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Evaluation of dermal corrosion and irritation by Cytoreg in rabbits

William Jimenez [a], Eduardo Gonzalez [a], Vincent A. Murphy [b], William Bauta [a,*]

[a] Cytorex Biosciences, 1710 West Lake Houston Pkway, Suite 260, Kingwood, TX 77339, USA
[b] STILLMEADOW, Inc., 12852 Park One Drive, Sugar Land, TX 77478, USA

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ABSTRACT

Cytoreg is an experimental therapeutic platform consisting of an aqueous solution of six acids (hydrofluoric, hydrochloric, sulfuric, phosphoric, citric, and oxalic) with oncolytic, antiviral, immune modulatory and antibacterial activities. Cytoreg may be formulated for topical, oral, and parenteral administration. In the present study, a skin corrosion/irritation screen was conducted on three albino rabbits for the Cytoreg topical formulation at three dilutions; one animal each received a dilution of 100 %, 4 %, or 2 % in physiological saline solution. Three intact skin test sites per animal/concentration were evaluated. Each test site was treated with 0.5 mL of the appropriate test substance solution. Site one was dosed for 3 min, then observed. Dose site two was wrapped for 1 h, then both first and second test sites were observed. Dose site three was wrapped for 4 h. One hour after unwrapping the third site, all three test sites were observed for skin irritation and/or corrosion, and again at 24, 48 and 72 h after final unwrap. Based on the 4 -h dose scores through 72 h, the primary irritation index (PII) for Cytoreg is 0.00 at 2 % and 4 %, with a descriptive rating of non-irritating, and 0.25 PII with slightly irritating rating at 100 %.

1. Introduction

Cytoreg is an experimental therapeutic technology platform consisting of an aqueous solution of hydrofluoric, hydrochloric, sulfuric, phosphoric, citric, and oxalic acids [1]. In vitro and in vivo studies have shown Cytoreg to have oncolytic activity [2–5]. In Wistar rats, oral administration of Cytoreg is well tolerated [6] and was recently shown to increase arterial blood oxygen pressure (pO_2) by iv administration [7]. We became interested in the potential use of Cytoreg topical solution for the treatment of diabetic foot ulcers, owing to the possible improvement of oxygenation to the damaged tissue. Diabetic foot ulcers are a major cause of hospitalization and amputation worldwide. Such ulcers result from disease-related vascular abnormalities that lead to bacterial infection, gangrene, and necrosis [8].

The concentrated form of the drug (Cytoreg concentrate) may be diluted for topical, parenteral, and oral administration. Oral and parenteral Cytoreg formulations have are well tolerated in rabbits [9], rats, and dogs [10]. Cytoreg is an acidic composition whose constituent acids, are known to cause skin corrosion, irritation, and inflammation [11]. Concentrated commercial solutions of hydrofluoric, hydrochloric, sulfuric, and phosphoric are classified under the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) system [12] as Class 1, 1B, 1A, and 1B skin corrosives, respectively [11]. Given this fact, and consistent with regulatory guidance for the testing of topical pharmaceuticals in models prior to human trials, we investigated the effect of topical Cytoreg solution in a rabbit skin corrosion/irritation screen.

2. Materials and method

Cytoreg concentrate was prepared according to established procedure by Cytorex Biosciences (Kingwood, TX) [1]. All testing was performed at Stillmeadow, Inc. (Sugarland, TX) in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 2011) [13] and the protocol was approved by the institutional animal care and use committee (IACUC) at Stillmeadow, Inc. Healthy albino rabbits were released from quarantine. Each animal was prepared on the day prior to treatment by clipping the dorsal area of the trunk free of hair to expose an area at least 8 × 8 cm^2. Care was taken to avoid abrading the skin. Only animals with exposure areas free of pre-existing skin irritation or defects were selected for testing.

Three intact skin test sites per animal were evaluated. One animal was dosed with test substance as received (100 %); a second animal was dosed with Cytoreg concentrate diluted to 4 % (v/v) in 0.9 % saline, and

* Corresponding author.
E-mail address: mailliwatuab@protonmail.com (W. Bauta).

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Table 1
Primary dermal irritation scoring scale (Draize technique).[a]

Erythema formation	Score	Edema formation	Score
None	0	None	0
Very slight (barely perceptible)	1	Very slight (barely perceptible)	1
Well-defined	2	Slight (edges well defined)	2
Moderate	3	Moderate (raised ~1 mm)	3
Severe (beet redness to eschar preventing scoring of erythema)	4	Severe (raised > 1 mm beyond test area)	4
Maximum possible	4	Maximum possible	4

[a] Other observations may be noted, for example: desquamation, necrosis, ulceration, blanching, bleeding, coriaceous, bruising.

Table 2
Irritation scores and descriptive ratings used in rabbit.

Descriptive Rating	PII	Descriptive Rating	PII
Non-irritating	0.0	Moderately Irritating	2.0–5.0
Slightly Irritating	0.1–1.9	Severely Irritating	5.1–8.0

Table 3
Observations for erythema and edema on individual rabbits.

Obs. Time after Treatment	Test Site	ERYTHEMA Animal #			EDEMA Animal #		
		12-M	18-M	19-F	12-M	18-M	19-F
3 min	RA	0	0	0	0	0	0
1 h	RA	0	0	0	0	0	0
1 h	RP	0	0	0	0	0	0
5 h	RA	0	0	0	0	0	0
5 h	RP	0	0	0	0	0	0
5 h	LA	0	0	0	0	0	0
*24 h	RA	0	0	0	0	0	0
*24 h	RP	0	0	0	0	0	0
*24 h	LA	0	0	1	0	0	0
*48 h	RA	0	0	0	0	0	0
*48 h	RP	0	0	0	0	0	0
*48 h	LA	0	0	0	0	0	0
*72 h	RA	0	0	0	0	0	0
*72 h	RP	0	0	0	0	0	0
*72 h	LA	0	0	0	0	0	0

RA–Right Anterior; RP–Right Posterior; LA–Left Anterior.
M–Male; F–Female; *–Observations were made 24, 48 and 72 h after final unwrap (4 h exposure).
Note: #12 = 2 %, #18 = 4 %, #19 = 100 %.

a third animal was dosed with substance diluted to 2 % (v/v) in 0.9 % saline. Each ~6 cm^2 test site was treated with 0.5 mL of the appropriate Cytoreg solution by introducing the diluted test substance beneath a surgical gauze patch. Each patch was secured in place with a strip of non-irritating adhesive tape. With the exception of the 3-min dosing, the entire trunk of each animal was then wrapped with clear plastic film to retard evaporation of volatile substances and to prevent possible ingestion of the test substance. Wrappings were held in place with non-irritating adhesive tape. Wrappings were removed at the end of the 1- and 4-h exposure periods. All test sites (including the 3-min exposure site) were washed with room temperature tap water and a clean cloth to prevent further exposure.

Test sites were observed and scored for signs of skin irritation, necrosis, or other defects after patch removal at 3 min and 1 h for the 3-min and 1-h exposure sites, respectively. All three test sites were observed at ~1, 24, 48, and 72 h after last patch removal. The scoring scale for signs of dermal irritation is presented in Table 1 [14]. The Primary Irritation Index (PII) was determined for each animal by adding all erythema and edema scores through 72 h for the 4-h exposure only and dividing the sum by 4 to obtain an individual irritation score [15]. The descriptive irritation rating was determined from the PII for each % dilution of test substance according to classifications shown in Table 2 [16].

Table 4
US EPA toxicity category criteria for dermal corrosion/irritation.

Toxicity Category	Criteria
I	Corrosive (anytime)
II	Severe irritation at 72 h
III	Moderate irritation at 72 h
IV	Non-irritating to slight irritation at 72 h

3. Results

Observations of dermal irritation or defects are presented in Table 3. Very slight erythema was present at 24 h on the 4-h site of the 100 %-dosed animal only. No edema was present at any time during the study. No other irritation/effect was observed. The PII values out of a possible 8.0 were 0.00, 0.00, and 0.25 for Cytoreg concentrations of 2 %, 4 %, and 100 %, respectively. The descriptive ratings per PII were non-irritating for 2 % and 4 %, and slightly irritating for 100 % [14]. The US Environmental Protection Agency Toxicity Category of IV (none to slight irritation) was determined for all concentrations from the irritation scores at 72 h using the criteria in Table 4 [15].

4. Conclusion

The primary irritation index (PII) for Cytoreg is 0.00 at 2 % and 4 %, with a descriptive rating of non-irritating, and 0.25 PII with slightly irritating rating at 100 %. Therefore, Cyotreg was non-corrosive and preliminarily assigned US EPA classification Category IV (non to slightly irritating) for all concentrations.

CRediT authorship contribution statement

William Jimenez: Conceptualization, Writing - Review & Editing, Funding acquisition. **Eduardo Gonzalez:** Project administration, Funding acquisition. **Vincent A. Murphy:** Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing, Writing - Review & Editing, Visualization, Supervision, Project administration. **William Bauta:** Conceptualization, Writing, Writing - Review & Editing, Visualization

Conflict of Interest

The research was funded by Cytorex Biosciences and experiments performed at Stillmeadow, Inc. WJ and EG are employees of Cytorex Biosciences. WB is a scientific advisor to Cytorex Biosciences. VM is an employee of Stillmeadow, Inc.

Declaration of Competing Interest

The authors report no declarations of interest.

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References

[1] D. Martucci, Pharmacologically active strong acid solutions. US Patent 7,141,251 (2006).

[2] M.M. Johnson, K.J. Kumi-Diaka, R. Zoeller, B.S. Graves, K.T. Merchant, V. P. Hörmann, M. Hassanhi, Therapeutic efficacy of Genistein-Cytoreg® combination in breast cancer cells, Funct. Foods Health Dis. 2 (5) (2012) 137–150.

[3] R. de Jesús, M. Cuervo, N.V. Fernández, A. Osorio, D. Stea, D. Martucci, L. Pozo, W. Jiménez, Estudio histológico del melanoma B16F1 inoculado en ratones hembra c57bl/6//biou tratados con la formulación Cytoreg, MedULA Revista de Facultad de Medicina 23 (2) (2014). Universidad de Los Andes. Mérida. Venezuela.

[4] J. Kumi-Diaka, M. Hassanhi, J. Brown, K. Merchant, C. Garcia, W. Jimenez, Cytoreg® inhibits growth and proliferation of human adenocarcinoma cells via induction of apoptosis, J. Carcinogen. 5 (2006) 1.

[5] R. de Jesús, N. Vicuña-Fernández, A. Osorio, M. Cuervo, D. Martucci, L. Pozo, W. Jiménez, Pruebas de efectividad in vivo de la formulación Cytoreg contra el melanoma B16F1en ratones hembras C57BL/6//BIO, Revista de Toxicología en Linea (RETEL), 2003, pp. 27–45. https://www.sertox.com.ar/wp-content/uploads/2020/01/43003.pdf.

[6] K. Villasana, W. Quintero, Y. Montero, et al., Effect of the ionic antineoplastic agent Cytoreg on blood chemistry in the Wistar rat model, Med. Gas Res. (2021) accepted for publication.

[7] D.F. Bandyk, The diabetic foot: pathophysiology, evaluation, and treatment, Semin. Vasc. Surg. 31 (2018) 43–48.

[8] R. de Jesus, N.V. Fernández, Y. Morales, A. Osorio, D. Martucci, L. Pozo, C. M. García, W. Jimenez, Chronic toxicity in Wistar rats of Cytoreg, an ionic antineoplastic therapeutic mix of strong and weak acids, Adv. Clin. Toxicol. 4 (4) (2019), 000170.

[9] R. de Jesús, N. Vicuña-Fernández, M. Romel, D. Martucci, L. Pozo, W. Jiménez, Estudio de la toxicidad crónica en conejas administrando la formulación Cytoreg® Revista de Toxicología en Linea (RETEL), 2003, pp. 52–67. https://www.sertox.com.ar/wp-content/uploads/2020/01/41004.pdf).

[10] Jimenez, W., Bauta, W., et al. Unpublished results.

[11] Material Safety Data Sheets (MDS) for acids may be downloaded from https://www.sigmaaldrich.com/US/en.

[12] https://pubchem.ncbi.nlm.nih.gov/ghs/.

[13] National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals, 8th ed., The National Academies Collection: Reports funded by National Institutes of Health; National Academies Press (US), Washington (DC), 2011.

[14] United State Environmental Protection Agency, Health Effect Test Guidelines OPPTS 870.2500 Acute Dermal Irritation, August, 1998.

[15] J.H. Draize, et al., Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes, J. Pharmacol. Exper. Ther. 83 (1944) 377–390.

[16] United State Environmental Protection Agency, Health Effect Test Guidelines OPPTS 870.1000 Acute Toxicity Testing – Background, December, 2002.