

Early nonsurgical removal of chemically injured tissue enhances wound healing in partial thickness burns

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Abstract

Chemical burns are slow healing injuries and their depth is difficult to assess. Tissue destruction continues as long as active material is present in the wound site. The routine therapy for treatment of full thickness chemical burns is early excision; it shortens hospitalization and reduces morbidity. However, presently there is no specific treatment for chemical burns of partial thickness. This study examined several treatment modalities for partial thickness chemical burns: surgical excision; laser ablation and chemical debridement with Debridase or trypsin-linked to gauze. Chemical burns were inflicted with nitrogen mustard (NM — a nitrogen analog to sulfur mustard — mustard gas) in an experimental guinea pig model. Debridase was most effective and reduced significantly lesion area of burns after 'humid' exposure to 2 mg NM.

The healing action of Debridase was also evident in the significantly higher histopathological score of biopsies from local tissue obtained on day 5. Laser ablation was most effective and accelerated healing of burn lesions after 'dry' exposure to 5 mg NM. The histopathology score of the laser treated burns was higher on day 4 compared to untreated controls. It is concluded that for partial thickness chemical burns early nonsurgical removal of the damaged tissues accelerates wound healing. © 1998 Elsevier Science Ltd for ISBI. All rights reserved

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1. Introduction

Testing of treatment procedures for potential hazards to skin from acute exposure to dermatotoxicants is routinely required for several classes of chemicals to which human beings may be exposed accidentally or deliberately. Chemical burns are caused by a large variety of materials produced for military, industrial, agricultural and domestic use. They comprise ca 4% of the burn casualties in developed countries [1,2] and may reach 14% in underdeveloped countries [3]. Chemical burns are a great concern in war situations, because although generally not fatal they cause long incapacitation and blocking of treatment centers [4].

The main difference between chemical and thermal injuries is that the former are progressive for as long as

some active compound is present in the wound lesion. Chemical burns are notorious for their slow healing course, and their hospitalization period is longer by 30% compared to thermal injuries of similar size [5]. This holds true especially for warfare chemicals such as white phosphorous [6] and mustard gas (MG) — the most widely used warfare vesicant [4].

Early topical decontamination with 0.5% sodium hypochloride solution is still the standard treatment for chemical burns in many military systems [7]. Nevertheless, it is generally accepted that heavy washing with pure water is also effective for primary treatment of chemical burns [7]. The healing pattern and outcome of adequately treated chemical burns are almost identical to that of thermal burns [6,8]. To this point the recommended treatment for deep chemical burns is early tangential excision, and it is similar for burns caused by several chemicals (i.e. phenol, hydrofluoric acid, Cl). This procedure is also the preferential treatment for full thickness thermal burns, since it proved to reduce effectively morbidity and mortality [10].

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Whereas superficial partial thickness thermal burns are still treated conservatively and generally heal within 3 weeks; for deep partial thickness or unclassified thermal burns, a whole range of procedures are used [10]. It is beyond the scope of this paper to review them all. It is presently still difficult to decide on surgical intervention for chemical burns that look superficial or are of mixed type [7–11].

The present experiments were undertaken to evaluate and directly compare treatment modalities for slow healing partial thickness chemical burns. Tangential excision was compared to laser ablation and to enzymatic debridement of Debridase gel or trypsin covalently linked to cotton gauze. Chemical burns were inflicted with nitrogen mustard (NM) in an experimental guinea pig model.

NM (Mechlorethamine HCLm MSD) is an antineoplastic nitrogen mustard, and an analogue of sulfur mustard (MG). NM was used in the present experiments because it is available for clinical practice and is soluble in water. Wound healing timecourse and pathology observations at the gross and at the light microscopy level are evaluated and compared.

2. Materials and methods

Female Hartley guinea pigs (350 ± 25 g) were used in this study ($n = 87$). They were climatized for 4 weeks in separate cages and received food and water ad libitum. The study was approved by the Institutional Animal Care and Use Committee. The animals were anesthetized with intramuscular injection of 12 mg/kg Ketamine (Parke–Davis Medical, Southampton, UK) and 1.5 mg/kg Droperidol (Abic Ltd, Ramat-Gan, Israel). Back hair was clipped and chemically depilated. Two target areas, 3 cm in diameter were marked on each side of the back midline, 4 cm apart. In a laminar air flow hood, two amounts of NM (Mechlorethamine HCl: Mustargen, Merck, Sharpe and Dohme, West Point, PA 19486, USA) were applied.

- (1) 2 mg NM in 0.8 ml aqueous solution. A sterile gauze disc, 3 cm in diameter was soaked with this solution, placed onto the skin, and covered with an occlusive plastic cap. The space within the cap was filled with slightly moist cotton wool to provide a uniform and humid exposure and to mimic skin folds and sweating body areas.
- (2) 5 mg NM in 0.8 ml aqueous solution, were applied in a similar manner, but the cotton wool within the cap was left *dry*, to mimic 'dry' body areas. The gauze discs and caps were removed after 6 h. Exposed animals were kept in the hood for 24 h and then decontaminated with 0.5% sodium hypochloride solution.

Table 1
Experimental design

Group No.	Treatment	No. of animals
<i>Group A. 2 mg NM ('humid exposure')</i>		
1.	Tangential excision	11
2.	Debridase debridement	12
3.	Trypsin debridement	12
4.	Nitrofurazone gauze dressing	8
5.	Untreated NM burns	10
<i>Group B. 5 mg NM ('dry exposure')</i>		
6.	Tangential excision	8
7.	Excimer laser ablation	8
8.	Nitrofurazone gauze dressing	8
9.	Untreated NM burns	10

The experimental design and treatment modalities are summarized in Table 1. Tangential excision of the eschar was performed with the Watson hand dermatome set at 0.012", down to bleeding dermis (Groups 1 and 6). Excimer laser (308 nm in xenon gas), ablation time ca 5 min (Group 7). Debridase gel (BTG, Nes-Ziona, Israel) was used for enzymatic debridement for a period of 4 h (group 2). Trypsin covalently linked to cotton dressing was applied onto the burn site and changed every 24 h for 2–4 days, until debridement was achieved (Group 3). Following eschar removal the exposed wound bed was dressed with Nitrofurazone gauze, as in the conservative treatment group. Untreated NM burns (Group 5 and 9) and Nitrofurazone dressing (Group 8) served as controls. Wound healing was assessed by plannimetry and histopathology of 3 mm punch biopsies taken on varying intervals. The biopsy preparates were stained with Hematoxylin-Eosin and evaluated quantitatively according to predetermined histologic criteria. These criteria were determined in preliminary experiments and scored 0, 1 and 2 according to approximation to normal of morphologic components of the tissues (Table 2).

3. Results

The experimental design to examine treatment modalities for chemical burns inflicted with NM is summarized in Table 1, and the histopathological criteria and scoring in Table 2. Surgical tangential excision was evaluated and compared to laser ablation and enzymatic debridement by Debridase or trypsin. Untreated NM burns and burns treated with nitrofurazone served as controls. The healing timecourse and histopathologic score of skin lesions after 'humid' exposure to 2 mg NM are summarized in Figs 1 and 2, respectively. The results clearly indicate that early

Table 2
Microscopic criteria for evaluation and scoring of lesions

Criterion	Score*		
	0	1	2
1. Epidermal structure	Total destruction/absence	Partial necrosis/ulcer	Normal
2. Dermal-epidermal junction and microblisters	<25% attachment of epidermis to dermis, blisters	25-75% attachment of epidermis to dermis, no blisters	Normal/no microblisters
3. Collagen bundles and dermal organization	Amorphic dermis, destruction of bundles	Edematous/disorganized bundles, partial necrosis of dermis	Normal collagen bundles
4. Epidermal regeneration	<25%	25-75%	>75%
5. Leucocyte infiltration†	>16	6-15	<5

*Maximal score = 10.

†Per field ($\times 400$ magnification).

removal of the injured tissues with Debridase gel accelerated the rate of wound healing compared to tangential excision. Trypsin debridement had no promoting effect compared to Nitrofurazone treated and selfhealing burns (Fig. 1). The healing action of Debridase is highly significant on day 7 ($P < 0.05$). At this time point lesion area of the Debridase treated burns was reduced by 70% of their original size, whereas those of the surgically excised wounds by only 30%. Trypsin dressing had no advantage compared to controls (ca 50% reduction). All wounds inflicted with 2 mg NM were closed by 21 days (Fig. 1).

Light microscopy changes in biopsies taken from the center of the exposed skin at varying intervals were documented and scored (Fig. 2). The histological results were in accordance with the lesion area curves and indicated that the promoting effect of Debridase was also demonstrated by a significantly higher histopathological score ($P < 0.001$) compared to tangential excision on day 5 and throughout the experiment, and compared to trypsin treatment on days 5, 11 and 21.

The next experiments were carried out to examine treatment modalities for chemical burns after 'dry' exposure to 5 mg NM. Ablation with Excimer laser was

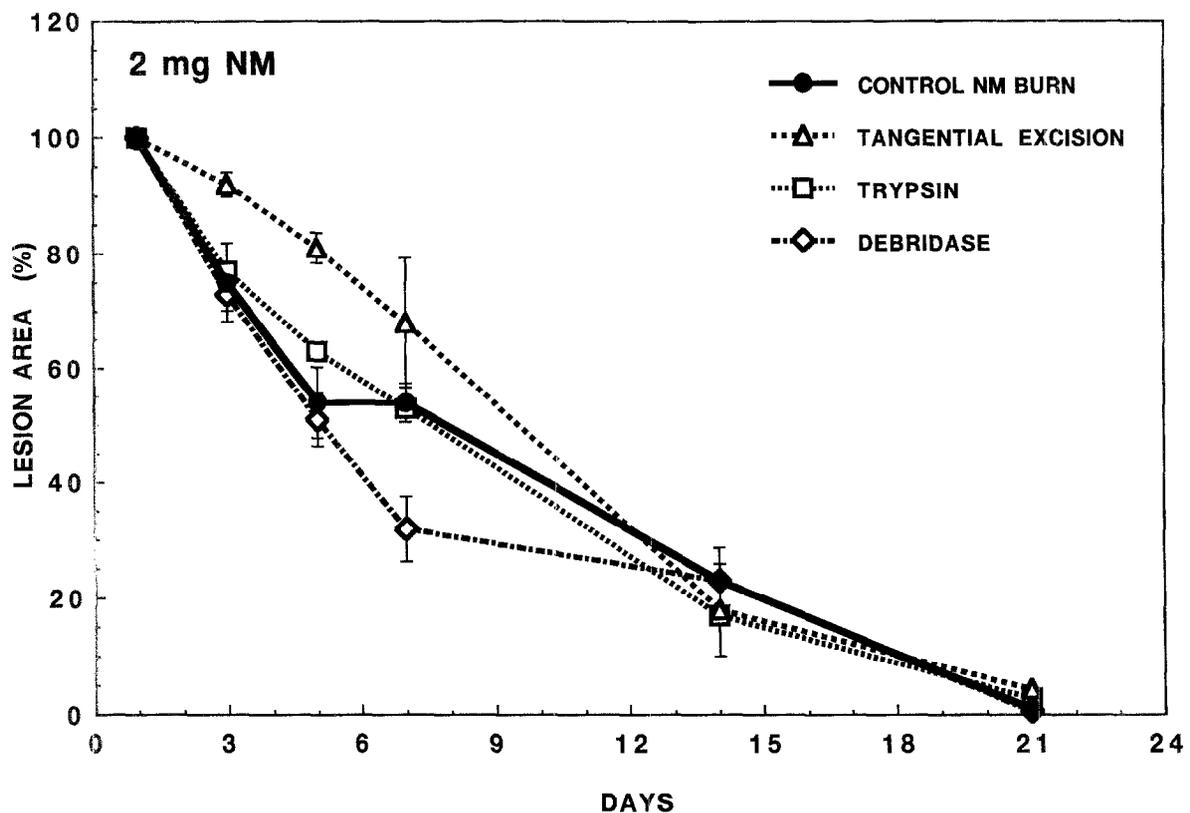


Fig. 1. Effect of various treatment modalities on lesion area after experimental burns with 2 mg NM, 'humid-exposure'.

compared to tangential excision and Nitrofurazone dressing; self healing burns served as controls. The

results clearly indicate that laser ablation accelerated significantly wound closure (Fig. 3). Lesion area of

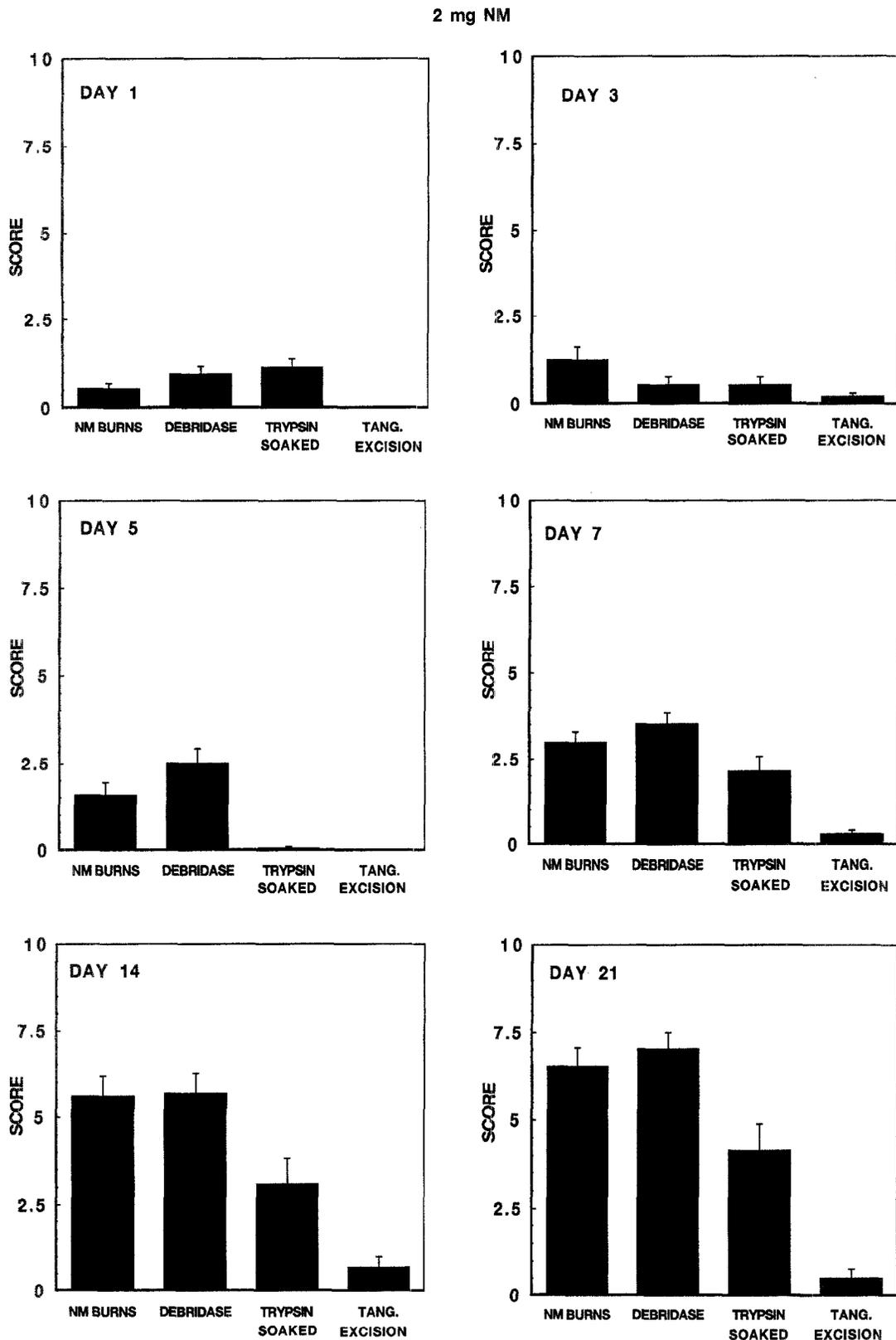


Fig. 2. Histopathologic changes in biopsies from wound bed after various treatments of NM burns ('humid exposure' to 2 mg NM).

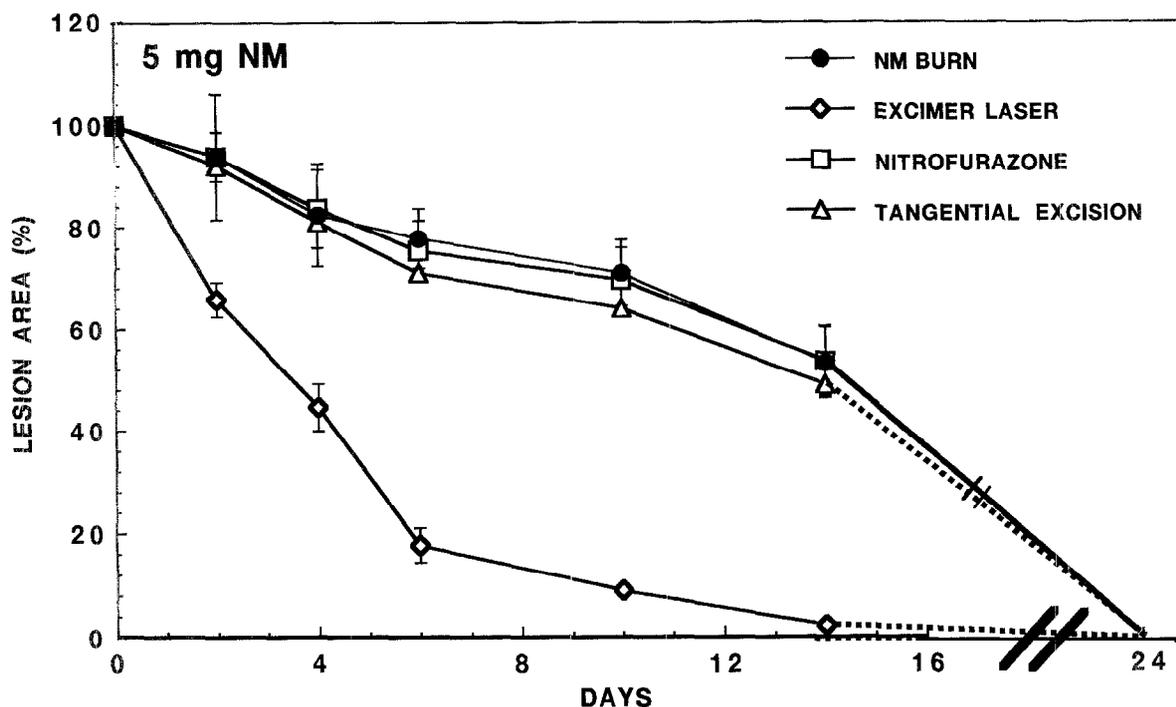


Fig. 3. Effect of various treatment modalities on lesion area after experimental burns with 5 mg NM, 'dry' exposure.

laser ablated burns was dramatically reduced by 80% of its original size within 6 days ($P = 0.001$), and closed within 2 days, whereas neither surgical excision nor Nitrofurazone had any promoting effect compared to untreated burns, at any particular timepoint for the duration of the experiment. All lesions were closed within 24 days (Fig. 3).

Light microscopy changes in the biopsies taken from the lesion center at varying intervals did not indicate consistent significantly higher score for a particular treatment (Fig. 4). However, all three procedures yielded significantly higher scores than untreated burns on day 4 and 6, and by day 14 all burn lesions exhibited similar histopathological scores (Fig. 4).

4. Discussion

The present results provide evidence that early nonsurgical removal of injured tissues is an effective treatment for chemical burns of partial thickness. Debridement or laser ablation accelerated wound healing as determined by the rate of lesion area closure and histopathological score. The choice of the experimental model and NM doses allows long term follow-up studies on healing rates and on assessing efficacies of potential therapeutic measures. NM — a nitrogen analog of MG gas was used as a model for chemical vesicant agents, because it is available for clinical practice and very soluble in water. Although chemical burns behave in much the same way as

comparable (in depth) thermal burns, they are slower to heal, cause long incapacitation and blocking of treatment centers [1,2,6,8–10].

Early excision of small and deep thermal burns is a treatment that was recommended by Lustgarten as early as 1891 [10]. The interest in this procedure was revived in 1970, after Janzekovic reported good results for early excision and grafting of full thickness burns [12]. Since then a dramatic improvement has been achieved in the management of thermal burns, and primary excision followed by prompt grafting became the routine therapy [13–16].

The basis for the tissue injuries caused by vesicant agents remains unclear. Like other alkylating agents they affect DNA synthesis and inhibit cell proliferation [17,18]. It has been shown that MG — the most widely used warfare agent induces alternations in the collagen fibrils participating in cell attachment to the basement membrane and may cause enhanced protease synthesis and release as a result of DNA damage [17,18].

It is postulated that early removal of the injured tissues in chemical burns of partial thickness might be of benefit to the management of these slow healing cutaneous injuries primarily by enhancing the rate of wound healing. In battlefield situations the skin lesions of the casualties start between 18 and 24 h after exposure, and because the exposure is generally to low doses there is no persistence of active compound in the blister fluid [4,7]. Nevertheless the risk of secondary infection of the blisters can occur very rapidly and must be avoided [4,7]. The fluid of bullous lesions clots and

adheres to its floor after 24 h, then drainage becomes difficult [4]. Therefore we aimed at an early removal of the injured tissues, while trying to preserve the healthy tissues as much as possible. Surgical tangential excision, a well established procedure, did not improve wound healing of partial thickness chemical burns in the experimental model. In addition, it was technically difficult to control the amount of tissue to be removed and thus standardize the procedure and obtain reproducible results. Laser ablation fulfilled these requirements, it enables control of the amount of tissue to be removed with minimal blood loss. However, with the present equipment this technique was time consuming: 5–7 min were needed to clear a lesion of 3 cm in diameter. Thus, although this therapy cannot be applied presently for large burns, it should certainly be

considered for treatment of small lesions. Still with laser technology advancing so fast, in all probability faster and powerful lasers will soon be available for this type of clinical practice.

Two procedures for enzymatic debridement of partial thickness chemical burns were examined in parallel: Debridase gel and trypsin covalently linked to cotton gauze. The results indicated that Debridase performed better than trypsin and tangential excision. Its beneficial effect was evident in the higher wound healing rate and histopathologic score of the treated lesions. The histopathologic score of the injuries after 'humid' exposure to 2 mg NM is lower compared to 'dry' exposure to 5 mg NM (Figs 2 and 4 respectively). This is in accordance with clinical observations in casualties with vesicant burn lesions over moist parts

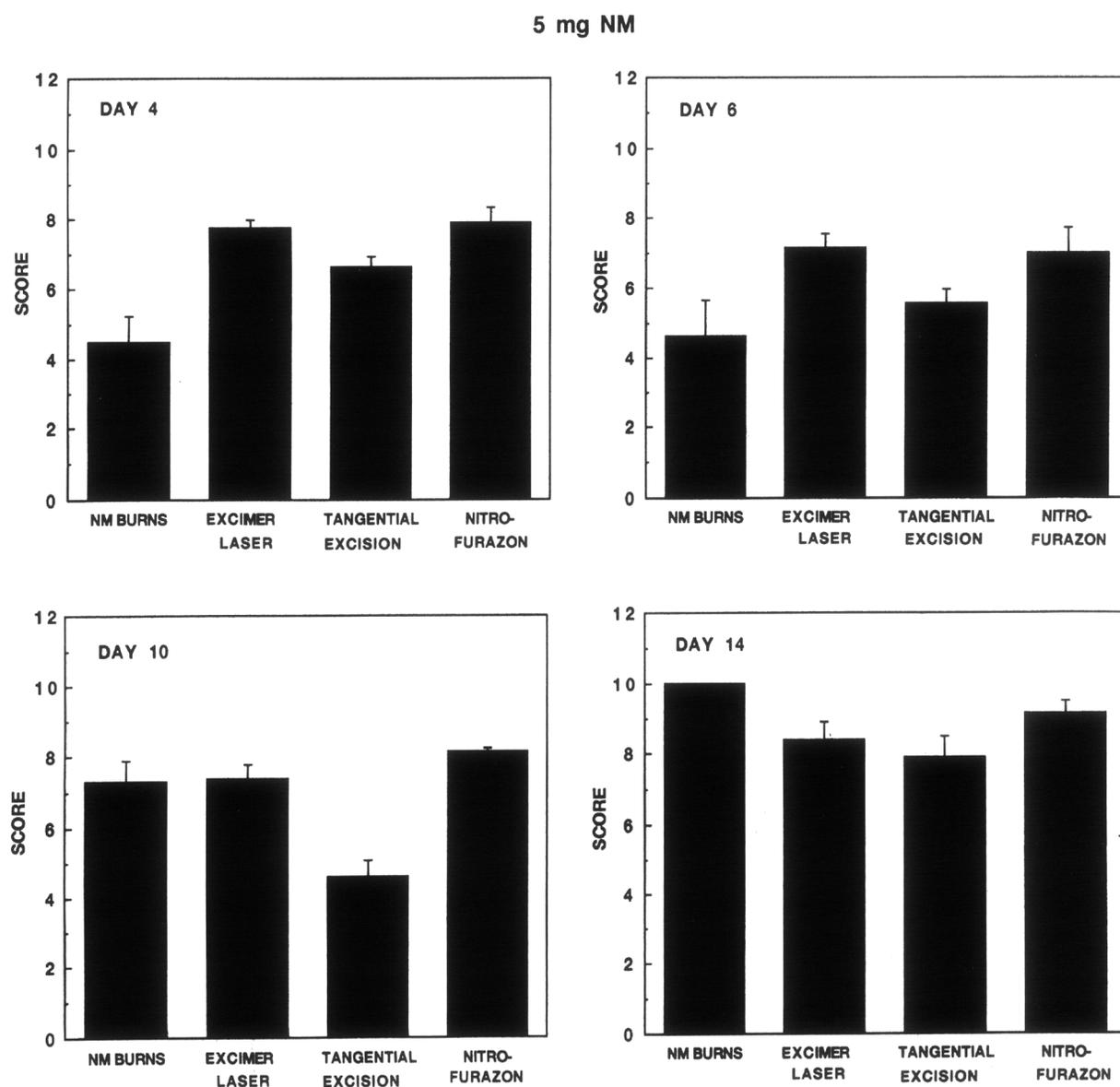


Fig. 4. Histopathologic changes in biopsies from wound bed after various treatments of NM burns ('dry-exposure' to 5 mg NM).

and skin folds of the body [1,2,4,6,7]. It is noteworthy that the clinical experience with Debridase treatment of partial thickness thermal burns was also encouraging, resulting in a graftable wound bed that healed within 10–14 days (A. Eldad, unpublished results).

Finally by offering this simplified in vivo model it is felt that questions of whether certain therapeutic procedures are effective and suitable for treatment of partial thickness chemical burns can be answered directly. Protocols and evaluation procedures are of importance since awareness that violence exists as a part of life and concern about these injuries is not over.

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