EFFECTS OF COMPOUND 48/80 AND EXOGENOUS HISTAMINE ON WOUND HEALING IN MICE[1]

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ABSTRACT. Compound 48/80 has previously been shown to improve wound healing in rats, presumably through stimulation of histidine decarboxylase activity and mobilization of histamine from mast cells. In the present study, C57Bl/6 mice were wounded by dorsal skin incision followed by treatment with compound 48/80, exogenous histamine, or the combination of 48/80 plus histamine. Skin-breaking strength was significantly increased over saline-injected controls by the combined treatment with 48/80 and histamine. Neither 48/80 or histamine alone had any influence on wound healing. Histamine content of skin at the wound site was significantly reduced by 48/80 treatment, but was unaffected by 48/80 plus histamine or histamine given alone. In contrast, stomach and leg muscle histamine levels were significantly increased beyond those of unwounded, wounded saline- or 48/80-injected mice. These results were also confirmed in CD mice, and are in contrast to findings in rats in which treatment with 48/80 alone significantly improved wound healing of similarly injured animals.

INTRODUCTION. The interrelationship between wound healing and histamine (HM) has been extensively studied (1). It has been shown that HM-forming capacity at the wound site is an important determinant of wound healing as measured by skin-breaking strength and the stimulation of collagen deposition (1).

Since both vasodilation and fibroplasia, stimulated by HM, are believed to be important for wound healing (2,3), endogenous or intracellular HM appears to play a crucial role in wound healing. However, the effect of exogenous HM is still unclear. Kahlson et al. (1) demonstrated that exogenous HM did not significantly influence wound healing as measured by tensile strength of sutured skin incisions, whereas Dabrowski et al. (4) observed that local injections of HM had a stimulatory effect on collagen deposition in low doses and an inhibitory effect in high doses.

Carnosine (ß-alanyl-L-histidine) is considered to be an important precursor for HM in animals subjected to stress. Fitzpatrick and Fisher (5) showed that carnosine in skeletal muscle decreased after hind leg fracture in rats, and Nagai et al. (6) indicated that local treatment with carnosine improved tensile strength of wounds, compared with those of untreated animals.

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In the present study, we evaluated tensile strength of wounds as well as HM concentrations in skin at the wound site, in stomach, and in muscle in C57Bl/6 mice, an inbred strain with low kidney carnosinase activity (7). The mice were treated with exogenous HM as well as with compound 48/80, which releases HM from mast cells and stimulates histidine decarboxylase (HDC) activity (5).

METHODS. Experiment 1. Male C57Bl/6 mice were maintained on a standard commercial laboratory diet (#5015, Purina Mills Inc.) and tap water ad libitum. Animals weighing 20-24 g were divided into five groups of seven mice each. They were anesthetized with pentobarbital (60 mg/kg body weight), and their dorsal region was shaved with an electric shaver. A 3 cm median incision was made and the wound closed with five interrupted sutures of 4-0 silk (Ethicon Inc.). The five groups were treated once daily for 7 days with intraperitoneal injections as follows: Group 1 control, unwounded, not injected; Group 2 wounded (day 1), saline (0.1 ml); Group 3 wounded (day 1), 2.5 mg/kg compound 48/80 (Sigma Chemical Co.) in saline (0.1 ml); Group 4 wounded (day 1), 20 mg/kg histamine dihydrochloride (Sigma Chemical Co.) in saline (0.1 ml); Group 5 20 mg/kg histamine dihydrochloride in saline (0.1 ml) and 2.5 mg/kg compound 48/80 in saline (0.1 ml). All solutions were freshly prepared daily. The last treatments were carried out 15 min before decapitation.

On the seventh day after wounding, the mice were decapitated, and skin of the wound site, stomach, and from leg muscles (gastrocnemius, soleus, and tibialis complex) were removed for the determination of HM concentration by the

Table 1. Effect of Compound 48/80 and of Exogenous Histamine on Wound-Breaking Strength and Tissue Histamine Content of C57BL/6 mice[1]

Animal treatment	Measurement			
	Skin-breaking strength (g)	Tissue histamine (μg/g)		
		Skin	Stomach	Leg Muscle
Not wounded		8.7 ± 1.0[a]	7.3 ± 1.5[a]	0.51 ± 0.17[a]
Wounded				
Saline, ip	84.9 ± 21.4[a,2]	9.7 ± 3.3[a]	10.5 ± 2.0[b]	0.53 ± 0.27[a]
48/80 (2.5 mg/kg), ip	73.8 ± 17.5[a]	5.2 ± 2.6[a]	8.5 ± 2.0[a]	0.51 ± 0.30[a]
Histamine (20 mg/kg), ip	85.4 ± 23.9[a]	10.5 ± 4.8[a]	13.6 ± 1.7[c]	2.60 ± 0.61[b]
48/80 + Histamine, ip	111.9 ± 22.7[b]	9.6 ± 3.2[a]	13.4 ± 1.9[c]	2.22 ± 1.00[b]

[1] Averages ± SD for seven mice, 7 days after wounding and daily injections of the substances listed.
[2] Values within each column with different letter superscripts are significantly different from one another with P varying from 0.05 to 0.0005.

isotope method described by Lee et al. (8). The skin-breaking strength was measured on duplicate strips of skin (1 by 3 cm) from each animal using an Instron tensiometer.

Experiment 2. Male C57Bl/6 mice (20-40 g body weight) were maintained on the commercial laboratory diet and tap water as in Experiment 1. They were assigned into one of three groups treated as follows: Group 1 unwounded, untreated, not injected; Group 2 wounded, saline (0.1 ml) i.p.; Group 3 wounded, 2.5 mg/kg compound 48/80 in saline (0.1 ml) i.p.

The mice were injected once daily in the morning. The last treatments were carried out 15 min before decapitation. Wounded and treated animals were killed on the first, fifth, and seventh day following wounding. The skin of the wound site was removed. HDC activity was determined by the method of Snyder and Epps (9).

RESULTS. Experiment 1. The skin-breaking strength and the HM contents of the skin of the wound site, stomach, and muscle are shown in Table 1.

Compound 48/80 treatment daily for 7 days did not alter skin-breaking strength, but it significantly ($p < 0.05$) reduced skin HM content compared with those of all other treatments. Compound 48/80 did not alter stomach and muscle HM contents compared with those of the unwounded control and saline-injected groups. Treatment with HM did not alter skin-breaking strength, and skin HM concentration was the same as for the control and saline-injected groups. Muscle and stomach HM contents were significantly increased by the HM injections. The combination of HM injection with compound 48/80 significantly increased skin-breaking strength compared with other treatment groups and maintained skin HM levels equal to those of uninjured controls. This was particularly noteworthy in the face of HM mobilization induced by compound 48/80. The muscle and stomach HM levels were increased by the HM plus compound 48/80 treatment to the same extent as observed in the group treated only with exogenous HM.

Experiment 2. HDC activities on days 1, 5, and 7 following wounding of mice is shown in Table 2.

Table 2. Histidine Decarboxylase Activity of Mice with or without Compound 48/80 Treatment for 7 Days following Wounding[1]

Time after wounding (day)	Histidine decarboxylase activity (nmol × 10^{-4}) hr/mg protein		
	Unwounded	Wounded	
		Saline	48/80
0	3.5 ± 0.6[a,2]		
1		21.9 ± 5.5[d]	30.0 ± 12.9[e]
5		12.1 ± 3.2[b]	17.7 ± 7.0[c]
7		9.5 ± 2.4[b]	9.5 ± 2.6[b]

[1] Averages ± SD for five to eight mice per group, 7 days after wounding and daily injections of compound 48/80 or saline.
[2] Values with different letter superscripts are significantly different from one another with P varying from 0.05 to 0.005.

By day 1, HDC activity of the saline-injected, wounded animals was 6.2 times the level of the unwounded controls. Compound 48/80 further elevated HDC activity significantly compared with that of the saline-injected group; its level was 8.6 times that of the unwounded controls. Five days after wounding, the saline- and 48/80-injected wounded groups had significantly decreased HDC activities, compared with the levels observed on the day after wounding. By the seventh day, HDC activities had further decreased significantly compared with the levels observed on the fifth day. However, both treatment groups still showed significantly higher HDC activities than did the unwounded controls.

DISCUSSION. Fitzpatrick and Fisher (5) reported that treatment of rats for 7 consecutive days with compound 48/80 significantly increased skin-breaking strength and increased collagen deposition as well as HDC activity. In confirmation of earlier observations (1), these results had suggested that 48/80 increased HM-forming capacity in tissues and played an important role in wound healing. The results of

this study, however, clearly showed that a similar treatment for 7 consecutive days with compound 48/80 in mice did not alter skin-breaking strength compared with wounded, saline-injected controls. This was surprising, since HDC activity was significantly increased by compound 48/80, but only initially. By day 5 after wounding, the HDC activities of the controls and 48/80-treated mice, although significantly higher than corresponding values for unwounded animals, were similar.

The skin HM levels of the mice treated with compound 48/80 were significantly lower than any of the other groups, including the unwounded animals. This is probably a reflection of the HM-liberating function of 48/80 from mast cells. This lower concentration of skin HM was not reflected in any adverse effect on wound healing.

In contrast to the lack of improvement in wound healing from compound 48/80 treatment alone, the combination of 48/80 plus exogenous HM significantly increased skin-breaking strength (Table 1). Again, it should be noted that this improvement was not linked to any increase in skin HM concentrations. In this regard, the positive results obtained from 48/80 plus HM stand apart, not only from the lack of response from 48/80 alone, but also from treatment with exogenous HM alone. It may well be that the exogenous HM replaced or augmented the HM liberated from mast cells by 48/80. Thus, it cannot be ruled out that a higher level of exogenous HM than administered in the present study (20 mg/kg body weight) might have improved wound healing to the same extent as the combination of 48/80 plus HM.

It was tempting to ascribe the absence of improved wound healing from treatment with compound 48/80 in the C57Bl/6 mice (compared with the earlier rat studies [5,6]) to the low carnosinase activity of this mouse strain. Thus, the results would be logical if carnosine could not be mobilized rapidly enough to provide histidine for HM synthesis following 48/80 stimulation of HDC activity (10). To rule out the possibility that the difference in wound-healing response between the mice in the present study and rats in earlier investigations is, indeed, peculiar to the C57Bl/6 strain, we carried out a similar wound-healing study with mice of the outbred, CD strain. Compound 48/80 treatment of wounded CD mice caused the same, significant reduction in skin HM level as found in C57Bl/6 animals (7.2 ± 1.6 48/80 vs. 11.6 ± 2.5 μg/g saline controls). Similarly, 48/80 treatment did not affect skin-breaking strength in the CD mice (56.9 ± 21.9 48/80 vs. 63.3 ± 26.2 g saline controls). Since the same results were obtained in C57Bl/6 and CD mice, carnosine mobilization as the determining factor in the difference observed between rats and mice is not supported.

The HM content of stomach and leg muscle was altered only by the administration of exogenous HM but not by compound 48/80. This latter finding may reflect the fact that the number of mast cells in skeletal muscle is fewer than that in skin (11) and the fact that compound 48/80 represents a poor stimulus of mucosal mast cell degranulation (12).

These studies support the concept that HM plays an important role in wound healing and that increased HM formation as well as exogenous HM can be beneficially utilized to improve this process.

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