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Review

Chemical burns: Diphoterine untangled

K. Skaria Alexander [a,*], Jason Wasiak [b], Heather Cleland [a]

[a] Victorian Adult Burns Service, The Alfred Hospital, Melbourne,Victoria, Australia
[b] Faculty of Medicine, Dentistry and Health Sciences, Melbourne School of Health Sciences, The University of Melbourne, Victoria, Australia

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ABSTRACT

Objective: Diphoterine is a hypertonic, amphoteric, polyvalent and chelating decontamination solution used in the treatment of cutaneous and ocular chemical burns. Due to infrequent use by emergency physicians along with the small number of available studies, its debate in the literature as to its efficacy and safety remains inconclusive.

Methods: A structured literature search was performed in MEDLINE, EMBASE BIOLOGICAL ABSTRACTS and TOXNET to June 2016 for original English-language studies reporting on the safety and effectiveness of Diphoterine. Methodological and reporting quality of pre-clinical animal studies was assessed using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias tool and Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. Clinical studies were assessed using Chambers' criteria.

Results: 13 studies (seven in the pre-clinical, five in the clinical setting and one mixed) met the study inclusion criteria.

Pre-clinical studies showed a faster resolution of pH and reduced tissue necrosis with Diphoterine. Clinical studies showed reduced tissue necrosis/severity of symptoms, faster pH resolution and a reduction in pain when using Diphoterine.

No adverse events were attributable to Diphoterine.

Reporting and methodology of the studies was poor or showed a high risk of bias.

Conclusions: Diphoterine appears to be safe to use and is probably superior to other rinsing solutions.

However, immediate decontamination is imperative and if Diphoterine is not available a different rinsing solution should be used.

The methodology of the published literature for Diphoterine is generally poor and future publications should use the frameworks given as templates.

Contents

* *Corresponding author.*
E-mail address: ksalexander@doctors.org.uk (K. S. Alexander).

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1. Introduction

Chemical burns represent a small but significant proportion of burn injuries, with the incidence varying from 3.4% in the United States [1] to 4.3% in Australia and New Zealand combined [2]. Similar to that of other trauma demographic and injury-specific populations, males were more prone to accidental exposure to a chemical spill (45.9% non work related, 45.2% work related).

Currently, there is debate regarding the ideal rinsing solution because there is a dearth of good evidence to recommend a particular treatment. As the incidence is low and there are a variety of chemicals (>25,000) that cause burns [3] with a range of severity of injury, it is impracticable to conduct a randomised controlled trial on humans [4]. The evidence base therefore relies upon in vitro and preclinical data which can then be progressed to human studies [4]. Whilst there are some genetic and physiological differences between humans and animals, which make translation of results difficult; the benefits of using preclinical trials include the ability to control potentially confounding factors, e.g. baseline differences in animals, burn mechanisms and specifics of treatments. Some authors rightly feel that the trials so far rely upon a change in pH as an outcome, but this does not accurately translate into the depth of tissue damage. Authors have also commented that as water is hypotonic to the cornea, there is a net influx of water into the anterior chamber, which may aid diffusion of the burning agent into the eye [5].

Diphoterine (Laboratoire Prevor, Valmondois, France) is an amphoteric, polyvalent, chelating, and slightly hypertonic decontamination solution, which can be used for either acid or alkali chemical splashes to the skin and eyes. Diphoterine has multiple binding sites, allowing to it to be effective with multiple chemicals, whilst not inducing a significant exothermic reaction [6]. It should be applied as soon as possible following the exposure for it to quickly eradicate the chemical, thereby reducing tissue necrosis. This can result in less morbidity for the patient, with fewer days in hospital, fewer operations and fewer days off work. Previous reviews have resulted in differing conclusions regarding the best rinsing solution [6–9], whilst other reviews have not concentrated only on Diphoterine [5,10–14].

It is necessary to perform a systematic review applying robust, validated frameworks to help evaluate all the evidence currently available on Diphoterine regarding both its safety and effectiveness at treating chemical burns, particularly in the pre-hospital or Emergency Department setting.

2. Methods

2.1. *Data sources and search strategy*

A structured literature search was performed in MEDLINE 1946-2016, EMBASE 1974-2016, Biological Abstracts 1980-2016, and the Toxicology Data Network from 1965 using a series of key words: ‘burns; chemical’; ‘eye burns’; ‘skin injuries’ and ‘diphoterine’. In addition; we searched abstract (Health and Safety Science Abstracts; National Institute for Occupational Safety and Health; Occupational Health and Safety Reference Collection) and drug company (www.prevor.com) websites to September 2016. Details of the MEDLINE search strategy are shown in Fig. 1.

1. exp burns, chemical
2. exp burns, chemical/drug therapy
3. burns.mp
4. exp eye burns
5. exp eye burns/chemically induced
6. exp eye burns/therapy
7. exp skin/drug effects
8. exp skin/injuries
9. or/1-9
10. diphoterine.mp
11. previn.mp
12. or/10-11
13. 9 and 12
14. Limit to English

Fig. 1 – OVID Medline 1946-2016.

2.2. Study inclusion and exclusion criteria

Pre-clinical (e.g. in vivo/ex vivo animal and in vitro) and clinical studies (e.g. randomized control trials, cohort or case series designs) that examined the safety and efficacy of Diphoterine application for chemical splashes to the skin and eyes were considered for inclusion in this review. Non-English-language studies, non peer-reviewed studies, narrative or systematic reviews, guidelines, commentaries were excluded.

2.3. Study selection process

All studies retrieved by the initial search underwent a scanned process by a single review author (SA) to exclude irrelevant studies then two authors (SA; JW) screened titles and abstracts against the inclusion criteria. Articles were retrieved and reviewed independently by two authors (SA; JW) to apply inclusion criteria. In all instances, differences of opinion were resolved by discussion among the authors.

2.4. Outcome measures

Primary outcome measures were depth of cutaneous burn and severity of ocular burn using the Roper Hall classification (Table 1). The negative primary outcome measure was any adverse effect from Diphoterine. Secondary outcome measures for in vitro and preclinical studies included pH change/buffering capacity and inflammatory response. Secondary outcome measures for clinical trials included pH change/buffering capacity, change in pain, number of days of hospitalisation, need for surgery and time off work.

2.5. Data extraction

Two reviewers (SA; JW) extracted data independently on (a) methods: study design, total duration of study, study location, study setting, withdrawals, and date of study; (b) participants: sample, mean age or age range, gender, severity of condition, diagnostic criteria if applicable, inclusion criteria, and exclusion criteria; (c) interventions: description of intervention, comparison, duration, intensity, content of both intervention and control condition, and co-interventions; (d) outcomes: description of primary and secondary outcomes specified and collected, and at which time points reported; (e) notes: funding for trial, and notable conflicts of interest of trial authors, country where trial was conducted.

2.6. Assessment of methodological quality (pre-clinical studies)

Two authors (SA; JW) assessed the risk of bias using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool [15]. Risk of bias was graded as either "high", "low" or "unclear". The SYRCLE tool is a modification of the Cochrane Risk of Bias tool [16] for randomised controlled trials. The adaptations have been made to the original tool to consider any further potential sources of bias that may be incurred when critiquing animal studies instead of RCTs.

2.7. Assessment of reporting quality (pre-clinical studies)

Two authors (SA; JW) evaluated the reporting quality of the included studies using the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines [18]. These guidelines consisted of a 20-item checklist describing the minimum information that all scientific publications should report when using animals for research. The guidelines were developed using the CONSORT statement as its foundation [19]. Each question was either scored out of 1 (0– clearly inaccurate, 1– clearly accurate) or 2 (0 – clearly inaccurate, 1 – possibly accurate, 2– clearly accurate). The maximum score attainable was 36 indicating the highest quality of reporting [20]. Reporting percentages for each question were calculated, and reporting for each question was rated as good (80–100%), average (50–79%) or poor (<50%).

2.8. Assessment of methodological quality (clinical studies)

Two authors (SA; JW) assessed the quality of non-randomized studies using the criteria described by Chambers et al. [17]. These are a series of eight questions assessing reporting

Table 1 – Roper hall classification for ocular surface burns.

Grade	Prognosis	Corneal alteration	Limbal ischemia (% limbal circumference)
1	Excellent	Epithelial alteration no corneal opacity	0
2	Good	Oedematous cornea but iris still visible	<33%
3	Reserved	Complete loss of corneal epithelium, stromal oedema disturbing visualization of details in iris	33–50%
4	Bad	Opaque cornea, iris and pupil not visible	>50%

Table 2 – Studies excluded from review.

Author	Reason for exclusion from review
Falcy (1993)	Optional questionnaire– poor methodology
Gerard (2000)	French language
Gerard (2001)	French language
Kuckelkorn (2002)	Review
Gerard (2002)	Case report
Hall (2002)	Review
Gerasimo (2003)	No peer review
Merle (2006)	Abstract of review
Hall (2006)	Review
Spoler (2007)	Not relevant
Lukey (2007)	Book chapter– no peer review
Merle (2008)	Review
Hall (2009)	Review
Coaquin (2010)	Poster– no peer review
Mathieu (2010)	Poster– no peer review
Fosse (2011)	Poster– no peer review
Schrage (2011)	Opinion
Mathieu (2011)	Poster– no peer review
Chau (2012)	Systematic review
Fosse (2012)	Poster– no peer review
Mbabisa (2013)	Thesis– no peer review
Kalson (2013)	Letter
Brent (2013)	Review
Scott (2015)	Opinion
Greenwood (2016)	Review
Lynn (2016)	Systematic review

quality and risk of bias (see Table 4). In this instance, a case series was considered: (a) good if it met all the criteria; (b) satisfactory if it fulfilled criteria 2, 4, 5, 6 and 7; or (c) or poor.

3. Results

The initial search strategy identified 39 studies. Independent scrutiny of the titles and abstracts identified 28 potentially relevant studies for full-text review. Of those, 15 articles were excluded for the following reasons: narrative or systematic reviews (n=10) etc. One article only had the abstract available, however this was from a review which therefore would have not met the inclusion criteria. Excluded studies with reasons are given in Table 2. Therefore, a total of 13 studies formed the basis of this review (see Fig. 2).

The general study characteristics of the included studies are summarized in Table 3. The earliest publications were in 2002 [21,22]. In particular, we identified seven pre-clinical studies, five clinical studies and one that had both preclinical and clinical arms. Most first authors resided in Germany (n=4, 31% [21–24]) or France (n=4, 31% [25–28]), followed by Italy (n=2, 15% [29,30]). The remaining 3 studies originated from Israel [31], Australia [32] and UK [33] (each n=1, 8%). Three of the preclinical studies had an in vitro arm [21,23,26], which involved investigating the capacity of different rinsing solutions.

3.1. *Preclinical studies*

There were 8 studies, 7 from Europe (3 from Germany, 2 from Italy and 2 from France), with the remaining study from Israel. Of the eight studies, the German studies investigated ocular burns (2 rabbit models and 1 pig model); the Israeli study investigated ocular burns in a rabbit model; both Italian studies were from the same laboratory investigating the physiological response to cutaneous burns in a rat model; the two French studies examined guinea pigs' skin tolerance to Diphoterine and cell viability after rinsing with different solutions using an Episkin (reconstituted human epidermis) model.

3.2. *Clinical studies*

In total there were 416 patients enrolled in 6 studies over 4 countries, with the majority (n=5) coming from Europe (2 each from France and Germany and 1 from UK), the remaining study was from Australia. Both Germany and France had one study investigating ocular burns only and one for both cutaneous and ocular burns. Both the Australian and UK studies reported cutaneous burns only.

3.3. *Study intervention*

One study [21] that stated benalkonium chloride was used as a preservative in both the Diphoterine and phosphate buffer solutions tested. Information from Prevor however does not state that Diphoterine contains this preservative.

All the studies used Diphoterine at full concentration apart from one [25] which tested different strengths in a skin sensitisation study.

The Diphoterine was applied at a variety of rates: 100 ml/min in 2 studies [21,22] 66 ml/min in 1 study [23] and roughly at a rate of 50 ml/min by one further study [30]. No rate was mentioned by the others [24,27,28,31,32,34]. There was no mention of washing the Diphoterine off and so it can be assumed that the Diphoterine was left on the surface. One study described an initial Diphoterine wash by the patient and a second wash performed by the onsite infirmary [24].

3.4. *Primary outcomes*

3.4.1. *Depth of cutaneous burn– 4 studies reported this outcome measure*

Fosse et al.'s study using Episkin (reconstituted human epidermis) exposed to 1 ml 25% tetramethylammonium hydroxide (TMAH) showed that Diphoterine when compared to water had 10.9% better cell viability after 10 s of TMAH exposure and 32.7% better after 30 s [26].

Cavallini et al. induced 52% HCl burns on the backs of Sprague-Dawley rats before washing with different solutions. He demonstrated after seven days that Diphoterine had the best wound healing, followed by saline, then calcium gluconate and no treatment was the worst [29].

Donoghue's cohort study of 180 alumina plant workers who, following alkali skin exposure, had initial washes with either Diphoterine or water [32]. Although Donoghue's study did not randomise patients, they showed more patients with no cutaneous damage when using immediate Diphoterine irrigation compared with water (52.9 vs 21.4%).

Viala et al.'s non randomised study examined 5 gendarmes who were exposed to CS gas in an exposure chamber [28]. One

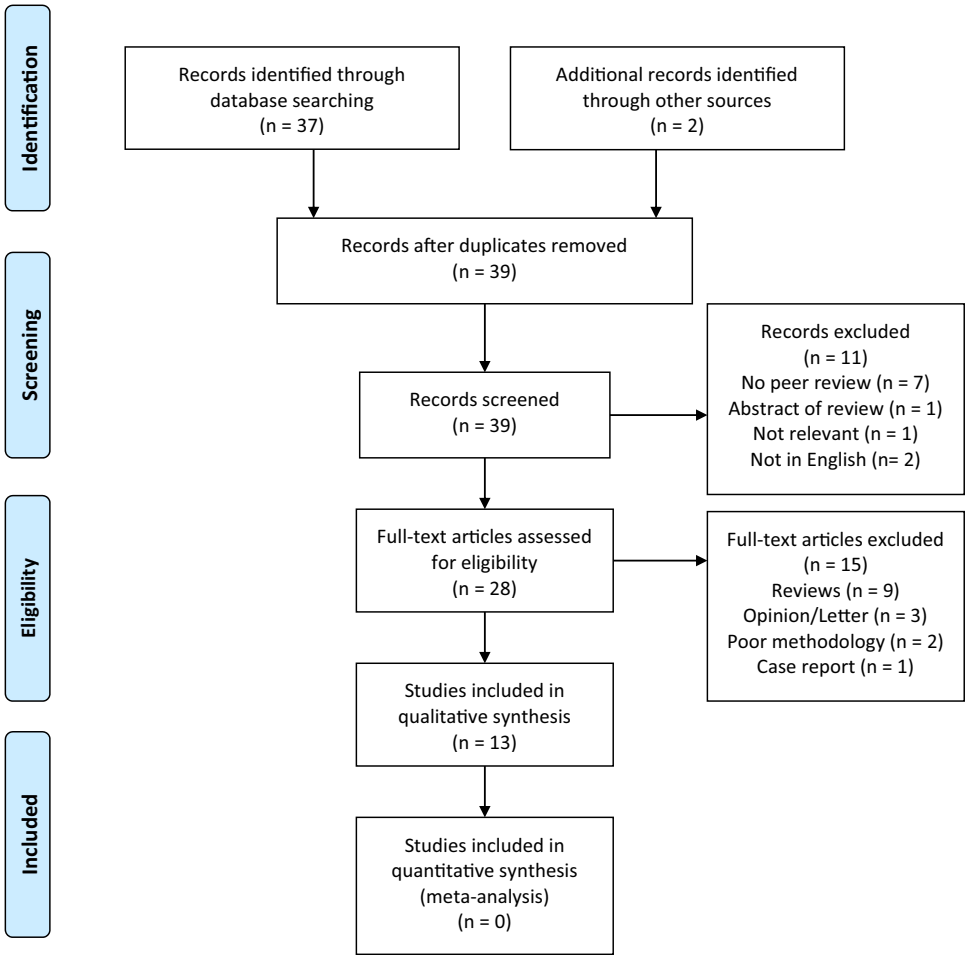

Fig. 2 – PRISMA flowchart.

gendarme used the Diphoterine before exposure and four gendarmes used Diphoterine after exposure. They showed no progression of cutaneous changes and a return to normal status within 7 min when using post-exposure Diphoterine. There were no cutaneous symptoms with pre-exposure Diphoterine.

3.4.2. *Severity of ocular burn – 5 studies*

Kompa et al. compared the osmolarity of Diphoterine to 0.9% NaCl, Ringer's lactate, balanced salt solution, phosphate buffer and tap water, and found Diphoterine was the only hyperosmolar solution as compared with normal ex vivo porcine corneas [21]. This in turn led to a reduction in corneal swelling as compared with other solutions.

Rihawi et al. burned rabbit eyes with sodium hydroxide [23]. He found that Diphoterine was the best of the rinsing solutions at reducing intracameral pH. Cederroth eye wash was the next best, whereas water and saline had little effect.

Schrage et al. burnt rabbit eyes with sodium hydroxide before decontaminating with either Diphoterine or saline [22]. He found the pHs of both the surface of the cornea and the anterior chamber returned better with Diphoterine. He showed that there was less opacification of both the iris stroma and lens. However, there was no histological evidence of improvement in healing on day 16 – this was an expected finding for a severe burn.

Goldich et al. found in his rabbit eye, nitrogen mustard burn model that rinsing with Diphoterine had less corneal damage and less increase in intraocular pressure [31].

Merle et al. showed a higher proportion of patients with Grade 1 injuries following Diphoterine use (62.5 vs. 35.4%) and less with Grade 4 injuries (0% vs. 16.7%) [27].

Viala et al. showed that pre exposure Diphoterine prevented ocular symptoms of CS gas (excessive lacrimation, eye irritation, and reflex blepharospasm) and immediate post-exposure irrigation with Diphoterine caused a cessation of its effects within 4 min [28].

3.4.3. *Safety of Diphoterine – 3 studies*

Mathieu's et al. skin sensitisation study in guinea pigs showed no changes with intradermal Diphoterine injections [25].

One arm of Kompa et al.'s study involved washing one eye of normal volunteers with either Diphoterine or phosphate solution [21]. 2 weeks later the other solution was used to wash the eye. This study was the only report that noted any irritation changes when using Diphoterine – mild burning 2/10 patients, and stipples in 1/10. However, this was less prevalent than in

Table 3 – Data extraction from included studies.

Author	Location	Methods	Study group	Burn agent	Skin/eye	Interventions	Measurements	Outcomes	Notes
PRE-CLINICAL									
Cavallini (2004)	Milan, Italy	Controlled trial	20 male Sprague-Daley rats – 250–300 mg, 4 per group	52%HCl 0.5 ml for 15 s onto back skin	Skin	30 s wash with diphoterine. Controls – no treatment, Ca gluconate, 0.9% NaCl	Blood measurements of IL6 and TNFα taken at 6 h, 72 h and 7 days	IL6 and TNFα significantly reduced with diphoterine at all timepoints	Later became medical advisor for Prevor
Cavallini (2004a)	Milan, Italy	Controlled trial	20 male Sprague-Daley rats – approx 250 mg, 4 per group	52%HCl 0.5 ml for 15 s onto back skin	Skin	30 s wash with diphoterine. Controls – no treatment, Ca gluconate, 0.9% NaCl. All washed at 50 ml/min	Blood measurements of substance P and β endorphin taken at 6 h, 72 h and 7 day	Substance P significantly lower at 6 h and 72 h, β endorphin significantly higher in diphoterine at 7 days. Wound healing at 7 days – diphoterine > NaCl > Ca gluconate > no treatment	Later became medical advisor for Prevor
Goldich (2013)	Tel Aviv, Israel	Controlled trial	16 albino rabbits – 250–350 mg	Ocular mustard injury 2%wt/vol to right eye for 5 min via a 6 mm vacuum trephine	Eye	500 ml diphoterine. Control – 500 ml 0.9% NaCl. 8 rabbits per group. Cointervention – chloramphenicol eye drops nightly for 22 days until euthenised	Slit lamp exam and intraocular pressure (days 1, 3, 7, 12, 18, 22) Ascorbic acid (days 1, 7, 22) – marker for systemic oxidative stress Histological examination (day 22)	Diphoterine more effective than immediate irrigation with saline in reducing corneal injury, intraocular pressure elevation and systemic oxidative stress	
Fosse (2010)	Valmondois, France	1. Controlled trial	Beaker experiment	25% tetramethylammonium hydroxide (TMAH)		1. Water and diphoterine titrated into TMAH pH measured by electrode	1. pH	1. 17 times volume of water needed to reach physiologically acceptable pH as compared with diphoterine	Conducted in Laboratoire Prevor
		2. Controlled trial	Episkin (3D human skin model)	25% tetramethylammonium hydroxide (TMAH)	Skin	2. Episkin exposed to 60 μl of 25% TMAH for 10 or 30 s. Then washed with 150 μl of diphoterine or tap water. Repeated washing 20 times for total 7 min 30 s. Episkin then placed in incubator and MTT assay	2. Cell viability	2. 10.9% better cell viability after 10 s TMAH exposure and 32.7% better after 30 s	

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Table 3 (*continued*)

Author	Location	Methods	Study group	Burn agent	Skin/eye	Interventions	Measurements	Outcomes	Notes
						performed for cell viability			
Mathieu (2007)	Valmondois, France	1. Trial	12 guinea pigs m:f 6:6 with backs shaved		Skin	Diphoterine applied onto guinea pigs backs at 5, 10, 25, 50, 75 and 100% concentrations onto gauze held by elastoplast	Any cutaneous reaction– graded 0-3	No guinea pigs showed any cutaneous reaction to diphoterine or water. However all bad reaction to DNCB	Conducted in Laboratoire Prevor
		2. Controlled trial	30 guinea pigs m:f 15:15 with backs shaved		Skin	20 animals in diphoterine group, 10 animals in control groups, had 6 injections of 0.1 ml diphoterine into skin of back. Negative control– sterile water, positive control DNCB			
Rihawi (2005)	Aachen, Germany	1. Controlled trial	Beaker experiment	5 ml 0.5N NaOH		Titrated with tap water, 0.9% NaCl, phosphate buffer solution, cederroth eyewash solution, Previn and diphoterine	pH	No difference between tap water, saline solution, phosphate buffer and Ringer's lactate. Previn and cederroth eye wash both had strong buffer effects	Study sponsored and materials supplied by Cederroth +Prevor. Senior author is Schrage
		2. Controlled trial	35 ex vivo rabbit eyes immediately taken after death in slaughterhouse (5 eyes per group)	25 μl of 2N NaOH on 10 mm diameter filter paper for 20 s onto one eye	Eye	Controls – tap water, PBS, 0.9% NaCl, cederroth eye wash, no wash. 2 types of diphoterine used – German (Previn) and international versions. All solutions irrigated at 66 ml/min for 15 min	Intracameral pH continually monitored	None of the rinsing solutions returned intracameral pH to its normal level, i.e., 7.4. Previn– 8.6, Diphoterine– 8.4, Cederroth Eye Wash Solution– 9.1, water – 10.7, isotonic physiological saline solution– 11.5	
Schrage (2002)	Aachen, Germany	1. Double blinded RCT	4 rabbits	3 ml 1N NaOH for 30 secs via plexiglas cylinder onto one eye	Eye	1. Washed with 500 ml of either diphoterine or NaCl at 100 ml/min for 5 min	Surface pH and aqueous humour (via aspiration of 0.1 ml via 20 gauge needle) pH	Surface pH increased after burn to 14 in both groups and was lowered by 0.9% saline rinsing to 12, and by Diphoterine® to 7.5.	No financial interest in Diphoterine Previn supplied products Author later became medical advisor for Prevor

Table 3 (*continued*)

Author	Location	Methods	Study group	Burn agent	Skin/eye	Interventions	Measurements	Outcomes	Notes
								Aqueous humor was initally pH 10 returned to 11 with saline and to 9 with Diphoterine	
		2. Double blinded RCT	16 rabbits (8 per group)	3ml 1N NaOH for 30 secs via plexiglas cylinder onto one eye	Eye	2. As above but continued with TDS NaCl washes with 160ml NaCl for 16 days	Daily examination and photographs day 16 histological examination	Less opacification of iris stroma and lens with diphoterine but no difference in healing as would be expected from a severe burn	
CLINICAL Donoghue (2010)	Perth, Australia	Prospective case series over 17 months	180 alumina workers	Cutaneous alkali burns, TBSA 0.1-38%	Skin	Diphoterine in a 100ml spray can carried by alumina workers on their work belt. Used after injury and compared with workers who used water first instead. If 100ml was insufficient to cover area then they sought extra cans form colleagues. All injuries were then assessed at the on-site medical facility	Severity of burn Time off work	52.9% of the patients treated with Diphoterine initially showed no evidence of cutaneous injury compared with 21.4% in those treated with water first. 7.9% in diphoterine group had blisters or worse, cf 23.8% in water group There was no significant change in days lost at work as one day had been lost by the water first group	No affiliation with Prevor
Merle (2005)	Martinique, French West indies	Prospective case series over 4 years	66 patients (30 first group, 36s group), age 38.2±14.8, male: female 45:21,	All ocular alkali burns presenting to the ED	Eye	First group had 500ml physiological solution, second group had 500ml diphoterine. Grade III/IV injuries admitted for rifamycin, 2% ascorbic acid, dexamethasone/ neomycin drops 6/ day, oral ascorbic	Post intervention eye examination, time to reepithelialisation and final visual acuity	Diphoterine group had less severe burns with Grade I/II 91.1% vs 68.7%, shorter reepithelialisation times (Grade I 1.9 vs 11.1days, Grade II 5.6 vs 10), better final visual acuity and less corneal opacification Although Grade III	Later became medical advisor for Prevor

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Table 3 (*continued*)

Author	Location	Methods	Study group	Burn agent	Skin/eye	Interventions	Measurements	Outcomes	Notes
						acid TDS and anit-symblepharon ring		burns had shorter reepithelialisation times with diphoterine (20.0 vs 45.2), this was not significant. There were no Grade IV burns in the diphoterine group	
Nehles (2006)	Remscheid, Germany	Retrospective case series over 60 months (1994–98)	24 male workers, age 21–62	Chemical burns to skin and eye in metallurgy facility	Mixed	Immediate decontamination with diphoterine by worker and then second wash by onsite infirmary. No controls used	Need for further medical or surgical treatment Days off work	None required any further treatment. 3 workers had loss of 1 day at work for observation only in hospital No adverse effects from diphoterine No sequelae	Funding for translation of data and preparation for presentation was from Prevor, 2 authors from Prevor
Viala (2005)	Versailles, France	Trial, not randomised, French gendarme training facility	5 gendarmes	3000 mg/m^3 CS gas in chamber	Mixed	4 had post exposure decontamination with diphoterine (2 administered by another person, 2 self administered), 1 had pre exposure decontamination. Gendarmes could choose which group they were in. Diphoterine came in a 250 ml low pressure spray can. No controls used	Self reporting and observation of ocular and facial skin symptoms	Gendarmes with post exposure diphoterine resolved symptoms in 7 min. Preexposure gendarme had no skin or eye changes. Coughing only as airway not covered by diphoterine	2 authors from Prevor. Diphoterine provided by Prevor
Zack-Williams (2015)	Birmingham, UK	Retrospective case series, 32 months (Jan 2010– Sep 2012)	131 patients with pH change and/or pain; m:f 104:26	All cutaneous chemical burns presenting to burns centre	Skin	Irrigated with diphoterine if within 24 h of burn, or water only if after 24 h	Times to surgery, heal and discharge. Length of stay, pH change	Better change in pH with diphoterine but no significant change in time to healing or need for surgery	
MIXED Kompa (2002)	Aachen, Germany	1. Controlled trial	Beaker experiment	0.1N HCl and 0.1N NaOH		Titration of tap water, 0.9% NaCl, Ringer's lactate, balanced salt solution, phosphate	pH	Only Previn and phosphate had a very high buffer capacity. All others had low or no buffer capacity	Senior author is Schrage

Table 3 (*continued*)

Author	Location	Methods	Study group	Burn agent	Skin/eye	Interventions	Measurements	Outcomes	Notes
						buffer and Previn in 100 μl graduations			
		2. Controlled trial	200 healthy ex vivo porcine eyes (100 in each group)	4N NaOH for 60 s onto each eye	Eye	Corneas fixed lyophilised, ground and rehydrated to determine osmolarity. Compared with Previn, tap water, NaCl 0.9%, Ringer lactate, balanced salt solution (BSS; Aqsia), phosphate buffer	Osmolarity	Previn only hyperosmolar solution compared with healthy corneas, but still hypoosmolar to burned cornea. All other solutions were hypo/isoosmolar to healthy corneas	
		3. Controlled trial	56 healthy ex vivo porcine eyes (8 in each group)	4N NaOH for 60 s onto each eye	Eye	burned corneas washed for 5 min with either 0.5 l tap water or NaCl at 400, 800 and 1200 mosmol/l, phosphate buffer, previn, 0.9% NaCl for 5 min	Corneal swelling	Corneal swelling inversely proportional to osmolarity	
		4. Double blinded controlled trial	10 healthy volunteers age 27 ±5 years		Eye	0.5 l or either Previn of phosphate buffer in one eye. 2/52 later contralateral eye was washed with other solution. Both solutions were mixed with the preservative benalkonium chloride	Before, immediately after, and 3 days after rinsing– sight test, slit lamp examination, pachymetric measurements, and confocal microscopy, subjective sensations	Stipples occurred in one case using diphoterine as opposed to 7 using phosphate. Complete restoration of prewash status in 3 days with Previn. Burning sensation in 2/10 Previn but 8/10 phosphate. Both negative findings probably as a result of benalkonium chloride	

Table 4 – Assessment of clinical study quality using Chambers criteria.

Author	Selection eligibility criteria reported	Selected population representative of normal practice	Variability reported	Loss to follow up reported or explained	At least 90% of those included at baseline followed up	Prospective recruitment	Consecutive recruitment	Relevant prognostic factors reported	Rating
Donoghue (2010)	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Poor
Kompa (2002)	Yes	Yes	No	No	No	Yes	Yes	Yes	Poor
Merle (2005)	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Poor
Nehles (2006)	Yes	Yes	No	No	No	No	Yes	Yes	Poor
Viala (2005)	Yes	No	No	No	No	Yes	Yes	Yes	Poor
Zack-Williams (2015)	Yes	Yes	No	No	No	No	Yes	Yes	Poor

Rating: (a) good if meets all the criteria; (b) satisfactory if it fulfilled criteria 2, 4, 5, 6 and 7; or (c) or poor.

the phosphate control group and was thought to be secondary to the benalkonium chloride preservative used.

Nehles et al. found no irritant or adverse events attributed to Diphoterine [24].

3.5. *Secondary outcomes*

3.5.1. *Buffering capacity – 4 studies*

All preclinical studies showed Diphoterine to have a greater buffering capacity when compared with other rinsing agents. Kompa et al.'s study found phosphate was the only other to have a strong buffering effect [21].

Rihawi et al.'s study found Cederroth (eyewash solution of borate and 0.9% NaCl) to be a strong buffer but phosphate was weak [23].

Fosse et al.'s study showed that for an amount of Diphoterine, seventeen times the volume of water was required to bring the pH to physiologically acceptable level when added to a strong base [26].

Zack-Williams' et al. retrospective study [33] of all cutaneous burns presenting to their burn centre showed a significantly greater change of pH was attained with Diphoterine when compared to water (1.076 vs. 0.4). However, the Diphoterine group had a higher starting pH than the water group (8.07 vs. 7.77), and although this difference was not significant, a greater potential for change in pH was therefore possible.

3.5.2. *Serum biochemical changes and pain – 3 studies*

Cavallini demonstrated lowered IL6 and TNFα at all time points (6 h, 72 h and 7 days) following irrigating rats with Diphoterine as compared with the controls [30]. This indicated a reduction in the systemic inflammatory response with Diphoterine. In another study using the same model of burn injury, Cavallini et al. noted that substance P was lowered at 6 h and 72 h post irrigation [29]. Substance P is a marker of inflammation and pain. In this study Cavallini et al. also reported raised β-endorphin levels at 7 days after treating rats

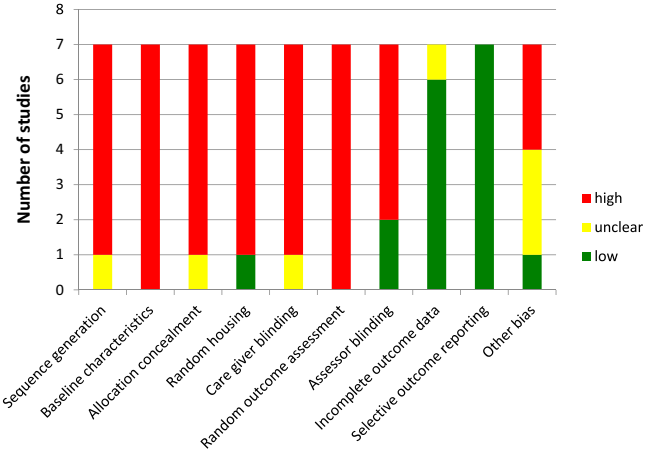

Fig. 3 – Risk of bias for preclinical studies using SYRCLE RoB tool.

Table 5 – Assessment of preclinical study quality using ARRIVE framework title (1), abstract (2), introduction-background (3), introduction-objectives (4), methods-ethical statement (5), study design (6), experimental procedure (7), Experimental animals (8), housing and husbandry (9), sample size (10), allocation (11), experimental outcomes (12), statistics (13), results-baseline data (14), number analyzed (15), outcome and estimation (16), adverse events (17), discussion-interpretation/scientific implications (18), general applicability/relevance (19) and funding (20).

Author	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total/36	%
Cavallini (2004)	1	1	2	1	2	0	1	1	0	0	0	2	2	0	0	0	0	1	1	0	15	42
Cavallini (2004a)	1	2	2	1	2	2	2	1	1	0	1	2	2	0	1	0	0	1	1	0	22	61
Goldrich (2013)	1	2	2	0	2	2	2	1	0	0	1	1	2	0	1	1	0	1	1	0	20	56
Kompa (2002)	1	1	2	0	0	0	1	0	0	0	0	2	0	0	1	1	0	1	1	0	11	31
Mathieu (2007)	1	1	2	1	2	1	2	1	0	0	0	2	0	1	1	0	0	1	2	1	19	53
Rihawi (2005)	1	1	2	0	0	0	2	0	0	0	0	2	0	0	1	1	0	1	2	2	15	42
Schrage (2002)	1	1	2	2	2	1	2	0	0	0	0	2	2	0	1	1	0	1	1	2	21	58
Total score	7	9	14	5	10	6	12	4	1	0	2	13	8	1	6	4	0	7	9	5	124	49
Max score	7	14	14	7	14	14	14	14	14	14	7	14	14	7	14	14	14	14	14	14	252	
%	100	64	100	71	71	43	86	29	7	7	29	93	57	14	43	29	0	50	64	36		

with cutaneous burns with Diphoterine as compared with the controls. This indicated a potential reduction in pain with Diphoterine.

In Viala et al.'s study [28] the gendarmes using the Diphoterine immediately after CS gas exposure showed a rapid reduction in pain. Whereas the gendarme who used the Diphoterine before CS gas exposure experienced no pain.

3.5.3. *Hospitalisation/need for surgery – 3 studies*

In Nehles' et al. non-controlled retrospective case series [24] of all workers suffering chemical burn injuries in a metallurgy facility, 3 of the 24 workers required observation only overnight in hospital after treatment with Diphoterine. Each of these workers required 1-day loss of work.

Zack-Williams' et al. series did not show any difference in length of hospitalisation or need for skin grafting [33].

The gendarmes in Viala et al.'s study were fit to return to work within 10 min of the Diphoterine use [28].

3.6. *Assessment of methodological quality of pre-clinical studies (SYRCLE tool)*

The results of the quality assessment of the 7 studies that used animal models are shown in Fig. 3. Poor reporting of essential methodological details led to an unclear risk of bias in the majority of assessments, except for incomplete outcome data and selective outcome reporting, which were most likely as a consequence of small sample sizes and short experiment durations. Only single studies reported on randomization [30] or random housing [30]. whilst only 4 studies [25,29–31] reported on the included general animal characteristics (e.g. age, sex, weight and strain), none reported on all of these variables. Only 2 studies [23,26] had some mention of the baseline health characteristics of the animals. For the ex vivo experiments, there were no details on baseline characteristics or husbandry, which only confounded the overall unclear risk of bias amongst the studies. In addition, only one study [31] was found where none of the study authors had some formal collaborative link with the manufacturer of Diphoterine at the time of print or at a later date.

3.7. *Assessment of reporting quality (ARRIVE guidelines)*

The percentage of items reported for the ARRIVE guidelines is listed in Table 5. Whilst the mean overall reporting quality was 17.57 (SD = 3.9), the lowest score was 11 [21] and the highest score was 22 [30]. Of the 20 items listed for the ARRIVE guidelines, only 4 items had reporting graded as good (80-100%). These items considered title (Q1), introduction and background (Q3), experimental procedure (Q7) and experimental outcomes (Q12). 6 items were reported averagely (50-79%), with most studies supporting an abstract (Q2), objectives (Q4), ethical statements (Q5), statistics (Q13), interpretation/scientific implications (Q18), general applicability/relevance (Q19). Poor reporting (<50%) was noted for 8 questions: study design (Q6), description of the experimental animals (Q8), housing and husbandry (Q9), sample size (Q10), allocation to groups of animals (Q11), baseline health status of animals (Q14), numbers analysed (Q15), outcomes and estimation, e.g. standard deviations (Q16), funding (Q20). No authors reported on sample size calculation (Q10) and adverse events (Q17).

3.8. *Assessment of methodological quality of clinical studies (Chambers criteria)*

The results of the quality assessment of the 6 clinical studies are shown in Table 4. All 6 studies adopted a case series design and evaluated as poor, with at least two studies recruiting patients using a retrospective design [24,33]. None of the studies included follow up in their study design. If these two questions were to be discounted, two studies would have been rated as good [27,32], and one would have been rated as satisfactory [21].

4. Discussion

Although we found a total of 13 studies (7 pre-clinical studies, 5 clinical studies and 1 mixed, involving 416 patients), we were unable to perform a meta-analysis because of the significant clinical heterogeneity amongst the studies. We observed from case series data that early application of Diphoterine had the

ability to prevent tissue damage to both skin and eye when compared with other rinsing agents. However, if severe ocular damage was already present (e.g. Grade 3/4), Diphoterine did not lead to better healing outcomes. Irrespective of these findings, we were cognizant of the poor methodological quality and reporting of these studies underpinning the evidence. In particular, we found that although studies were generally able to describe why they were doing experiments, they lacked precision in reporting their methodology and the study findings. Nevertheless, we chose to include them because the reported study outcomes were of interest to the practicing clinician. None of the clinical studies included follow up, and whilst it is probable that Diphoterine's favourable outcomes in the short term lend to good long-term outcomes, no data is provided on scarring, functional problems, etc. to make that assessment. The lack of high quality evidence was suggestive of an 'evidence and reporting gap', which may have been ameliorated if higher methodological design approaches (e.g. quasi-randomised or cohort studies) and reporting was adhered to by the individual authors. No harm was caused by the Diphoterine.

This present study is believed to be the first of its kind to evaluate the methodological and reporting quality of pre-clinical and clinical studies using a variety of validated instruments such as SYRCLE and ARRIVE. Thereby owing to the absence of any known reported studies, direct comparisons from other systematic reviews within the field of burns could be drawn upon, but not directly compared. In the first three reviews [6–8] most of the included original studies were unpublished, written in a language other than English and associated with the manufacturer making it difficult to draw any firm conclusions about the applicability of their findings to our present study. Whilst having connections with the manufacturer may not have any bearing on the outcome of research, a recent meta-analysis found that industry sponsored studies were less likely to report negative or harmful effects [35]. In other reviews, such as Lynn et al. [36] and Brent [9], we found an inconsistency in reporting trial data. For example, Lynn et al. observed an earlier study by Zack-Williams et al. [33] that a better change in pH would theoretically lead to a faster healing time. However, Zack-Williams et al. reported no difference in healing time. In the second example, we noted that when Brent critiqued the study by Donoghue [32], he reported that there was a significant difference in time to decontamination and suggested that patients with larger burns would have self-selected water as the decontamination solution as they were more familiar with it. However, a response by Donoghue in 2014 [37] stated that both of these criticisms were misinterpretations of their data. Specifically, that it was the time to first Diphoterine use that was different– the time to first wash with either Diphoterine or water was similar, and that there was no statistical difference in TBSAs between the two groups. These variations would suggest that our ability to compare liked reviews was not only impossible, but potentially misleading for clinicians.

This study ability to evaluate the methodological and reporting quality of pre-clinical and clinical studies using a variety of validated instruments such as SYRCLE and ARRIVE was considered a key strength. Information gleaned from these tools would allow the clinician to accurately assess the methods and the results in a clinically meaningful way. Secondly, SYRCLE and ARRIVE were recommended by the National Centre for the Replacement, Refinement & Reduction of Animals in Research when assessing methodological and reporting qualities [38].

However, when using these tools, we were presented with a number of limitations. Firstly, we were cognizant that the various animal models included in this review did not match the full clinical situation, therefore it was difficult to assess the relevance of the result findings. Secondly, the methodological details in many of the animal studies were poorly reported. This in turn hampered our ability to undertake a thorough risk of bias assessment and make firm conclusions from the included animal studies. Poorly reported studies were included in this review because papers that did not report on essential details may not have necessarily been methodologically impaired as per the SYRCLE tool. Furthermore, most of studies within this review were of a single-centre case series design, which sat low within the evidence hierarchy, and were considered to be hypothesis generating rather than engaged in the process of evidence generation. Given the lack of studies with comparator arms, the true effect of the intervention could not be precisely determined.

Limitations also existed within the search strategy in that it was restricted to electronic databases that may not have been representative of all indexed studies. Other medical databases, national clearinghouse and guideline sites, conference proceedings, national registries, and non-published studies were not systematically searched. Only studies written in English were included which would have excluded some of the original European studies. However, on review of one paper by Gerard [39] where the abstract had been translated into English, the results are similar to those obtained in other papers and no new information was gleaned.

The best outcomes are delivered by prompt decontamination of the chemical. As there is an absence of evidence to show that Diphoterine has adverse effects when compared with other products and that it probably is therapeutically superior, we proffer that if Diphoterine is freely available early after the injury it should be used. There is no strong evidence to say what is defined as early, but Zack-Williams et al. [33] showed that up to 24h may still have some benefit. However, if Diphoterine is not available, then the burn should be immediately irrigated with either water or saline. We concur with other authors that Diphoterine should be available for use in emergency departments for the treatment of complex chemical injuries under the guidance of plastic and burn surgeons [40]. We would suggest that workplaces that use toxic chemicals or mange toxic chemical injuries have Diphoterine available.

Since our treatment practice recommendations were driven by limited evidence, there is an urgent need for more consistent and improved methodological and reporting quality regarding pre-clinical and clinical studies by researchers and journal editorial processes.

The results of our study demonstrate that it is important to exercise caution in translating the results of the pre-clinical and clinical studies, however, Diphoterine appears to be a good treatment option for cutaneous and ocular chemical burns. Future publications would be advised to adhere to the given

methodological and reporting guidelines to improve the validity of their research.

Conflicts of interest

The authors have no affiliation to Prevor or its distributors and have no conflicts of interest to declare. We confirm there are no financial and personal relationships with other people or organisations that could inappropriately influence (bias) our work.

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