Diphoterine : skin sensitization study in the guinea pig

MATHIEU L[1], BURGHER F[1], HALL AH[2]

Researches and communications Department, PREVOR Laboratory, Valmondois, France; TCMTS Inc., Elk Mountain, WY, USA

Introduction

Diphoterine is a rinsing solution for eye/skin chemical splashes. It has been used since several years at industrial workplaces in Europe. The emergent use of Diphoterine decreases or avoids the appearance of the chemical burn, the need for medical treatment and the loss of work. A survey of the market has been organized by PREVOR Laboratory since 1999 in order to collect all the claims from users and no sentizisation to Diphoterine has been reported from industries.To confirm this observation and bring new proof of its innocuity, a skin sensitization study has been performed .

Material and methods

The possible delayed sensitizing capacity 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 n°20030418ST). The experimental technique is based on those of Magnusson-Kligman and Guillot and coll. The sensitivity and the reliability of the experimental method are verified 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 test and 15 males and 15 females for the main study. For preliminary study, an area of approximately 24 cm2 (4 cm x 6 cm) on the retro-scapular region on either side of the vertebral culumn (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 Diphotérine was tested undiluted and diluted in sterile water. Concentrations expressed as percentage volume/volume (v/v) were 100 v/v, 75 v/v, 50 v/v, 25v/v, 10 v/v, 5 v/v

Determination of the maximum concentration causing a slight to moderate irritation by intradermal injection was performed on 2 males and females. Each animal received intradermal injections of 0.1mL in the retroscapular region at 6 sites (one concentration per site). The skin reaction was graded approximately 24 hours after injection (following OECD Guideline n°406, see Table 1). Determination of the maximum concentration causing a slight to moderate irritation by cutaneous application was performed on 2 males and 2 females. Each animal received a cutaneous application of 0.5 mL over an area of 8 cm2 (4 cm x 2 cm), using one concentration on each flank, two concentrations per animal. The tested substance Diphotérine was deposited on a gauze piece of size 4 cm x 2 cm. The gauze was held in place for 24 hours using an Elastoplaste semi-occlusive dressing. The concentration tested were Diphotérine undiluted and diluted at 75 v/v. The skin reaction at each concentration was graded approximately on hour after the removal of dressings. Determination of the Maximum Non-Irritant Concentration (MNIC) by cutaneous application was performed on 2 males and 2 females which received a cutaneous application of 0.5 mL over an area of 4 cm2 (2 cm x 2 cm), using one concentration on each flank, two concentrations per animal. Diphotérine was deposited on a gauze piece of size 2 cm x 2 cm. The gauze was held in place for 24 hours using an Elastoplaste semi-occlusive dressing. The tested concentrations were Diphotérine undiluted and diluted 75 v/v. The skin reaction was graded approximately 24 and 48 hours after the removal of dressings.

The main study (Table 2) concerning the evaluation of sensitization activity of Diphotérine involved 30 animals, 20 animals (10males/10females) treated with Diphotérine and a negative control group of 10 animals (5 males/5 females). The preparation of animal skin is similar to the preliminary study. For the intradermal induction on day 1, each pig received 6 injections in the retroscapular region on either side of the vertebal column on a 24 cm2 area (4 cm x 6 cm), free of hair. The treatment of the animals is presented in Table 3. For the topical induction on day 9, the area is managed with 0.5 mL of a suspension of 10% sodium lauryl sulfate in minearl oil if no irritation appears due to the maximum of concntration by intradermal induction. On the day before topical induction (day 8), this suspension was applied topically to the skin at 6 injections sites utilised on day 1, over a 8 cm2 area, clipped free of hair, to create a local irritation. Topical induction, second induction at day 9 involved cutaneous application at the 6 injections sites od day 1. 0.5 mL of diphotérine was applied at the concentration determined by cutaneous application during the preliminary study, on a piece of absorbent gauze held in place for 48 hours by an Elastoplaste semi-occlusive dressing. Negative controls animals received 0.5 mL of the vehicle and positive control group received 0.5 mL of 1% DNCB solution (dinitrochlorobenzene (1-chloro-2,4-dinitrobenzene)). During the expression phase (from day 11 to day 21), animals remained untreated. On day 22, for topical challenge, animals of the treatment group received topical application of 0.5 mL of Diphotérine at the concentration determined by the MNIC during the preliminary study to the right flank region aver an 4 cm2 (2 cm x 2 cm)previously free of hair on a piece of absorbent gauze which remained in place for 24 hours using an Elastoplaste semi-occlusive dressing. Under the same conditions, negative control animals received 0.5 mL of Diphotérine 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 times 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 on the latter.The classification of the degree of sensitising capacity according to Magnusson and Kligman is based upon the percentage of animals showing a reaction, rather than the severity of the individual reaction (Table 4).

OBSERVATIONS	SCORE
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent Erythema	2
Intense erythema and swelling	3

Table 1: Grading system of skin reactions

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

Table 4: Degree of sensitising capacity

Study day	Phase	Administration route	Concentration of the substance % (v/v
D1	Primary induction	Intradermal injection	100
D9	Sensitization phase	Topical application	100
D22	Challenge	Topical application	100

Table 2:: Main study

Site	Negative controls	Positive controls	Test substance 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% DNCBand 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 Diphotérine

Table 3: Treatments of groups during intradermal induction on day 1

Results

Under the experimental conditions adopted, results obtained were as follows :

The preliminary test showed that the application of the test substance Diphotérine did not induce coloration of the application site. Grading of any skin lesions was therefore possible. No skin reaction was observed in each animal treated with cutaneous application of Diphotérine undiluted or diluted at 75v/v in sterile water. The maximum concentration causing a slight to moderate irritation determinated by intradermal administration was Diphotérine undiluted. It was the same concentration for the maximum concentration determinated for cutaneous application and determination of the maximum non-irritant concentration (MNIC). Then, For the main study, Diphotérine undiluted was used for the primary induction phase on D1, the second induction phase or sensitization on D9 and the challenge phase on D22. Animals were monitored daily throughout the study period and the behaviour of animals treated with Diphotérine was normal and was not different from that of the control group. Mean body weight gain in males and females treated with Diphotérine differed significantly from that of males or femùales of the negative group, at the thresholfd of respectively 1% and 5%. No irritation reaction was noted at times 24 and 48 hours in animals of the negative control group and in animals treated during the challenge phase with the test substance Diphotérine® at the Maximum Non-Irritant Concentration (M. N. I. C.) (Table 5). The test substance showed no allergenicity at 24 and 48 hours. Under the experimental conditions adopted, the test substance Diphotérine (batch D430611A*) showed no allergenicity of Class at 24 hours and 48 hours. According to the terminology employed, it is considered that the test substance Diphotérine® is free of sensitizing capacity in the Guinea pig.

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

Table 5 : Summary of results of cutaneous reactions

Conclusion

Under the experimental conditions adopted, the test substance Diphotérine showed no allergenicity of class at 24 hours and 48 hours. according to the terminology employed, it is considered that the test substance is free of sensitising capacity in the guinea pig. This result brings a new proof of the non sensitising capacity of Diphotérine already observed in industries.

References

1. Hall AH, Blomet J, Mathieu L Vet Hum Toxicology 2002, 44, 4, 228-231

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