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Original Article

A prospective, randomized, blind comparison between saline, calcium gluconate and diphoterine for washing skin acid injuries in rats: effects on substance P and β-endorphin release

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Summary

Background and objective: A randomized, blind study to evaluate the effects on β-endorphin and substance P release after washing acid burns with 0.9% saline, calcium gluconate or diphoterine in a model of chemical burn in rats.

Methods: Twenty Sprague–Dawley rats (approximate weight 250 g) were anaesthetized with ketamine (30 mg kg^{-1} intramuscularly) and then given an acid injury on the back skin with 0.5 mL of hydrochloric acid 52%. The rats were then randomly allocated to receive no washing (control group, $n = 5$), washing with normal saline (0.9% NaCl) ($n = 5$), 10% calcium gluconate ($n = 5$) or diphoterine ($n = 5$). Blood concentrations of substance P and β-endorphin were measured 6 h, 48 h and 7 days after the chemical burn. An independent blinded observer evaluated wound healing at the 7th day.

Results: Seven days after burn wound healing was almost complete only in rats treated with diphoterine. Plasma concentrations of substance P were lower in rats receiving skin flushing with diphoterine compared to the other groups at 6 and 48 h after acid burn ($P < 0.05$ and $P < 0.05$, respectively); this was also associated with higher concentrations of β-endorphin at day 7 ($P < 0.05$).

Conclusions: Skin flushing with diphoterine reduced substance P release during the first 48 h after burn, and was associated with better wound healing and higher concentrations of β-endorphin 7 days later when compared with normal saline or 10% calcium gluconate.

Keywords: INTERCELLULAR SIGNALLING PEPTIDES AND PROTEINS, kinins, tachykinins, Substance P; OPIOID PEPTIDES, endorphins; WOUNDS AND INJURIES, burns, chemical.

Skin burn is an acute injury with protracted debilitating course, and chemical burns represent a relevant proportion of these injuries [1,2]. The extent of the damage caused by a chemical agent depends on its concentration, amount, duration of contact with the skin and tissue permeability to the agent [1,2].

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The first step for emergency treatment for chemical skin burn is to remove the causative substance as quickly as possible by flushing the skin with large volumes of fluids [1–3].

Acute burn activates a complex inflammatory cascade [1,2], including several sensory neuropeptides and pain modulating hormones, such as substance P and β-endorphin [4–7], which play a significant regulatory role in the immune, pain, inflammatory and wound healing outcome of burned patients [8,9]. Accordingly, it has been reported that adequate emergency washing of localized skin burns may improve

the relief of the associated pain, and this may be used as an end-point for adequacy of treatment [10].

Several substances have been proposed for emergency skin washing after chemical burn, including normal saline (0.9% NaCl) or calcium gluconate, which is the gold standard agent to flush acid skin burns [2,3,11,12]. Diphoterine® (Prevor, Valmondois, France) is a hypertonic, polyvalent, amphoteric compound developed as a water-based decontamination solution for chemical burns [13,14]. The objective of this prospective, randomized, blind study was to compare normal saline, calcium gluconate or diphoterine for skin flushing after acid skin burn in terms of wound healing and effects on β-endorphin and substance P release in an animal model of chemical burn in rats.

Methods

The Ethics Committee of the Institute of Microsurgery and Experimental Surgery, University of Milano, Monteggia Department, Milan approved the study, and all experimental procedures and protocols complied with the 'Guiding principles in the care and use of animals' [15]. The animals were never subjected to unnecessary pain. The study was conducted on 20 Sprague–Dawley rats, each weighing approximately 250 g. During the study period, the animals were kept in a stabularium with a normal light/darkness period (12 h/12 h). Each animal was in a single box, and external factors such as light, fights or bites were controlled. Before the experiment, back interscapular region of the rats was shaved, taking care to avoid abrading the skin. Before inducing the acid skin burn, the rats were anaesthetized by an i.m. injection of ketamine 30 mg kg^{-1}. The acid injury was induced by applying hydrochloric acid (HCl) 52% 0.5 mL to the skin over a circular area with a diameter of 2 cm for 15 s. Then the rats were randomly allocated to receive no washing (group control, $n = 5$), skin washing with normal saline (group saline, $n = 5$), calcium gluconate 10% (group calcium gluconate, $n = 5$) or diphoterine® (group diphoterine, $n = 5$). The injured skin was washed for a 30 s period at a flow of approximately 50 mL min^{-1}.

Blood samples were drawn from the caudal vein 6 h, 48 h and 7 days after the chemical burn. Before blood sampling, the studied rats were anaesthetized with ether by inhalation. Blood concentrations of substance P and β-endorphin were measured using enzyme and radioimmunoassay techniques. To determine substance P concentrations, 50 μL of blood was added to 50 μL of blue alkaline phosphatase conjugate, then incubated for 2 h and washed three times with 550 μL Buffer (Peninsula Laboratories, California, USA). For β-endorphin concentrations, 100 μL of blood was added to RIA Buffer (Peninsula Laboratories) and rabbit antibodies anti-β-endorphin, and then incubated for one night at 4°C. Afterwards, 500 μL RIA Buffer was added to the samples and centrifuged for 20 min at 3000 rpm and 4°C. The radioactivity was measured after removing the supernatant.

Seven days after the chemical burn an independent blinded observer evaluated the wound healing of the injured skin using a five degrees scale:

1. presence of eschar;
2. disappearance of eschar;
3. detersion of eschar;
4. wound re-epithelization and;
5. complete healing.

Statistical analysis was performed using the program Systat® 7.0 (SPSS Inc, Chicago, IL, USA). Changes with time of the plasma concentrations of substance P and β-endorphin were analysed with the analysis of variance for repeated measures, while Tukey's test was used for *post hoc* comparisons. Unless otherwise indicated, results are presented as median (range) or as number (percentage). $P \leqslant 0.05$ was considered as significant.

Results

All rats survived through the study period. At the 7 day evaluation of wound healing rats of group control showed the worst degree of evolution of the wound injury, with the presence of eschar in all injured rats related to the deep penetration of the burning agent to the deep dermis. Rats of group calcium gluconate showed a 2–3 degree of wound healing, with disappearance of the eschar and initial elimination of the foreign, exogenic or endogenous elements, and of the cellular and tissue structures (detersion) of the wound. Rats of group normal saline showed a 3–4 degree of wound healing, with detersion of the wound and initial re-epithelization. All rats of group diphoterine showed 5 degree wound healing, with complete or almost complete healing of the burn injury.

Figure 1 shows the changes in both substance P and β-endorphin plasma concentrations in the four treatment groups. Plasma concentrations of substance P were lower in rats receiving skin flushing with diphoterine compared to the other groups at 6 and 48 h after acid burn ($P < 0.05$ and $P < 0.05$, respectively). This was also associated with higher plasma concentrations of β-endorphin 7 days after the injury in group diphoterine compared to the other groups ($P < 0.05$).

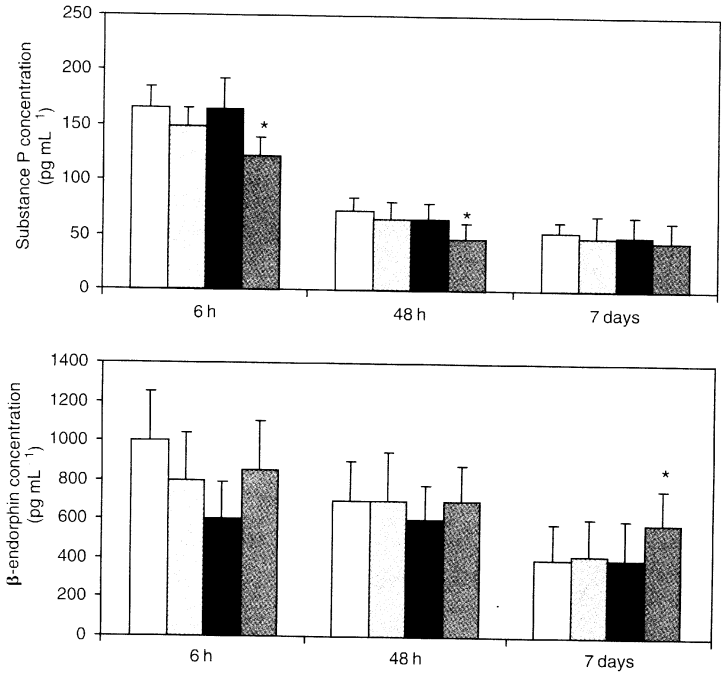

Figure 1.
*Changes with time of plasma concentrations of substance P (above) and β-endorphin (below) in rats receiving no washing after the acid burn (group control, n = 5), or skin washing with normal saline (group saline, n = 5), 10% calcium gluconate (group calcium gluconate, n = 5) or diphoterine (group diphoterine, n = 5). *P < 0.05 compared to groups control, saline and calcium gluconate. □: Control; ▨: saline; ■: calcium gluconate; ▩: diphoterine.*

Discussion

The incidence of chemical injuries related to accidental contact of acid agents with the skin has markedly increased in the last few years, resulting in a large number of patients treated as emergencies [16]. When acid substances come into direct contact with the skin, hydrogen ions react with protein in the skin to form acid proteinate, which in turn causes coagulation necrosis – the tissue damage continues as long as the acid is in contact with the tissue [11]. Accordingly, the first mandatory step for the emergency treatment of accidental acid burn is based upon immediate flushing of the injured skin with large volumes of fluids [1–3,12]; positive results have been reported with water [11] and calcium gluconate, which is now the most frequently used agent for emergent treatment of acid skin burn [12,17].

The most striking finding of this prospective, randomized, blinded study is that immediate skin washing with diphoterine resulted in significant improvement of wound healing with reduced plasma concentrations of substance P 48 h after the experimental burn compared to the control group and the other two forms of treatment. This was also associated with higher plasma concentrations of β-endorphin 7 days after the injury.

It has been demonstrated that release of substance P increases significantly after burns [5]. Substance P enhances vascular permeability and promotes regeneration of sensory peptide nerve fibres in the injured skin [6,7], playing a significant role in inflammation, immune response, pain and wound healing [5,9]. Saria and colleagues [18] also demonstrated that substance P contributes to the development of oedema in the rat hind paw after thermal injury, suggesting that the lower plasma concentrations of substance P observed in our present study in rats, treated with diphoterine, could be related to a reduction in the local injury produced by the acid contact because of a better HCl elimination from the skin, with a less deep injury. This hypothesis is also corroborated by the observation of a faster evolution of wound healing together with higher plasma concentrations of β-endorphin 7 days after acid burn in those rats treated with diphoterine.

Populations of C fibres contain several other substances that could be involved in the mediation of inflammation after chemical injury, such as calcitonin gene-related peptide (CGRP) and glutamate [19]. In this investigation only substance P was evaluated, while β-endorphin was measured to evaluate the effects of acid skin injury on pain modulation. Other studies could be advocated to better evaluate changes in other substances modulating inflammation and pain after acid skin burn.

Bromberg and colleagues [20], evaluating HCl injures in mice, demonstrated that the degree of skin

injury was strictly related to the duration of exposure to the toxic agent; and similar findings were also reported by other investigators [21]. Yano and colleagues [11] demonstrated that washing with water before subcutaneous tissue pH reached a minimum value effectively suppressed the fall in pH produced by the acid injury, resulting in better outcome of the skin lesion. On the other hand, positive results have been also demonstrated in several models of acid burn with using topical or iontophoretic calcium gluconate [12,17]. Diphoterine is a relatively new eye/skin chemical splash decontamination solution [14]. It is a polyvalent, slightly hypertonic, amphoteric, water-soluble molecule that binds acids, bases, oxidizing agents, reducing agents, solvents, irritants, alkylating agents and radionuclides. *In vitro* and *in vivo* studies have demonstrated that diphoterine actively decontaminates more than 600 chemical toxic agents more effectively than water lavage, also resulting in immediate pain relief [14]. As a water-soluble compound, diphoterine also has a passive rinsing effect; however, its active chemicophysical properties make diphoterine a very effective eye/skin decontamination solution [14].

In a model of chemical burn in rabbits, Gerard and colleagues [13] demonstrated that washing the skin with diphoterine prevented the development of stromal oedema at cytopathological analysis, which was not similarly prevented when flushing the skin with normal saline. These findings are in agreement with results of our present study, which demonstrated that the faster evolution of wound healing after HCl burn in rats treated with diphoterine was also associated with a better evolution of the biohumoral responses induced by burn itself as compared to the other considered treatments.

Further evaluation with extensive clinical use should be advocated to better evaluate the usefulness of this new agent; however, results of this prospective, randomized, blinded study demonstrated that, in a model of HCl burn in rats, immediate skin flushing with diphoterine improves the evolution of wound healing and is associated with lower concentrations of substance P during the first 48 h after burn, and higher concentrations of β-endorphin 7 days after the lesion compared with the use of normal saline or 10% calcium gluconate.

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