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Use of an amphoteric lavage solution for emergency treatment of eye burns First animal type experimental clinical considerations

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Abstract

Purpose: Severe eye burns occur rarely, but are related to a poor prognosis in rehabilitation. As emergency treatment has been identified as decisive factor for the prognosis of eye burns, new first aid rinsing solutions should be considered carefully in their clinical action. In a first approach, the new drug Diphoterine® was subjected to a comparison with saline solution to evaluate the effects in a model of severe eye burns.

Methods: In a double-masked experiment 16 rabbits underwent a severe eye burn of one cornea followed by immediate rinsing with 0.9% sodium–chlorine solution ($n = 8$) or Diphoterine® ($n = 8$). During 16 days after burn, an irrigation therapy with 0.9% saline solution three times daily 160 ml was applied in both groups following the recommendation of prolonged irrigation therapy performed in our clinic. In a similar setup, 16 eyes were subjected alkali burns with measurements of aqueous humor pH within 30 s after burn and after a period of 5 min rinsing with 500 ml saline 0.9% or Diphoterine®, respectively.

Results: The result of the severe eye burn with an opaque cornea was similar in both groups. During rinsing no fibrin precipitates occurred in the Diphoterine® rinsed group whereas this was detectable in all eyes rinsed with saline solution. After 16 days there was no difference between both groups indicating no harmful effect of Diphoterine® as emergency treatment compared to saline 0.9%. After 30 s of burn with 1N NaOH and rinsing with 500 ml of the specified solutions the anterior chamber pH was 10 ± 0 in the saline group and 9.35 ± 0.3 in the Diphoterine® group showing efficacy of the buffering capacity of Diphoterine®.

Conclusion: Diphoterine® proves to be efficient in the primary treatment of burns. The anterior chamber pH could be lowered by 5 min of rinsing. No harmful effects of Diphoterine® could be observed compared to rinsing with saline solution in the course of an severe alkali burn of the cornea.

Keywords: Diphoterine®; Adverse effects; Cornea; Burns; Emergency; Amphotere

1. Introduction

Many solutions for emergency treatment of eye burns are recommended and been used without verifying their innocuous effect. This leads to some established first aid systems derived from phosphate buffer in combinations with saline solution, for example Eyesaline®, Phosphate buffer (Isogutt®) and Tima-oculav®. Other rinsing fluids have no buffering effect using solutions like tap water showers, Isogutt-akut® and saline 0.9% solution. There is no doubt, that fastest possible emergency rinsing is the best treatment for any kind of burn, but there are differences in efficacy of treatments [1]. And if possibility of choice is given, e.g. replacing the agents in emergency eye showers used before, the most efficient and innocuous substance should be chosen.

This story was planned to investigate the effects of different types of rinsing solutions in a severe eye burns in an exponential situation. Two different endpoints are relevant: efficacy of rinsing, roughly estimated by means of intraocular pH measurements after burn and detection of side effects identified after a long-term treatment according to the limitations of the products indications. Three different types of solutions are on the market. Type 1: saline and tap water as non-buffering substances, Type 2: phosphate-, acetate- and borate-buffers with agent-dependent limitations in the alkali or acid burn treatment and Type 3: amphoteres with special binding characteristics by

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means of divalent acid and alkaline binding sites like Ethylene-diamine-tetraacetate (EDTA) or Diphoterine®. The essential concept of acute washout of the burning substance is represented in all three types of applications. The concept of buffering in the second group is related to a weak acid–buffer or alkali–buffer combined with an exothermic reaction producing "heat" and leaving remnants of salts. Type 3 group amphoteres like ethylen–diamin–tetraacetate or highly modified derivatives of this like Diphoterine® act in a different way without exothermic reaction by capture of ions and neutralizing by means of amphophilic reaction. This difference makes them interesting in therapy of burns especially because of a polyvalent action towards alkalis and acids without exothermic reaction. Their capability of binding in a wider range of pH depends on two different dissociation constants in water defined by the chemical pK_a or pK_b meaning the base or acidic dissociation. The connections between both is the total dissociation of water with 14. Thus, $pK_a + pK_b$ in watery solution always equals 14. The dissociation constant is the decisive factor in thermodynamic development of chemical reactions. Therefore, substances like aminic acids as main constituents of proteins are under pH of 7.4 never fully dissociated as acids or bases. Amphotheric rinsing solutions provide binding sites for acids and bases so that the decision on treatment is independent of the type of burning agent. Diphoterine® has acid binding sites with a pK_a of about 5 and a alkaline binding sites with a pK_a around 9.

A new product in this field is Diphoterine® which has been introduced to the German market (Previn®) since 1995. To evaluate the potential of prevention and harmful effects, we tested the action of two different substances in a double masked setup: Diphoterine® versus saline solution 0.9% on alkali burn.

2. Materials and methods

Proof of pH change by rinsing with saline or Diphoterine® in the anterior chamber of the eye and on the corneal surface.

2.1. Experiment I

Saline solution 0.9% and Diphoterine® (preserved by sodium-*para*-hydroxyl-benzoat-dimethyl, 0.5 g/l) were tested in a double masked study. In deep general anesthesia, the right cornea including 1 mm of the limbus of each four rabbits was firmly covered with a plexiglas cylinder which was filled with 3 ml 1N NaOH for 30 s. After 30 s the alkali was removed in one go with a syringe and an immediate irrigation therapy started with 500 ml of Diphoterine® or saline solution for 5 min started. Samples for pH measurements were taken immediate after eye burns and at the end of the rinsing period. We took samples by aspiration of 0.1 ml aqueous humor via a 20 gauge needle from anterior chamber through the posterior sclera through the iris to prevent rinsing fluid contamination into the eye. The samples were subjected to pH meter (radiometer) measurements with a syringe mini pH electrode. Tears were taken from the corneal surface fluid directly after burns and after rinsing and measured in the same way. All measurements were checked due to the small amounts of fluid for plausibility by means of filter paper strips put on the surface directly or measuring the remaining droplet directly from the tip of the pH meter after measurement. The aspiration of anterior chamber fluid and surface measurements were repeated immediately after 5 min of rinsing with saline or Diphoterine®.

2.2. Experiment II

Another 16 rabbits in two groups of eight animals were subjected eye burns as described earlier in deep general anesthesia. The immediate to the eye burn following therapy consisted of irrigation with 500 ml Diphoterine® or saline 0.9%, respectively during 5 min. The continued therapy for 16 days consisted in 3 times daily 160 ml saline 0.9% solution corresponding to the rinsing therapy after eye burns in our clinic.

Details of epithelial erosion, ulceration and opacification were recorded with the help of Na-fluorescein stains (Fluorescein Thilo® unpreserved) and photographs were taken daily followed by planimetrical analysis on a digitizer board (Genius) connected to a computer analysis using Tek-Illustrator® software for area measurements. After 16 days the corneas were excised, shock frozen between two cooled steel blocks at −196 °C, halfed and stored at −80 °C for further analysis. The anterior chamber was prepared carefully and pathology of lens and iris were recorded in a clinical grading. Iris synechies were graded (0: no synechia, 1: single synechia, 2: sectoral synechia and 3: secclusio pupillae). Iris atrophy was graded following the pigment loss of the iris (0: no atrophy, 1: small atrophy, 2: sectoral atrophy and 3: total atrophy). Cataract grading was 0: no cataract, 1: small anterior capsule opacification, 2: anterior capsule and cortical clouding and 3: severe cortical lens clouding.

The frozen corneas were processed to sections of 10 μm cut on a cryomicrotome at −32 °C and prepared for scanning electron microscopy as described by Schrage et al. [2]. The other half of the corneas were weighed in hydrated and dehydrated state to determine the water content.

All animal research was done according to the ARVO guidelines for the use of animals in ophthalmic and vision research and local laws of the Federal Republic of Germany.

Conflict of interest: The expenses of purchasing and housing the animals were met by the manufacturers. No payments were made to the experimenters or the authors involved in this story.

Table 1
Experiment I pH of extra- and intraocular fluid directly after 30 s

	Corneal surface	Anterior chamber	Significant Student's *t*-test
Directly after eye burns	13 ± 0	10 ± 0	
After 5 min rinsing saline	9 ± 0	10 ± 0	
After 5 min rinsing with Previn (Diphoterine®)	7.5 ± 0	9.34 ± 0.59	$P < 0.05$ vs. NaCl

Of 1N NaOH eye burns and after 5 min of rinsing with saline, phosphate buffer or Diphoterine®.

3. Results

3.1. Experiment I

Surface pH increased after burn to 14 in both groups and was lowered by 5 min of 500 ml of 0.9% saline rinsing to 12. The same amount of Diphoterine® lowered the corneal surface pH to 7.5. Aqueous humor showed pH of 10 after 30 s after eye burns and returned to 11 with 500 ml of saline and to 9 with 5 min of rinsing with 500 ml of Diphoterine® (Table 1). All results with the pH microelectrode were in the order of magnitude detected with pH paper measuring stripes so that measuring errors due to low volumes are in the order of magnitude less than 0.5 pH.

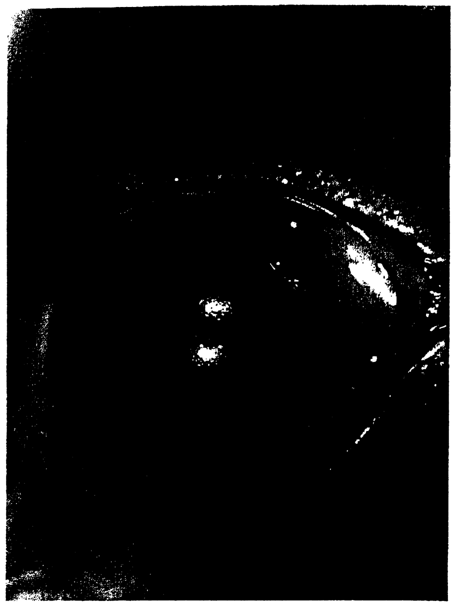

Fig. 2. Corneal clouding after alkali burn and treatment with 500 ml Diphoterine® solution.

3.2. Experiment II

During rinsing fibrin clots appeared on the surface of the saline rinsed animals whereas such clots could never been observed in Diphoterine® rinsed animals. Corneal opacification (Figs. 1 and 2), development of the epithelial healing, breakdown of the epithelial healing process (Fig. 3) and corneal ulcerations (Fig. 4) were similar in both groups. There was no significant difference in surface extension or depth of ulcerations in both groups (Fig. 4). Overall both groups showed a similar reaction severe burn and no healing. There was no significant advantage from Diphoterine® rinsing unless it gave proof of effectiveness in pH measurements. This result was expected in this type of injury. There was an insignificant less severe iris and lens alteration in the Diphoterine® treated group. Also the iris stroma

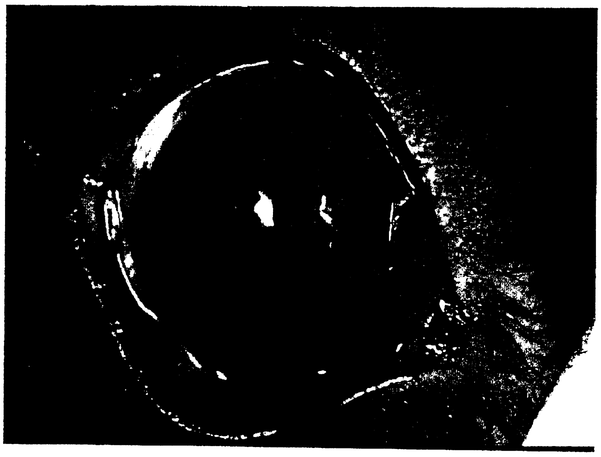

Fig. 1. Corneal clouding after alkali burn and treatment with 500 ml saline.

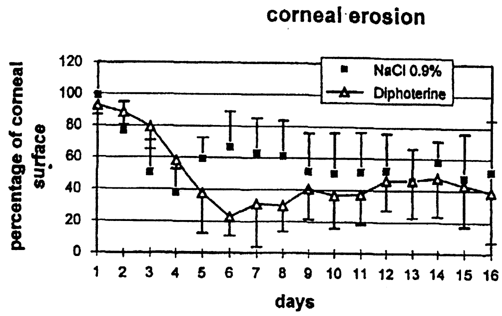

Fig. 3. Development of epithelial healing measured by the surface area of erosion as percent of the total corneal surface data point represent means of the erosion surface marked by fluorescein stains, as percentage of the total corneal surface in eight animals.

atrophy and the lens opacification were slightly milder in the Diphoterine®-group (Fig. 5, Table 2).

4. Discussion

Saline 0.9% is one of the recommended solutions in emergency treatment of eye burns (ANSI Z358.1-1990 Standards). New rinsing solutions as Diphoterine® have to be compared to this international standard. Although the buffering capacity of saline 0.9% is negligible, it is one of the standards in emergency rinsing of burned eyes. Other therapy regimes like treatment with buffers like boron-, ascorbate-, citrate- or phosphate-buffer have been successfully tested in former studies [3–7]. These buffers have properties buffering well in the alkaline region (phosphate, ascorbate, citrate) or in the acid region (boron-buffer). Most of these buffers have been tested in sodium hydroxide eye burn animal or in vitro models, where the specific buffering capacity is maximal. Chemical accidents are not stereotype like these experiments and especially hydrofluoric acid or epoxides and the vast spreading of accidents with new chemical substances fail in treatment with these recommendations. Useful studies subjecting this were presented by McCulley et al. [8], Reim et al. [9] and were summarized by Wagoner recently [10].

Therefore, fundamental requirements to a superior rinsing fluid systems are the unspecified buffering and neutralization of all types of acids and bases in the range of pH 5–9, the function as radical scavenger and as non-ionic attractant of reactive chemicals. One type of chemicals fulfilling these requirements are amphoteric substances with two different groups of pK in the acid and alkali region. Diphoterine® fulfills this requirement with a $pK1 = 5.1$ and a $pK2 = 9.3$. A new substance like this one must be checked for its side effects as a pharmaceutical proof of being innocuous to replace former standards by a better one following the regulations of ethical treatment in medicine. Systematic research has to test the agent's toxicity in the indicated application. If there is no toxicity or other undesired side effect, the substance can be taken under consideration for test persons and in clinical trials.

According to former results [11,12] continued phosphate buffer applications in eye burns have to be considered as delicate in respect to corneal calcifications. Calcification due to local factors have been argued before [13]. Phosphate buffer as ionic solution is not at all a part of the physiological composition of the normal cornea [14]. Local factors

Table 2
Lens opacification within the scores after corneal removal in comparison between saline 0.9% and Diphoterine®

Fisher's exact test	Lens opacification >1	Lens opacification ≤1	Total
Saline solution (0.9%)	6 (38)	2 (13)	8 (50)
Diphoterine®	4 (25)	4 (25)	8 (50)
Total	10 (63)	6 (38)	16 (100)

The two-sided P-value is 0.6084, considered not significant. There is not a significant association between rows and columns. Values given in parenthesis are in percentage.

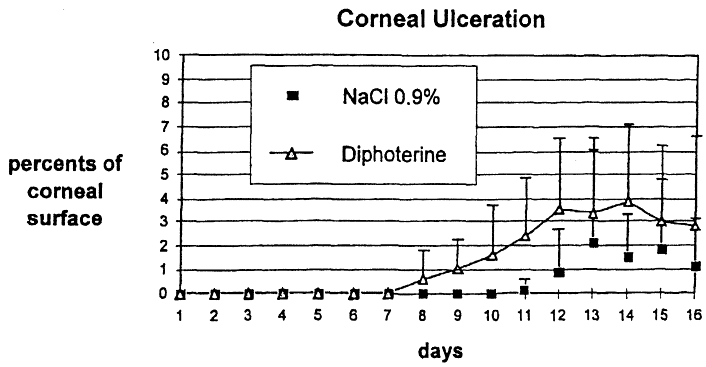

Fig. 4. Corneal ulceration measured in their surface extend related to the total corneal surface, both group showed similar corneal ulcerations. Data points represent means of the ulcerated area as percentage of the total corneal surface in eight animals.

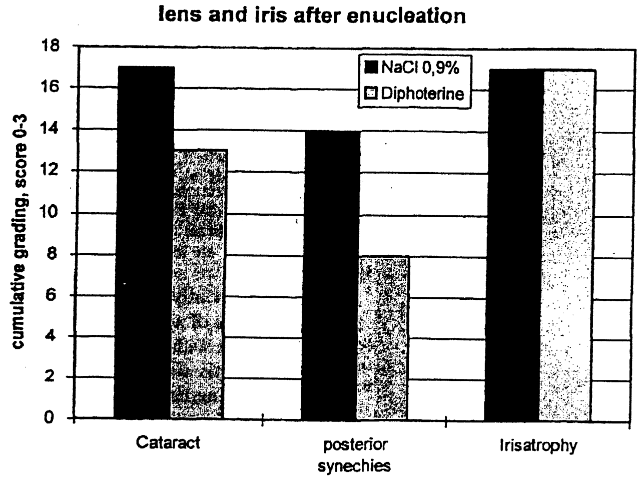

Fig. 5. Distribution of iris pathology and lens opacification in both therapy groups. Cumulative scores of each eight animals per column. Median scores: saline solution 0.9% = 2, Diphoterine® solution = 1.5.

like inflammation, denuded stroma and proteolytic activities with cell death [15] combined with an unphysiological phosphorus supply by external therapy result in local calcifications of the cornea [1]. Therefore, due to demands on the security of an emergency treatment rinsing agent with effects in the later development of corneal pathology it is necessary to evaluate which type of rinsing solutions is accessible to effectively rescue burned eyes with no additional harmful side effects for the later prognosis. We think that a systematic examination of Diphoterine® has to be undertaken to further evaluate the effectiveness of action. According to our results from our momentary animal experiments, we believe that Diphoterine® is a useful rinsing solution in first aid therapy of alkali burns with a substancial possibility of treating other types of alkalines, acids [16] and radicals.

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