulsions as a nanostructured delivery vector for antidote components. In order to control the release of the active substances that were proposed for the

formulation of the antidote, we suggested encapsulating them in the aqueous phase of microemulsions prepared from components with high biocompatibility. Skin absorption during transdermal administration of drugs is favored by the presence of surfactants that stabilize microemulsions.

Preliminary tests were performed for the preparation of uniphasic microemulsions with bicontinuous structure. Thus, we selected a composition which leads to obtaining bicontinuous structured Winsor IV microemulsion systems: sample 4 composition: 32% aqueous phase; 33% oil phase (IPM); 35% Surfactant-Cosurfactant (Tw80 + Sp80). The surfactant/cosurfactant molar ratio that gave the best results regarding the size of the microemulsion area on the phase diagram was 1:1, thus being selected for the final formula in the series. Dynamic light diffusion measurements were performed using the undiluted microemulsion, with a standard cell Nano Zetasizer Malvern instrument. The mean size of the liquid droplets was 19.54 ± 2.56 nm.

Establishment of the analytical dosing method for the antidote components in solutions and microemulsions

In order to study the release of active substances from the antidote formulations, we used the quantitative dosing of the antiinflammatory components of the proposed antidote preparation, namely dexamethasone and ketoprofen (inflammation mediation through oxidative stress being the main pathogenic mechanism involved in skin toxicity of vesicants). Tests were performed using the HPLC LC 4500 JASCO INC system. Chromatographic data were obtained using the ChromNAV 2.0 HPLC software.

Study of the release profile of antidote components

To evaluate the release profile of the antidote's components during transdermal administration, a permeability study was performed for the aqueous solution and for the selected microemulsion, taking into consideration it had the composition with the highest biocompatibility, due to the characteristics of surfactants used for stabilization. The in vitro permeation study was performed using a Strat-M® membrane as a material that mimics the properties of the skin and the Franz cell technique. The in vitro skin permeability study using ketoprofen and dexamethasone was performed using a vertical Franz cell (PermeGear, Inc., Hellertown, PA, USA). Each experiment was performed three times.

The effective diffusion area of the membrane mounted on the Franz cell was 0.99 cm^2. The 8 mL receptor compartment was filled with medium consisting of phosphate-buffered saline (PBS; pH 7.4). These experimental conditions are considered to be sink, thus assuming that the concentration of the drug in the receptor solution is negligible compared to that in the donor compartment. The diffusion cell was maintained at 37° C, and the receptor compartment was continuously homogenized using a magnetic stirrer. Then, 0.5 g of aqueous or microemulsion was added to the donor system compartment, which was sealed with Parafilm® to prevent evaporation of the sample. After certain time intervals (0.5, 1, 2, 4, 6, 10 and 24 h), 0.5 mL samples were extracted from the receptor medium and supplemented with a new receptor medium to maintain a constant volume. Samples of the analyte receptor medium extracted at said time intervals were analyzed using the methods described above, after appropriate prior dilution.

Results

In vitro evaluation of the cytotoxicity of the vesicant compound 2-chlorodietylethyl sulfide (CEES) and of the complex antidote optimized solution (A1), using the MTT cell viability test.

The following formula is used to interpret the results:

% cell viability = (DO positive control – DO blank)/(DO negative control – DO blank) x 100

Where:

DO = optical density read at 570 nm
positive control = cells + CEES and treatment + CEES + MTT + MTT solvent
negative control = cells + MTT + MTT solvent
blank = medium (complete DMEM) + MTT + MTT solvent

Antidote treatment stock solution was formed by: Acetylcysteine: 10%; Dexamethasone: 4%; Ketoprofen: 5%; 1/5 and 1/10 dilutions were prepared from the stock antidote solution (Table 1).

Table 1. The distribution in wells on the cell culture plate

1	2	3	4	5	6	7	8	9	10	11
Blank	Cells control	Cells + Stock treatment	Cells+ CEES1	Cells+ CEES1	Cells+ CEES1+ stock treatment	Cells+ CEES1+ stock treatment	Cells+ CEES1+ 1/5 treatment	Cells+ CEES1+ 1/5 treatment	Cells+ CEES1+ 1/10 treatment	Cells+ CEES1+ 1/10 treatment
Blank	Cells control	Cells+ Stock treatment	Cells+ CEES2	Cells+ CEES2	Cells+ CEES2+ stock treatment	Cells+ CEES2+ stock treatment	Cells+ CEES2+ 1/5 treatment	Cells+ CEES2+ 1/5 treatment	Cells+ CEES2+ 1/10 treatment	Cells+ CEES2+ 1/10 treatment

The samples (positive control) were processed in 2 wells for each treatment concentration. At the end, the arithmetic mean of the optical densities readings read at a wavelength of 570 nm was calculated. The negative control

and the blank were distributed in duplicate, and the arithmetic mean of the read optical densities was calculated afterwards (Table 2). The average values of the cell viability (calculated in percentages) and which are determined by the tested compounds are represented in Table 3, as well as in Figures 1 and 2.

Table 2. The average values of optical density (DO) at 570 nm

Toxic	1	2	3	4	5	6	7	8	9	10	11
CEES 1	0,054 ±0,0037	1,578± 0,067	1,389± 0,049	0,064 ±0,002		0,823 ± 0,004		0,587± 0,0025		0,291± 0,001	
CEES 2				0,070 ±0,0015		0,944 ± 0,0035		0,700± 0,0025		0,343± 0,003	

Footnote. CEES 1: mean optical density (DO) values for cell exposure to CEES 1, concentration 7.78 × 10-2M; CEES 2: mean optical density (DO) values for cell exposure to CEES 2, concentration 7.78 × 10-3M; Column 1 blank DO; Column 2 control untreated cells DO; Column 3 cells + stock treatment;
Columns 4, 5 average DO values: CEES + cells; Columns 6, 7 average DO values: CEES + stock treatment + cells; Columns 8, 9 average DO values: CEES + 1/5 dilution stock treatment + cells; Columns 10, 11 average DO values: CEES + 1/10 dilution stock treatment + cells

Table 3. Cell viability in different solutions

	1	2	3	4	5	6	7	8	9	10	11
CEES 1		100	87,570±2,67	0,668 ±0,05		50,432 ± 0,49		34,989 ± 0,89		15,20± 0,9	
CEES 2				1,010±0,07		50,387± 0,55		42,384± 0,98		18,980± 0,73	

Footnote. CEES 1 mean cell viability values for cell exposure to CEES 1, concentration 7.78 × 10-2M
CEES 2 is the mean cell viability values for cell exposure to CEES 2, concentration 7.78 × 10-3M;
Column 2 was considered reference (100%) for the value of cell viability for control untreated cells; corresponding DO 1,578 ± 0,067; Column 3 cells + stock treatment; Columns 4, 5 average cell viability values: CEES + cells. Columns 6, 7 average cell viability values: CEES + stock treatment + cells. Columns 8, 9 average cell viability values: CEES + 1/5 dilution stock treatment + cells. Columns 10, 11 average cell viability values: CEES + 1/10 dilution stock treatment + cells

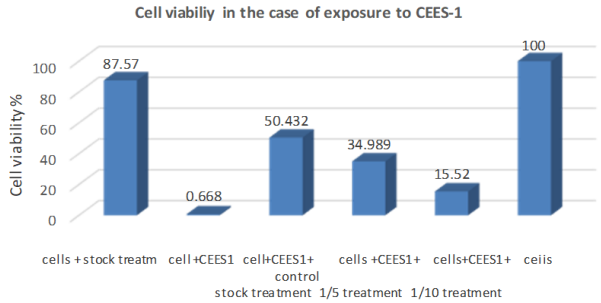

Figure 1. Cell viability after exposure to CEES-1

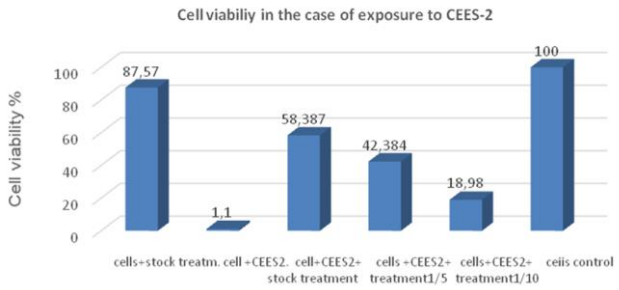

Figure 2. Cell viability after exposure to CEES-2

The CEES 2 formulation (concentration 7.78 * 10-3M) showed a lower toxicity compared to CEES1 (concentration 7.78 * 10-2M). The 1/1 dilution treatment reduced cyto-toxicity of the toxic compound for both CEES formulations. The reduction of the treatment effect with the increase of its dilution (1/5 and 1/10 respectively) can be observed.

The evaluation of the statistically significant difference between the averages of the optical density values correlated with cell viability for the groups intoxicated with 2-chloroethyl-ethyl sulfides and the control group, performed by applying the T Student test, highlighted the following aspects presented in Tables 4 and 5.

Table 4. Highlighting the cytotoxicity of the CEES solution 1

No.	Control	Average optical density values (nm)	CEES Concentration	P Probability (T test)	Observations
1	Cells Control	1,578±0,067	0	0,002	< 0,05
2	CEES 1 Positive Control	0,064 ±0,002	7,78 *10 -2M		

Table 5. Highlighting the cytotoxicity of the CEES solution 2

No.	Control	Average optical density values (nm)	CEES Concentration	P Probability (T test)	Observations
1	Cells Control	1,578±0,067	0	0,0035	< 0,05
2	CEES 2 Positive Control	0,070 ±0,0015	7,78 *10 -3M		

Optical density correlated with cell viability differs in a statistically significant way in groups exposed to CEES concentrations of 7.78 * 10-2 M and 7.78 * 10-3 M and untreated groups compared to the control cell group. The evaluation of the statistically significant difference between the average density values for the groups that were intoxicated with the two concentrations of 2-chloroethyl-ethyl sulfides and those that received treatment was performed by applying the Student T test, these aspects being highlighted in Tables 6 and 7.

Table 6. Comparative cytotoxicity of CEES-1 solution in the absence and in the present of treatment stock solution

No.	Control	Average optical density values (nm)	CEES Concentration	Treatment stock solution (percentage concentration)	P Probability (T test)	Observations
1	CEES1 Positive Control and treatment	0,823 ± 0,004	7,78 *10 -2M	Acetylcysteine: 10%; Dexamethasone: 4%; Ketoprofen: 5%;	0,00039	<0,05
2	CEES 1 Positive Control	0,064 ±0,002	7,78 *10 -2M	0		

Table 7. Comparative cytotoxicity of CEES-2 solution in the absence and in the present of treatment stock solution

No.	Control	Average optical density values (nm)	CEES Concentration	Treatment stock solution (dilution)	P Probability (T test)	Observations
1	CEES2 Positive Control and treatment	0,944 ± 0,0035	7,78 *10 -2M	Acetylcysteine: 10%; Dexamethasone: 4%; Ketoprofen: 5%;	0,00018	<0,05
2	CEES 2 Positive Control	0,064 ±0,002	7,78 *10 -2M	0		

Optical density correlated with cell viability differs in a statistically significant manner in the groups exposed to CEES1 7.78 * 10-2 M and CEES 2 7.78 * 10-3 M compared to the groups exposed to the same CEES concentrations and treated with the optimized antidote stock solution (in a dilution ratio of 1/1). The obtained data therefore demonstrate the effective therapeutic effect of the studied antidote in vitro.

Obtaining and characterizing a delivery and controlled release system for the formulation of an antidote for the treatment of chemical burns caused by vesicants

Ketoprofen was dosed using the Mediterranean Sea8 chromatographic column (Teknokroma), a column with a stationary phase of silica gel type modified with C8 groups. Chromatograms were recorded by measuring the absorbance at 233 nm (Figures 3 and 4).

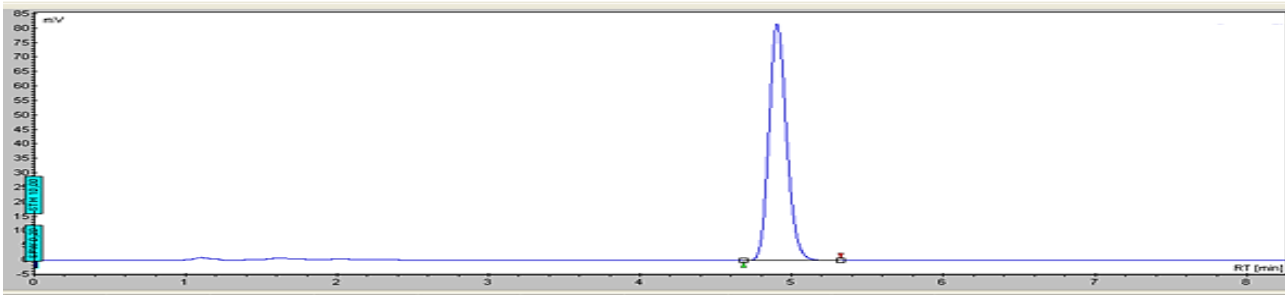

Figure 3. Chromatogram obtained for ketoprofen, the associated retention time being 4.902 minutes

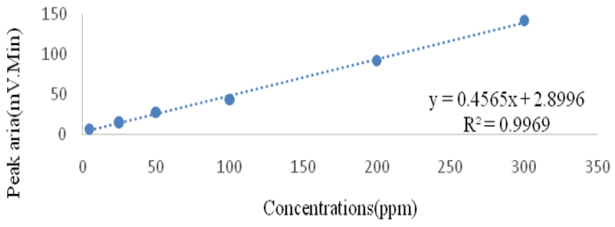

Figure 4. Calibration line associated with ketoprofen

Dexamethasone Dosing

The dosing method for dexamethasone in the receptor liquid was spectrophotometric, implemented using a UV-VIS Jasco 650V spectrophotometer. Measurements were made in quartz cells, reading the maximum absorption at a wavelength of 244 nm. A calibration curve linear over the range 4–16 µg/ml was drawn, with these data of the fitting equation: y = 10.973x + 39.695, R2 = 0.9902 (Figure 5).

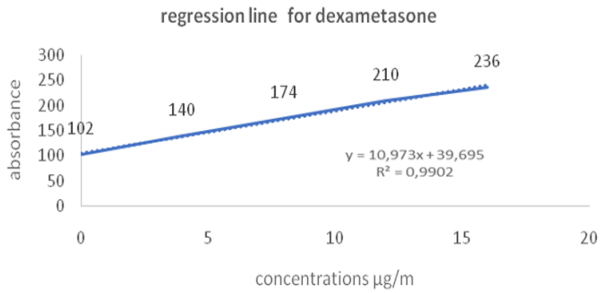

Figure 5. Calibration curve for dexamethasone

Study of the release profile of antidote components

Experimental data were used to determine the cumulative release of the active substances (KET and DEX), calculated using specialized literature equations. The total amount of release drug at harvest, expressed as a percentage of the total amount in the sample that was subjected to analysis (Figures 6 and 7).

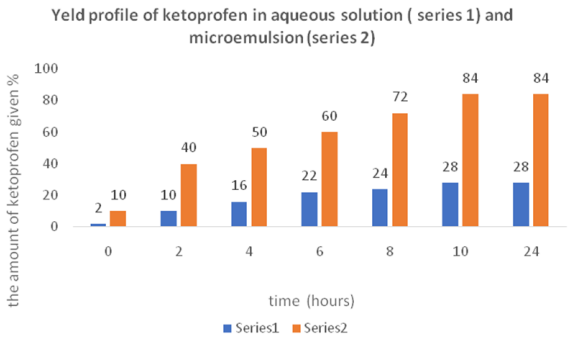

Figure 6. Ketoprofen release profiles from aqueous antidote solution compared to P6 microemulsion

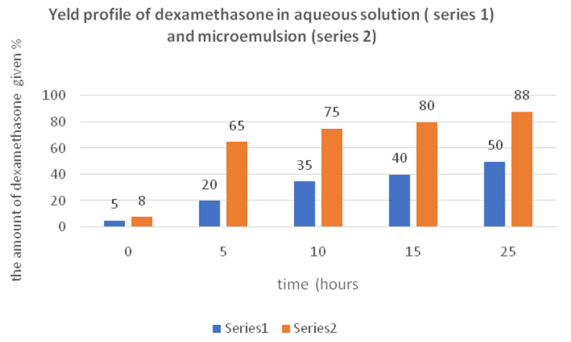

Figure 7. Dexamethasone release profiles from aqueous antidote solution compared to P6 microemulsion

Discussion

The main pathogenic mechanism of vesicant compounds is correlated with their alkylating property [8] and with their effect on glutathione (GSH), a nucleophilic antioxidant whose depletion leads to oxidative stress and macromolecular aggressions [9,10].

Another important pathogenic mechanism is the activation of some inflammation mediators: prostaglandins derived from cyclooxygenases [11]. Histopathologically, vesicant compounds mainly affect the epidermis. Cytotoxic effects have been observed mainly in basal keratinocytes (proliferative basal layer). Separation of the epidermis from the dermis and the appearance of edema was observed a few hours after exposure. The morphology of the basal layer was characterized by karyolysis and pyknosis. The dermis was less affected and showed only signs of discrete necrosis, along with a low number of fibroblasts and histiocytes. The erosive areas did not present an epidermis, but necrosis and massive cellular infiltration, with a lower degree of leukocyte infiltration [9,10]. Several studies found that serum levels of inflammatory mediators such as Il-1, Il6, IL-8, IL-10, IL-12, IL-13 mustard vesicants may be correlated with the severity of lesions induced by vesicants [12]. Interleukin-6 targets multiple cell types and induces a broad array of responses [13]. IL-6 might be involved in the early event of structural changes of the signal transducer glycoprotein that indirectly initiates the cascade of events such as skin irritation and blister formation observed in the pathophysiology of HD injury [14]. Exposure to vesicants can be fatal, especially when pulmonary airways are affected, causing profound inflammation, hyper-coagulation, and oxidative stress. Inflammation can influence coagulation by increasing cytokine levels of IL-6, IL-1, and IL-12, diminishing activated protein C (APC), decreasing fibrinolysis, and increasing platelet activation [15-17]. With an interdependent relation between inflammation and coagulation, the increased cytokine production led to the expression of the tissue inhibitor of plasminogen-1, and a tissue factor, and subsequently triggering the coagulation system through binding to the clotting factor VIIa [18]. The fluido-coaglant balance is extremely important in managing inflammation and wound healing. The inadequate removal of fibrin can hinder the normal healing process of wounds and may lead to the formation of fibrous adhesions [19]. Modeling coagulation and fibrinolysis could be future direction of research in wound healing [20].

The cutaneous toxic clinical effects of vesicants are correlated with the absorbed dose and the time of exposure, appearing late (7-24 hours after exposure) and being represented by edema, hyperpigmentation and blisters evolving into phlyctenules [21,22].

The usual medical countermeasures in case of cutaneous exposure to vesicants mainly involve the removal of the toxic compound by applying general decontamination: removal of clothes, thorough rinsing of the contaminated region with 0.1% benzalkonium bromide solution, applying antiseptics (silver sulfadiazine) and local antibiotics, occlusive bandages, and surgical treatment in case of severe injuries [23-26]. Current studies indicate that the effect of monotherapy is clearly inferior to therapies targeted at the multiple pathogenic mechanisms of vesicants. Thus, it is considered that the antioxidant antiinflammatory medication, anticytokines, represents a beneficial alternative in the treatment of this type of lesions [5,27,28]. In addition, the use of acetylcysteine, sodium thiosulfate and vitamin E were used in experimental studies and proved potential benefits due to their antioxidant properties [29-31]. The treatment of scars is still a controversial issue, mainly due to unpredictable results. Therapeutic options include lasers and fractioned radio frequency [32].

To elucidate pathogenic mechanisms and identify effective therapeutic solutions, studies were performed

using a chemical vesicant, namely 2-chloroethyl-ethyl sulfide (CEES) [1,33,34]. It has pathogenic mechanisms similar to those seen with acute exposure to vesicants (induction of oxidative stress and inflammatory response), but is less toxic, constituting a valid experimental alternative for scientific research studies in the laboratory [35-37].

By evaluating the experimental data obtained for the drug release through the skin-like model membrane, we found that for both tested substances, ketoprofen and dexamethasone, the transfer is facilitated by their incorporation in microemulsions. In the case of ketoprofen, the release occurs with the presence of the initial acceleration zone from both aqueous solution and microemulsion. As expected for the chemical structure of the ketoprofen molecule, very little is released from the aqueous vehicle, up to a maximum 32%, with the release occurring rapidly, with the plateau stabilizing after about 8-10 hours. From the microemulsion, it is released relatively quickly in the first 6 hours, up to approximately 60%, and within 24 hours it is released to up to 79% of the total amount. For dexamethasone, the release from the aqueous solution has no acceleration zone and has an almost linear appearance. A total amount of approximately 50% of what is present in the sample is released after the first 10 hours from the antidote solution. The amount released from the microemulsion is significantly higher, up to 75%, and the transfer through the skin is slightly prolonged, up to 24 hours.

The novelty element is represented by the therapeutic association of epidermal growth factors and niacinamide-type Poly (ADP-ribose) polymerase (PARP) inhibitors, as well as the inclusion of the complex antidote in microemulsions.

The epidermal growth factor (EGF) family is important in regulating growth, maturation, function, and maintenance in epithelial tissues. EGF stimulates the growth of various epidermal and epithelial tissues in vivo and in vitro1 and of some fibroblasts in cell culture [10,38] The EGF family is comprised of 13 members, which are all membrane-anchored proteins, in addition to their properties as ligands that activate the epidermal growth factor receptor (EGFR), bearing tyrosine kinase activity [39].

The PARP-1 monoclonal antibody recognizes native and/or cleaved polymerase. PARP cleavage is an early indicator of apoptosis (the cleaved form of caspase) as well as of DNA repair. The administration of optimized complex antidote treatment is correlated with inhibition of PARP expression, which can be explained by its effect on maintaining the integrity of the nuclear apparatus, which, under the protection of the pharmacological agents, is no longer stimulated to secrete PARP for its repair [40,41].

Microemulsions are the best novel drug delivery system due to their improved drug solubilization, and ease of preparation and administration [20,27,28,42]. They have emerged as novel vehicles for drug delivery, which allow controlled or sustained release for topical, transdermal administration of drugs [43-45]. Microemulsions are bicontinuous systems that are essentially composed of bulk phases of water and oil separated by a surfactant/ cosurfactant-rich interfacial region [46]. With the ability to carry both lipophilic and hydrophilic drugs, the dispersed phase, which is lipophilic or hydrophilic (O/W, or W/O microemulsions), can act as a potential reservoir for lipophilic or hydrophilic drugs, respectively. The use of microemulsions as delivery systems can improve the efficacy of a drug, allowing the total dose to be reduced, thus minimizing side effects [47].

Conclusions

Optimization of the complex antidote formula was achieved by developing and in vitro and in vivo testing of a fixed combination of active substances with anti-inflammatory and antioxidant effects, formulated as a solution with cutaneous administration Also, the complex antidote was formulated as a gel in 2020. In vitro cytotoxicity tests on fibroblast cultures revealed the protective effect of the newly developed antidote solution, a dose-related effect, in case of exposure to vesicants. Treatment applied as a 1/1 dilution reduced cytotoxicity for both CEES formulations.

The complex curative antidote that was developed and optimized in the study has superior therapeutic efficiency in case of exposure to sublethal doses of vesicants simulating mustard gas in experimental conditions. The novelty element is represented by the realization of a complex antidote formula, with anti-inflammatory, antioxidant, healing, hydrating and epithelizing effects following daily percutaneous administration for 7 days on the cutaneous chemical burn. Experimental studies have shown the optimization of transdermal transfer through encapsulation in microemulsion-type controlled-release vectors.

Highlights

- ✓ Chemical burns induced by vesicant agent are severe and the therapeutic management is challenging.
- ✓ Using a fixed combination of active substances with anti-inflammatory and antioxidant effects in a topically administrated solution revealed a protective effect on fibroblast cultures exposed to different concentrations of CEES solution.

Conflict of interest disclosure

There are no known conflicts of interest in the publication of this article. The manuscript was read and approved by all authors.

Compliance with ethical standards

Any aspect of the work covered in this manuscript has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

References

1. Aricò F, Tundo P. Mustard carbonate analogues. *Pure Appl Chem*. 2016;88(1-2):3-16. https://core.ac.uk/download/pdf/41144328.pdf
2. Ghanei M, Poursaleh Z, Harandi AA, et al. Acute and chronic effects of sulfur mustard on the skin: a comprehensive review. *Cutan Ocul Toxicol*. 2010 Dec; 29(4):269-77. doi: 10.3109/15569527.2010.511367
3. Jabbour RE, Salem H, Sidell FR. Blister agents/ Vesicants. Enciclopedya of Toxicology (Third edition), Academic Press, 2014; pp:522-525, ISBN: 9780123864550, doi: 10.1016/B978-0-12-386454-3.00590-X
4. Sayer NM, Whiting R, Green AC, Anderson K, Jenner J, Lindsay CD. Direct binding of sulfur mustard and chloroethyl ethyl sulphide to human cell membrane-associated proteins; implications for sulfur mustard pathology. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2010 May 15;878(17-18):1426-32. doi: 10.1016/j.jchromb.2009.11.030
5. Rose D, Schmidt A, Brandenburger M, Sturmheit T, Zille M, Boltze J. Sulfur mustard skin lesions: A systematic review on pathomechanisms, treatment options and future research directions. *Toxicol Lett*. 2018;293:82-90. doi: 10.1016/j.toxlet.2017.11.039
6. Lang S, Popp T, Kriegs CS, Schmidt A, Balszuweit F, Menacher G, Kehe K, Thiermann H, Gudermann T, Steinritz D. Anti-apoptotic and moderate anti-inflammatory effects of berberine in sulfur mustard exposed keratinocytes. *Toxicol Lett*. 2018 Sep 1;293:2-8. doi: 10.1016/j.toxlet.2017.09.004
7. Tewari-Singh N, Jain AK, Inturi S, White CW, Agarwal R. Clinically-relevant cutaneous lesions by nitrogen mustard: useful biomarkers of vesicants skin injury in SKH-1 hairless and C57BL/6 mice. *PLoS One*. 2013;8(6):e67557. doi: 10.1371/journal.pone.0067557
8. Sun X, Fu K, Hodgson A, Wier EM, Wen MG, Kamenyeva O, Xia X, Koo LY, Wan F. Sam68 Is Required for DNA Damage Responses via Regulating Poly(ADP-ribosyl)ation. *PLoS Biol*. 2016 Sep 16; 14(9):e1002543. doi: 10.1371/journal.pbio.1002543
9. Abel EL, Bubel JD, Simper MS, Powell L, McClellan SA, Andreeff M, MacLeod MC, DiGiovanni J. Protection against 2-chloroethyl ethyl sulfide (CEES)-induced cytotoxicity in human keratinocytes by an inducer of the glutathione detoxification pathway. *Toxicol Appl Pharmacol*. 2011 Sep 1;255(2):176-83. doi: 10.1016/j.taap.2011.06.012
10. Behm B, Babilas P, Landthaler M, Schreml S. Cytokines, chemokines and growth factors in wound healing. *J Eur Acad Dermatol Venereol*. 2012 Jul; 26(7):812-20. doi: 10.1111/j.1468-3083.2011.04415.x
11. Inturi S, Tewari-Singh N, Gu M, Shrotriya S, Gomez J, Agarwal C, White CW, Agarwal R. Mechanisms of sulfur mustard analog 2-chloroethyl ethyl sulfide-induced DNA damage in skin epidermal cells and fibroblasts. *Free Radic Biol Med*. 2011 Dec 15;51(12): 2272-80. doi: 10.1016/j.freeradbiomed.2011.08.020
12. Malaviya R, Sunil VR, Venosa A, Vayas KN, Businaro R, Heck DE, Laskin JD, Laskin DL. Macrophages and inflammatory mediators in pulmonary injury induced by mustard vesicants. *Ann N Y Acad Sci*. 2016 Jun; 1374(1):168-75. doi: 10.1111/nyas.13123
13. Brănescu C, Serban D, Dascălu AM, Oprescu SM, Savlovschi C. Interleukin 6 and lipopolysaccharide binding protein - markers of inflammation in acute appendicitis. *Chirurgia (Bucur)*. 2013 Mar-Apr; 108(2):206-14.
14. Arroyo CM, Broomfield CA, Hackley BE Jr. The role of interleukin-6 (IL-6) in human sulfur mustard (HD) toxicology. *Int J Toxicol*. 2001 Sep-Oct;20(5):281-96. doi: 10.1080/109158101753253027
15. Ahmad S, Ahmad A. Emerging targets for treating sulfur mustard-induced injuries. *Ann N Y Acad Sci*. 2016 Jun;1374(1):123-31. doi: 10.1111/nyas.13095
16. Serban D, Papanas N, Dascalu AM, Stana D, Nicolae VA, Vancea G, Badiu CD, Tanasescu D, Tudor C, Balasescu SA, Pantea-Stoian A. Diabetic Retinopathy in Patients With Diabetic Foot Ulcer: A Systematic Review. *Int J Low Extrem Wounds*. 2021 Jun;20(2):98-103. doi: 10.1177/1534734620982237
17. Weinberger B, Malaviya R, Sunil VR, Venosa A, Heck DE, Laskin JD, Laskin DL. Mustard vesicant-induced lung injury: Advances in therapy. *Toxicol Appl Pharmacol*. 2016 Aug 15;305:1-11. doi: 10.1016/j.taap.2016.05.014
18. Serban D, Tribus LC, Vancea G, Stoian AP, Dascalu AM, Suceveanu AI, Tanasescu C, Costea AC, Tudosie MS, Tudor C, Gangura GA, Duta L, Costea DO. Acute Mesenteric Ischemia in COVID-19 Patients. *J Clin Med*. 2021 Dec 30;11(1):200. doi: 10.3390/jcm11010200
19. Fometescu SG, Costache M, Coveney A, Oprescu SM, Serban D, Savlovschi C. Peritoneal fibrinolytic activity and adhesiogenesis. *Chirurgia (Bucur)*. 2013 May-Jun;108(3):331-40.
20. Opneja A, Kapoor S, Stavrou EX. Contribution of platelets, the coagulation and fibrinolytic systems to cutaneous wound healing. *Thromb Res*. 2019 Jul; 179:56-63. doi: 10.1016/j.thromres.2019.05.001
21. Taglialatela A, Alvarez S, Leuzzi G, Sannino V, Ranjha L, Huang JW, Madubata C, Anand R, Levy B, Rabadan R, Cejka P, Costanzo V, Ciccia A. Restoration of

Replication Fork Stability in BRCA1- and BRCA2-Deficient Cells by Inactivation of SNF2-Family Fork Remodelers. *Mol Cell*. 2017 Oct 19;68(2):414-430.e8. doi: 10.1016/j.molcel.2017.09.036

22. Poursaleh Z, Ghanei M, Babamahmoodi F, Izadi M, Harandi AA, Emadi SE, Taghavi NO, Sayad-Nouri SS, Emadi SN. Pathogenesis and treatment of skin lesions caused by sulfur mustard. *Cutan Ocul Toxicol*. 2012 Sep;31(3):241-9. doi: 10.3109/15569527.2011.636119
23. Jenner J, Graham SJ. Treatment of sulphur mustard skin injury. *Chem Biol Interact*. 2013 Dec 5;206(3): 491-5. doi: 10.1016/j.cbi.2013.10.015
24. Marinescu SA, Bejinariu GC, Boiangiu AM, Georgescu Balaceanu A, Gherghiceanu F, Giuglea C. Study on the concentrate of proteolytic enzymes enriched in bromelain and its effects on intermediate and extensive burns. *Farmacia*. 2019;67(3):522-530. doi: 10.31925/farmacia.2019.3.22
25. Neamtu M, Rusu RN, Ababei DC, Bild W, Gavrilovici C, Stana B, Bild V. Molecular backgrounds of individualised pharmacotherapy. *Farmacia*. 2020; 68(3):396-405. doi: 10.31925/farmacia.2020.3.4
26. Horodinschi RN, Stanescu AMA, Bratu OG, Pantea Stoian A, Radavoi DG, Diaconu CC. Treatment with Statins in Elderly Patients. *Medicina (Kaunas)*. 2019 Oct 30;55(11):721. doi: 10.3390/medicina55110721
27. Achanta S, Chintagari NR, Brackmann M, Balakrishna S, Jordt SE. TRPA1 and CGRP antagonists counteract vesicant-induced skin injury and inflammation. *Toxicol Lett*. 2018;293:140-148. doi: 10.1016/j.toxlet.2018.03.007
28. Mititelu M, Hîncu L, Ozon EA, Baconi DL, Paunica I, Bălălău OD. Analysis of potentially toxic contaminants in milk powder. *J Mind Med Sci*. 2021;8(2):237-244. doi: 10.22543/7674.82.P237244
29. Etemad L, Moshiri M, Balali-Mood M. Advances in treatment of acute sulfur mustard poisoning - a critical review. *Crit Rev Toxicol*. 2019 Mar;49(3):191-214. doi: 10.1080/10408444.2019.1579779
30. Pantea Stoian A, Mitrofan G, Colceag F, Suceveanu AI, Hainarosie R, Pituru S, Diaconu CC, Timofte D, Nitipir C, Poiana C, Serafinceanu C. Oxidative Stress in Diabetes A model of complex thinking applied in medicine. *Rev. Chim. (Bucharest)*. 2018;69(9):2515-19. doi: 10.37358/RC.18.9.6566
31. Laskin JD, Black AT, Jan YH, Sinko PJ, Heindel ND, Sunil V, Heck DE, Laskin DL. Oxidants and antioxidants in sulfur mustard-induced injury. *Ann N Y Acad Sci*. 2010 Aug;1203:92-100. doi: 10.1111/j.1749-6632.2010.05605.x
32. Pantea Stoian A, Hainarosie R, Pietrosanu C, Rusescu A, Andronache LF, Paunica, S, Balalau C, Pituru TS. Modern concepts in non-surgical esthetics; a review. *J Mind Med Sci*. 2018;6(2):190-5. doi: 10.22543/7674.62.P190195
33. Tewari-Singh N, Gu M, Agarwal C, White CW, Agarwal R. Biological and molecular mechanisms of sulfur mustard analogue-induced toxicity in JB6 and HaCaT cells: possible role of ataxia telangiectasia-mutated/ataxia telangiectasia-Rad3-related cell cycle checkpoint pathway. *Chem Res Toxicol*. 2010 Jun 21;23(6):1034-44. doi: 10.1021/tx100038b
34. Lomash V, Jadhav SE, Ahmed F, Vijayaraghavan R, Pant SC. Evaluation of wound-healing formulation against sulphur mustard-induced skin injury in mice. *Hum Exp Toxicol*. 2012 Jun;31(6):588-605. doi: 10.1177/0960327111429139
35. Bonomi PD, Gandara D, Hirsch FR, Kerr KM, Obasaju C, Paz-Ares L, Bellomo C, Bradley JD, Bunn PA Jr, Culligan M, Jett JR, Kim ES, Langer CJ, Natale RB, Novello S, Pérol M, Ramalingam SS, Reck M, Reynolds CH, Smit EF, Socinski MA, Spigel DR, Vansteenkiste JF, Wakelee H, Thatcher N. Predictive biomarkers for response to EGFR-directed monoclonal antibodies for advanced squamous cell lung cancer. *Ann Oncol*. 2018 Aug 1;29(8):1701-1709. doi: 10.1093/annonc/mdy196
36. Gerami P, Kim D, Compres EV, Zhang B, Khan AU, Sunshine JC, Quan VL, Busam K. Clinical, morphologic, and genomic findings in ROS1 fusion Spitz neoplasms. *Mod Pathol*. 2021 Feb;34(2):348-357. doi: 10.1038/s41379-020-00658-w
37. Dimitriu M, Socea B, Ionescu CA, Ples L, Gheorghiu DC, Constantin VD, Cirstoveanu CG, Bacalbasa N, Furau CG, Davitoiu DV, Gheorghiu N. Iodoform (triiodomethane)- An Old, but Still Extremely Efficient Antiseptic. *Rev. Chim*. 2019;70(4):1248-1250. doi: 10.37358/RC.19.4.7102
38. Rose M, Burgess JT, O'Byrne K, Richard DJ, Bolderson E. PARP Inhibitors: Clinical Relevance, Mechanisms of Action and Tumor Resistance. *Front Cell Dev Biol*. 2020 Sep 9;8:564601. doi: 10.3389/fcell.2020.564601
39. Reisländer T, Lombardi EP, Groelly FJ, Miar A, Porru M, Di Vito S, Wright B, Lockstone H, Biroccio A, Harris A, Londoño-Vallejo A, Tarsounas M. BRCA2 abrogation triggers innate immune responses potentiated by treatment with PARP inhibitors. *Nat Commun*. 2019 Jul 17;10(1):3143. doi: 10.1038/s41467-019-11048-5
40. Tomsic M, Podlogar F, Gasperlin M, Bester-Rogac M, Jamnik A. Water-Tween 40/Imwitor 308-isopropyl myristate microemulsions as delivery systems for ketoprofen: small-angle X-ray scattering study. *Int J Pharm*. 2006 Dec 11;327(1-2):170-7. doi: 10.1016/j.ijpharm.2006.07.035
41. Tatu AL, Radaschin DS, Constantin VD, Stana P, Ardeleanu V. Laser therapy in superficial morphea lesions – indications, limitations and therapeutic alternatives. *J Mind Med Sci*. 2020;7(1):46-51. doi: 10.22543/7674.71.P4651
42. Paromov V, Kumari S, Brannon M, Kanaparthy NS, Yang H, Smith MG, Stone WL. Protective effect of

liposome-encapsulated glutathione in a human epidermal model exposed to a mustard gas analog. *J Toxicol.* 2011;2011:109516. doi: 10.1155/2011/109516

43. Stone WL, Smith M. Therapeutic uses of antioxidant liposomes. *Mol Biotechnol*. 2004 Jul;27(3):217-30. doi: 10.1385/MB:27:3:217
44. Gupta S, Moulik SP. Biocompatible microemulsions and their prospective uses in drug delivery. *J Pharm Sci*. 2008 Jan;97(1):22-45. doi: 10.1002/jps.21177
45. Elboukhani I, Essadouni C, Mchachi A, Benhmidoune L, Chakib A, Rachid R, Elbelhadji M. Topical anesthesia versus peribulbar anesthesia in phacoemulsification cataract surgery and intraocular lens implantation *J Clin Invest Surg*. 2020;5(2):100-103. doi: 10.25083/2559.5555/5.2/100.103
46. Dutta C, Svirida A, Mammetkuliyev M, Rukhadze M, Benderskii AV. Insight into Water Structure at the Surfactant Surfaces and in Microemulsion Confinement. *J Phys Chem B.* 2017 Aug 10;121(31): 7447-7454. doi: 10.1021/acs.jpcb.7b04733
47. Souto EB, Cano A, Martins-Gomes C, et al. Microemulsions and Nanoemulsions in Skin Drug Delivery. *Bioengineering (Basel)*. 2022 Apr 5;9(4):158. doi: 10.3390/bioengineering9040158