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DIPHOTERINE® CHEMICAL SPLASH DECONTAMINATION SOLUTION: SKIN SENSITIZATION STUDY IN THE GUINEA PIG

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Diphoterine® is an active eye/skin chemical splash decontamination solution. It was evaluated for sensitization potential in the guinea pig with primary induction (day 1, intradermal injection), sensitization (day 9, topical application), and challenge (day 22, topical application). Allergenicity degree at 24 and 48 hours was based on the percentage of animals showing a reaction. Under these conditions, no irritation was noted at 24 and 48 hours in negative controls and in animals treated with Diphoterine during the challenge phase. Diphoterine showed no allergenicity at 24 and 48 hours. In this study, Diphoterine lacked sensitizing capacity in the guinea pig.

Keywords: Diphoterine®; Skin, chemical splash; Skin, decontamination; Skin, sensitization

INTRODUCTION

Diphoterine® is a sterile, water-based, active decontamination solution for eye/skin chemical splashes (1). It has been extensively used for several years in European industrial workplaces. The emergent use of Diphoterine prevents or decreases the severity of eye/skin chemical burns and decreases the need for medical or surgical burn treatment, sequelae, and lost worktime (1). The manufacturer, Laboratoire PREVOR, Valmondois, France, has instituted a post-marketing surveillance program in order to collect all user experiences, and no sensitization to Diphoterine has been reported

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in European industrial sites using this decontamination solution. To confirm this observation and provide further documentation that Diphoterine is innocuous, a skin sensitization study in the guinea pig was performed.

MATERIALS AND METHODS

The delayed sensitizing potential of Diphoterine was evaluated in the guinea pig in accordance with the general requirements of OECD Guideline and Directive 67/548/EEC. The study was performed at the CERB Laboratory, Baugy, France (CERB Report No. 20030418ST), in accordance with all applicable regulations and standards for protecting animal welfare. The experimental technique was based on those of Magnusson and Kligman (2) and Guillot et al. (3). The sensitivity and the reliability of the experimental method are verified for at least six months by use of a positive control group in which animals are treated with DNCB (dinitrochlorobenzene; 1-chloro-2,4-dinitrochlorobenzene) used as a 1% alcoholic solution. The study involved 6 males and 6 females for a preliminary study and 15 males and 15 females for the main study.

For the preliminary study, an area of approximately 24 cm^2 (4 cm × 6 cm) on the retro-scapular region on either side of the vertebral column (induction area) or on both flanks (challenge area) was clipped free of hair using an electric clipper. Only healthy animals with an intact skin were used for the experiment. Sterile water was chosen as vehicle. The test substance, Diphoterine (Laboratoire Prevor, Valmondois, France, Batch Number D430611A*), was tested either undiluted (at the same concentration used for chemical splash decontamination in the industrial setting) or diluted in sterile water. Concentrations expressed as percentage volume/volume (v/v) were 100%, 75%, 50%, 25%, 10%, and 5%.

Determination of the maximum concentration causing a slight to moderate irritation by intradermal injection was performed in 2 males and 2 females. Each animal received intradermal injections of 0.1 mL in the retroscapular region at 6 sites (one concentration per site). The skin reaction was graded approximately 24 hours after injection (following OECD Guideline No. 406, see Table 1).

Determination of the maximum concentration causing slight to moderate irritation by cutaneous application was performed in 2 males and 2 females. Each animal received a cutaneous application of 0.5 mL over an area of 8 cm^2 (4 cm × 2 cm), using one concentration on each flank, two concentrations per animal. The test substance, Diphoterine, was placed on a 4 cm × 2 cm gauze piece. The gauze was held in place on the skin for 24 hours using an Elastoplast semi-occlusive dressing. The Diphoterine concentrations tested were undiluted and diluted at 75% v/v. The skin reaction at each concentration was graded approximately one hour after dressing removal.

Table 1 Grading system for skin reactions

Observations	Score
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

Table 2 Main study

Study day	Phase	Administration route	Diphoterine concentration % (v/v)
D1	Primary induction	Intradermal injection	100
D9	Sensitization phase	Topical application	100
D22	Challenge	Topical application	100

Determination of the Maximum Non-Irritant Concentration (MNIC) by cutaneous application was performed in 2 males and 2 females that received a cutaneous application of 0.5 mL over a 4 cm^2 (2 cm × 2 cm) area, using one concentration on each flank, two concentrations per animal. Diphoterine was placed on a 2 cm × 2 cm gauze piece. The gauze was held in place on the skin for 24 hours using an Elastoplast semi-occlusive dressing. The tested Diphoterine concentrations were undiluted and diluted at 75% v/v. The skin reaction was graded approximately 24 and 48 hours after the removal of dressings.

The main study (Table 2) concerning evaluation of the sensitization potential of Diphoterine involved 30 animals, 20 animals (10 males/10 females) treated with Diphoterine and a negative control group of 10 animals (5 males/5 females). The preparation of animal skin was similar to that in the preliminary study. For the intradermal induction on Day 1, each guinea pig received 6 injections in the retroscapular region on either side of the vertebral column in a 24 cm^2 area (4 cm × 6 cm) cm) free of hair. The treatment of the animals is shown in Table 3.

Table 3 Treatments of groups during intradermal induction on Day 1

Site	Negative controls	Positive controls	Diphoterine-treated animals
1	2 injections of 0.1 mL of complete Freund's adjuvant diluted 50% in sterile and pyrogen-free isotonic sodium chloride solution	2 injections of 0.1 mL of complete Freund's adjuvant diluted 50% in sterile and pyrogen-free isotonic sodium chloride solution	2 injections of 0.1 mL of complete Freund's adjuvant diluted 50% in sterile and pyrogen-free isotonic sodium chloride solution
2	2 injections of 0.1 mL of sterile water	2 injections of 0.1 mL of 1% DNCB	2 injections of 0.1 mL of Diphoterine® at the maximum slight to moderate irritant concentration by intradermal injection as determined during the preliminary study
3	2 injections of 0.1 mL of an emulsion of equal volume of sterile water and of complete Freund's adjuvant diluted 50% in sterile water and pyrogen-free isotonic sodium chloride solution	2 injections of 0.1 mL of an emulsion of equal volume of 1% DNCB and of complete Freund's adjuvant diluted 50% in sterile water and pyrogen-free isotonic sodium chloride solution	2 injections of 0.1 mL of an emulsion of equal volume of sterile water and of complete Freund's adjuvant diluted 50% in sterile water and pyrogen-free isotonic sodium chloride solution and of Diphoterine

Table 4 Degree of sensitizing capacity

Percentage of sensitized animals	Grade (degree of allergenicity)	Classification
0	-	Non-sensitizer
>0–8	I	Weak sensitizer
9–28	II	Mild sensitizer
29–64	III	Moderate sensitizer
65–80	IV	Strong sensitizer
81–100	V	Extreme sensitizer

For topical induction on Day 9, the area was treated with 0.5 mL of a suspension of 10% sodium lauryl sulfate (SLS) in mineral oil if no irritation appeared due to the maximum of concentration by intradermal induction. On the day before topical induction (Day 8), this SLS suspension was applied topically to the skin at the 6 injections sites utilized on day 1, over an 8 cm^2 area, clipped free of hair, to create local irritation. Topical induction, second induction at Day 9, involved cutaneous application at the 6 Day 1 injection sites. Diphoterine, 0.5 mL, was applied at the concentration determined by cutaneous application during the preliminary study, on a piece of absorbent gauze held in place on the skin for 48 hours by an Elastoplast semi-occlusive dressing. Negative controls animals received 0.5 mL of the vehicle topically and the positive control group received 0.5 mL of 1% DNCB solution topically.

During the expression phase (from Day 11 to Day 21), the animals remained untreated. On day 22, for topical challenge, animals in the treatment group received topical application of 0.5 mL of Diphoterine at the concentration determined to be the MNIC during the preliminary study to the right flank region over a 4 cm^2 (2 cm × 2 cm) area previously free of hair on a piece of absorbent gauze which was held in place for 24 hours using an Elastoplast semi-occlusive dressing. Under the same conditions, negative control animals received 0.5 mL of Diphoterine at MNIC determined during the preliminary study and positive control animals received 0.5 mL of 1% DNBC solution.

The determination of the degree of allergenicity at 24 and 48 hours after removal of the dressing was based upon the percentage of animals in the group showing a reaction, rather than on the severity of the reaction. The classification of the degree of sensitizing capacity was also based upon the percentage of animals showing a reaction, rather than the severity of the individual reaction(s) following the method of Magnusson and Kligman (2) (Table 4).

RESULTS

The preliminary study showed that the application of the test substance, Diphoterine®, did not induce discoloration of the application site. Grading of any skin lesions was therefore possible. For intradermal injections of Diphoterine, the test substance applied either undiluted or diluted at 75% v/v induced a moderate erythema (score 2) in the animals. At 50% v/v and 25% v/v concentrations, a slight

Table 5 Summary of results of cutaneous reactions

Treatment	Time	Number of animals score 0	Number of animals score 1	Number of animals score 2	Number of animals score 3	% of Sensitized animals
Negative control (sterile water)	24 h	10	0	0	0	0
	48 h	10	0	0	0	0
Diphotérine	24 h	20	0	0	0	0
	48 h	20	0	0	0	0
Positive control (DNCB)	24 h	0	0	7	3	100
	48 h	0	4	5	1	100

erythema (score 1) or moderate (score 2) erythema was observed. At 10% v/v concentration, a slight erythema (score 1) was observed in all animals and at 5% v/v, no erythema (score 0) or slight erythema (score 1) was recorded in the animals.

For cutaneous applications of Diphoterine, no skin reaction was observed in animals treated with cutaneous application of undiluted Diphoterine or diluted at 75% v/v in sterile water. The maximum concentration causing a slight-to-moderate irritation determined by intradermal administration was undiluted Diphoterine. It was the same concentration for the maximum concentration determined for cutaneous application and determination of the maximum non-irritant concentration (MNIC). Therefore, undiluted Diphoterine was used in the main study for the primary induction phase on Day 1, the second induction phase (or sensitization) on Day 9, and the challenge phase on Day 22.

Animals were monitored daily throughout the study period. The behavior of animals treated with Diphoterine was normal and not different from that of the control group. Mean body weight gain in males and females treated with Diphoterine differed significantly from that of males or females of the negative control group, at the threshold of, respectively, 1% and 5%.

No irritation reaction was noted at 24 and 48 hours in animals in the negative control group and in animals treated during the challenge phase with the test substance, Diphoterine, at the Maximum Non-Irritant Concentration (MNIC) (Table 5). Under the adopted experimental conditions, Diphoterine showed no allergenicity at 24 and 48 hours. In these study conditions, it is considered that Diphoterine lacks sensitizing potential in the guinea pig.

DISCUSSION

Laboratoire Prevor, the manufacturer of Diphoterine®, has a post-marketing surveillance program in place to collect any adverse effects of this eye/skin chemical splash decontamination solution as used in the European industrial setting. To date, no adverse effects, and specifically no incidences of sensitization to the product, have been reported.

Diphoterine is an amphoteric, slightly hypertonic, chelating compound which can actively bind to and inactivate a wide variety of chemical compounds splashed

into the eyes or on the skin, such as both acids and bases, oxidizers and reducing agents, irritants, lacrimators, and solvents (1). In Europe, it is regulated as a medical device because it has no physiological effect on the cornea/conjunctiva or skin.

Diphoterine was not irritating to the eyes of normal human volunteer subjects (4). In experimental animal studies performed by contract laboratories, it was not irritating to rabbit eyes (Safepharm Laboratories, UK, 1987), was only slightly irritating to rabbit skin in some, but not all animals (Safepharm Laboratories, UK, 1987), was not irritating to rat skin and was non-toxic by the oral and dermal routes in rats with $LD_{50}s > 2{,}000$ mg/kg (Centre International de Toxicologie, France, 1990; Safepharm Laboratories, UK, 1988) (1). When Diphoterine was reacted *in vitro* with strong acid (hydrochloric acid, HCl) or base (sodium hydroxide, NaOH), the reaction residues were not irritating to rabbit eyes (Centre International de Toxicologie, France, 1990) (1). A comparative study in pig eyes of Diphoterine versus a phosphate buffered eye decontamination solution showed the lack of deleterious effects of Diphoterine (5).

Diphoterine has been shown to be particularly efficacious for decontamination of eye/skin splashes with corrosive chemicals (1). Experimental studies in the rabbit have shown the interest of even delayed Diphoterine eye decontamination (6). A case report supported the potential benefits of delayed Diphoterine eye decontamination when combined with hospital treatment using a protocol designed to decrease inflammation and promote tissue healing (7).

A comparative study of chemical skin burns with hydrochloric acid (HCl) in the rat demontrated a significantly better effect of Diphoterine versus normal saline decontamination on wound extent and healing, as well as on biomarkers of inflammation and pain (8,9). The rapid amelioration of pain during Diphoterine decontamination of chemical splashes has also been noted in a human occupational chemical exposure study (10). This effect of rapid pain relief following the initiation of Diphoterine decontamination can aid in optimal decontamination, especially of the eyes, but also requires that workers and rescuers be trained so that an adequate volume of Diphoterine solution is used (10).

A single case of possible sensitization to Diphoterine was found in the manufacturer's post-marketing surveillance program. However, on further evaluation it was proven to be a case of a splash with cinnamic alcohol, which itself is known to be an allergen. On subsequent testing, the patient was found to be sensitized to cinnamic alcohol and *not* to Diphoterine.

The current sensitization study was done in comparison to DNCB, a known allergen, and sterile water which has no known adverse effects on the skin as well as no skin sensitization potential. The results of the current study in the guinea pig were in accordance with the findings of the Diphoterine manufacturer's post-marketing surveillance program.

CONCLUSION

Under the experimental conditions adopted, Diphoterine® showed no allergenicity at 24 hours and 48 hours and it is considered that it lacks sensitizing potential in the guinea pig. This result is consistent with the lack of sensitization in workers in industrial settings noted in the manufacturer's post-marketing

surveillance program. Should an individual worker experience multiple eye or skin chemical splashes requiring emergent decontamination over a period of time, the results of this study, together with those of the post-marketing surveillance program, suggest that the development of sensitization with a subsequent allergic reaction if Diphoterine decontamination is required in the future is unlikely.

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