Int J Legal Med (1998) 111 : 6–9

ORIGINAL ARTICLE

K. Laiho

Myeloperoxidase activity in skin lesions

I. Influence of the loss of blood, depth of excoriations and thickness of the skin

Received: 16 December 1996 / Received in revised form: 28 May 1997

Abstract The measurement of the myeloperoxidase activity was used to quantify the acute granulocyte reaction. A 35% loss of blood resulted in a clear decrease of the myeloperoxidase activity at the edges of experimental incision wounds in rat skin in the first day after inflicting the wounds. After 12 h only about one-third and after 24 h about half of the activity observed in the wounds without the loss of blood remained. After 3 days however the activity had returned to the same level as in the wounds without any loss of blood. In very deep excoriations of the rat skin where only a narrow zone of the dermis was left about double the activity increase was observed after 12 and 24 h when compared to values observed in very superficial or moderate excoriations. When the same type of excoriations were made in both thin inguinal skin and thick dorsal skin then a much higher peroxidase activity increase was observed over 4, 12 and 24 h in the thin inguinal skin than in the excoriations made in the thick dorsal skin.

Key words Granulocyte reaction · Myeloperoxidase activity · Skin lesions · Loss of blood · Depth of excoriation · Thickness of skin

Introduction

According to the early reports in the literature [1] a reduction in the inflammatory reaction and healing of wounds is observed e.g. in shock, cachexia, loss of blood etc. As far as the influence of the loss of blood is concerned, an experimental study was published later [2] which reported that the beginning and progression of dermal leucocytosis were not influenced by blood loss but the activation of different enzymes was significantly delayed and decreased. Because of this contradiction it was decided to reinvestigate experimentally some of the factors which could influence the acute granulocyte reaction in traumatic lesions. According to the literature there is a good correlation between the number of granulocytes and the myeloperoxidase activity in the tissues [3–8]. It therefore seemed possible to quantitate the acute granulocyte reaction in the lesions by measuring the levels of myeloperoxidase activity. In the present paper the influence of the loss of blood on the granulocyte reaction measured by myeloperoxidase activity at the edges of the experimental wounds in the rats skin was studied. In addition myeloperoxidase activity measurements were done on experimental excoriations in which the depth of the excoriation varied and which were also made on two different areas of rat skin where the thickness of the skin was different.

K. Laiho (✉)
Department of Forensic Medicine, P.O. Box 40 (Kytösuontie 11), SF-00014 Helsinki, Finland
FAX: +358 (9) 1912 7518

Material and methods

In the experiments male Sprague-Dawley rats were used with a average weight of 500–550 g. The blood loss of 35% of the total blood volume was induced by the method of Strawitz et al. [9] which is a combination of tail bleeding and heart puncture. The procedure usually did not result in any rat mortalities. Prior to starting the tail bleeding, a 5 cm long incision wound perforating the skin was made on the dorsal skin of rats. In the control rats the same type of incision wound was made without any bleeding. Six rats with and without bleeding were sacrified at each of the following times after wounding: 4, 12, 24 h and 3 days. Zones approx. 1 mm thick were removed from the edges of the wounds and these specimens were used for myeloperoxidase activity determination.

To test the influence of the depth of the excoriation very superficial, moderate and very deep excoriations were made on the thick dorsal skin using coarse sandpaper and by holding the skin fixed with forceps during the procedure. In very superficial excoriations, only the epidermis was removed, in moderate excoriations the epidermis and a superficial part of dermis and in very deep excoriations the epidermis and most of the dermis was removed so that only a narrow zone of dermis was left. The excoriated area on the skin was in average about 3 cm^2.

In the studies on the influence of the thickness of the skin, the thick dorsal skin and the thin inguinal skin of the rats were used as the sites for the investigation of the variation of skin thickness. The excoriations on both sites were of same type (moderate) made using coarse sandpaper as above. In another series of experiments compression lesions were made at the same sites of the skin with

forceps, which were always closed to the same clasp and pulled off without opening. In excoriations and compression lesions the whole thickness of the skin which was left was taken as the specimen for the myeloperoxidase determinations. All specimens for peroxidase activity measurements were stored at –70° C until analysis. The rats were anesthetized with ether throughout the experimental procedures described above. Six rats with each type of excoriation, skin thickness or compression lesion were sacrified at each of the following times after infliction: 4, 12 and 24 h. The experiments were approved by the local committee for animal experiments.

The myeloperoxidase determinations were done as previously described [10] The enzyme activity was expressed as units per g dry weight of the tissue (U/g dw), and for this purpose a sufficient aliquot of the homogenate was dried in an oven at 120° C for at least 24 h and weighed. In excoriations and compression lesions the enzyme activity was also expressed as units per cm^2 of the skin surface. The surface area of the skin in the specimens was measured for that purpose. The statistical analysis of the results was performed using Student's *t*-test. In the figures $n = 6$ indicates that in each curve at each time point presented the mean represents observations from six experimental animals.

Results

Loss of blood

Figure 1 shows the effect of a 35% loss of blood on the peroxidase activity at the edges of the wound. After 4 h the mean peroxidase activity was about two-thirds, after 12 h about one-third and after 24 h about one-half of the activity in the control wounds. After 3 days however the activity had increased back to the same level as in the wounds without loss of blood.

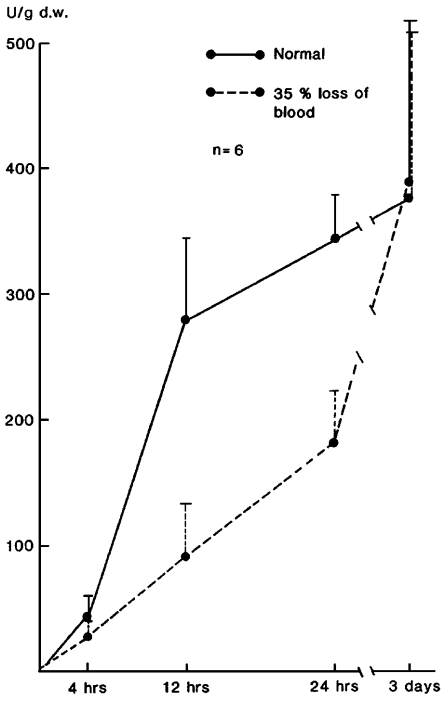

Fig. 1 Influence of 35% loss of blood on the myeloperoxidase activity at the edges of the incision wounds as compared to the wounds of the normal animals without loss of blood. Means and standard deviations are given. The mean values for wounds 12–24 h old are significantly different ($P < 0.001$)

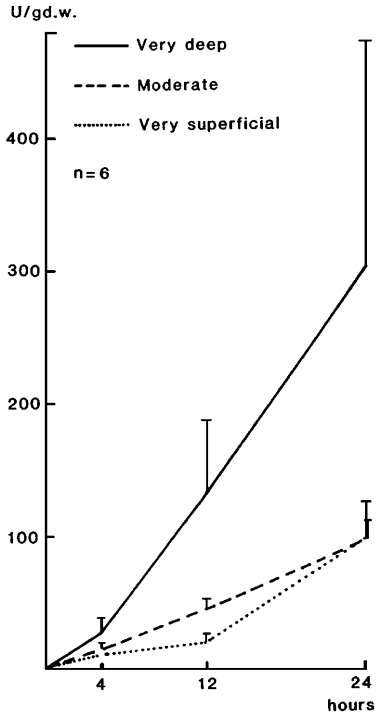

Fig. 2 Myeloperoxidase activity per gram dry weight in different types of excoriations. Means and standard deviations are given. The mean values for very deep excoriations aged 12–24 h are significantly higher compared to moderate and very superficial ones ($P < 0.05$)

Variation of the depth of the excoriations

In the very deep excoriations the myeloperoxidase activity expressed per g dry weight of tissue, increased much more rapidly than in the very superficial or in the moderate ones. In the deep lesions the mean values were about 2 times or more higher after 4, 12 and 24 h (Fig. 2). If the activities were expressed as units per cm^2 the mean values were still about 2 times higher ($P < 0.05$) after 12 and 24 h in the very deep excoriations.

Variation of the thickness of the skin

When the same type of excoriations (moderate) were made on the thick dorsal skin and on the thin inquinal skin the myeloperoxidase activity increase was much more apparent in the thin skin. (Fig. 3) In the compression lesions the difference was nearly the same or somewhat smaller (Fig. 4). If the myeloperoxidase activities were expressed as units per cm^2 the difference was somewhat smaller the mean values in the excoriations of the thin skin being about 3 times higher ($P < 0.01$) after 4 and 12 h and 1.5 times higher ($P < 0.05$) after 24 h. In compression lesions of the thin skin the myeloperoxidase mean values were about 2 times higher ($P < 0.01$) after 12 and 24 h if the activities were expressed as units per cm^2.

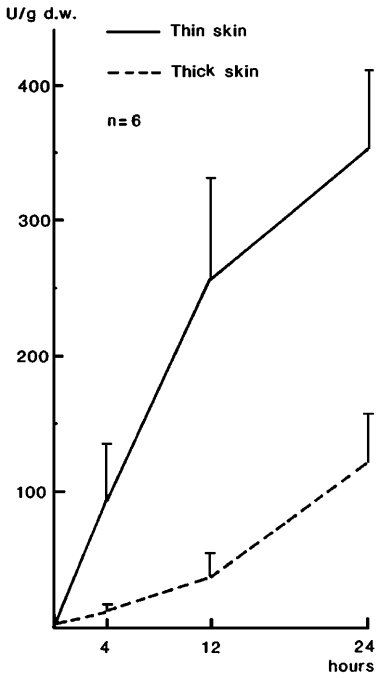

Fig. 3 Myeloperoxidase activity per gram dry weight in the same type of excoriations made on the thick dorsal skin and on the thin inguinal skin. Means and standard deviations are given. The mean values for excoriations aged 4–24 h are significantly different ($P < 0.001$)

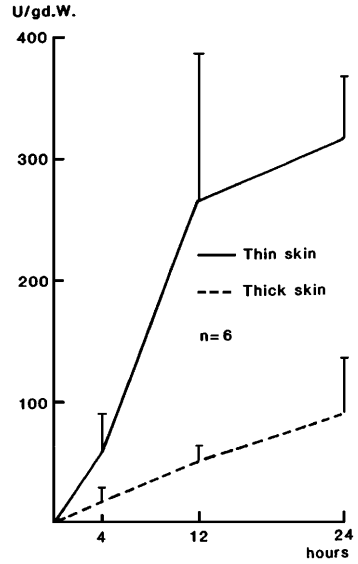

Fig. 4 Myeloperoxidase activity per gram dry weight in the compression lesions made on the thick dorsal skin and on the thin inguinal skin. Means and standard deviations are given. The mean values for lesions aged 12–24 h are significantly different ($P < 0.01$)

Discussion

Loss of blood

In rats hemorrhagic shock was observed at a blood pressure of about 35 mm Hg with an average loss of blood of about 41% of the total blood volume. However if this condition continued over 4 h 90% of the rats died within 48 h [11]. A 53% loss of blood resulted in the death of the rats in about 80–85% of cases within 4 h [9].The 35% loss of blood used in this work was estimated to result in hemorrhagic shock but usually most of the rats tolerated this procedure without mortality during the observation period. In the study of Berg et al. [2] one-third of the total blood volume was removed.

In the present experiments it was observed that when the rats had lost 35% of their total blood volume a remarkable reduction of the myeloperoxidase activity at the edges of the wounds were observed during the first day compared to the wounds without any blood loss. In hemorrhagic shock it has been described that significant vasocontraction and reduction of blood flow in the skin and a decrease in the number of the granulocytes in the peripheral blood occur both of which might retard the inflammatory reaction [12, 13]. The results obtained in this work are in accordance with these shock features and those of Walcher [1]. However in the present work this view is based on the myeloperoxidase activity measurements.

Variation of the thickness of the skin and of the depth of the excoriations

A significant difference in the increase of the myeloperoxidase activity was observed in the thin inguinal skin and in the thick dorsal skin of the rats. The increase in the former was much higher after the same type of excoriations or compression lesions in the skin. The inflammatory cells usually formed a zone against the abraded surface of the skin. If the excoriation is on the thin skin or if the abrasion deeply penetrates, a smaller amount of tissue is obtained as the baseline probably for the same amount of inflammatory cells. The final result expressed per dry weight of tissue is then larger than in thick skin or in superficial excoriations where a greater amount of tissue is taken as the baseline. This however seemed not to be the only reason for the results mentioned above because if this baseline difference was eliminated by expressing the myeloperoxidase activity per surface area again higher values were obtained in the thin skin or in the deep excoriations. At present a great number of different proinflammatory substances and chemical mediators have been described which have the ability to increase vascular permeability, augment adherence of circulating leukocytes to vascular endothelium and promote migration of leukocytes into tissues. Some of the most often mentioned are fibrinopeptide A and B, complement factor C5a, bradykinin, (haemorrhage, coagulation), platelet derived growth factor, platelet factor 4, platelet activating factor, prostaglandin E2 (activated platelets), transforming growth factor beta-1 (activated platelets, endothelial cells, keratinocytes, fibroblasts), interleukin 1, interleukin 8 (endothelial cells, tissue macrophages, mast cells, keratinocytes, fibroblasts), leukotriene B4, tumor necrosis factor (tissue macrophages, mast cells). Where the sources of the mediators are given in

parentheses [14–16] Recently, studies concerning the dynamics of the chemical mediators and mediator-associated antigen activation in the inflammatory cells for a forensic wound age estimation have been published [17–19] It could be speculated that in the deep injuries which result in more tissue damage and haemorrhage, greater amounts of different inflammatory mediators are liberated than in the superficial ones. On the other hand in injuries to thin skin the amount of tissue damage might not be as great as in the deep injuries of the thick skin. However in injuries of the thin skin the distance of the lesion to the subcutaneous tissue and to a zone rich in vascularity between the dermis and subcutaneous tissue [20] might be the same as in the deep injuries of the thick skin. It could be speculated that in both cases the inflammatory mediators are then liberated in the vicinity of rich vascularity so that greater amounts reach their probable main target points i.e. vessel wall and endothelium to which the adhesion and emigration of the granulocytes is connected. The perivascular location of mast cells able to liberate inflammatory mediators may also play some role. Also it is well known that the inflammatory reaction is more rapid in subcutaneous tissue than in the skin [21, 22].

References

1. Walcher K (1930) Über vitale Reaktionen. Dtsch Z Gerichtl Med 15:16–57
2. Berg S, Bode G, Garbe G, Sillus U (1977) Der Einfluss von Blutverlust und Alkohol auf die frühen Wundreaktionen. Z Rechtsmed 80:39–49
3. Smith AL, Rosenberg I, Averill DR, Moxon ER, Stossel T, Smith DH (1974) Brain polymorphonuclear leucocyte quantitation by peroxidase assay. Infect Immun 10: 356–360
4. Bradley PP, Priebat DA, Christensen RD, Rothstein G (1982) Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 78: 206–209
5. Bailey PJ, Sturm A (1983) Immune complexes and inflammation. A study of the activity of anti-inflammatory drugs in the reverse passive Arthus reaction in the rat. Biochem Pharmacol 32:475–481
6. Lundberg C, Arfors KE (1983) Polymorphonuclear leucocyte accumulation in inflammatory dermal sites as measured by 51Cr-labeled cells and myeloperoxidase. Inflammation 7:247–255
7. Lundberg C, Lebel L, Gerdin B (1984) Inflammatory reaction in an experimental model of open wounds in the rat. The role of polymorphonuclear leucocytes. Lab Invest 50:726–732
8. Krawisz JE, Sharon P, Stenson WF (1984) Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. Gastroenterology 87:1344–1350
9. Strawitz JG, Hift H, Temple RL, Ehrhard A, Rozansky N (1961) Irreversible hemorrhagic shock in rats: method and critical bleeding volume. Am J Physiol 200:257–260
10. Laiho K (1989) Removal of contaminating hemoglobin from peroxidase in traumatic skin lesions. Z Rechtsmed 103:101–109
11. Whigham H, Weil MH (1966) A model for the study of hemorrhagic shock in the rat: development of the method. J Appl Physiol 21:1860–1863
12. Shires TG, Canizaro PC, Carrico CJ (1984) Shock. In: Schwartz SI (ed) Principles of surgery. McGraw-Hill, New York, pp 115–164
13. Regel G, Dwenger A, Gratz KF, Nerlich ML, Sturm JA, Tscherne H (1989) Humorale und zellulare Veränderungen der unspezifischen Immunabwehr nach schwerem Trauma. Unfallchirurg 92:314–320
14. Chensue WS, Ward PA (1996) Inflammation. In:Damjanov I, Linder J (eds) Andersons pathology. Mosby, St.Louis, pp 387–415
15. Christophers E (1996) Regulatory factors in cutaneous wound healing. In: Oehmichen M, Kirchner H (eds) The wound healing process – forensic pathological aspects. Schmidt-Römhild, Lübeck, pp 25–34
16. Flad HD (1996) Chemokines and proinflammatory cytokines in wound healing. In: Oehmichen M, Kirchner H (eds) The wound healing process – forensic pathological aspects. Schmidt-Römhild, Lübeck, pp 49–57
17. Kondo T, Oshima T (1996) The dynamics of inflammatory cytokines in the healing process of mouse skin wound: a preliminary study for possible wound age determination. Int J Legal Med 108:231–236
18. Schulz-Schaeffer WJ, Brück W, Püschel K (1996) Macrophage subtyping in the determination of age of injection sites. Int J Legal Med 109:29–33
19. Amberg R (1996) Time-dependent cytokine expression in cutaneous wound-repair. In: Oehmichen M, Kirchner H (eds) The wound healing process – forensic pathological aspects. Schmidt-Römhild, Lübeck, pp 107–121
20. Wheater PR, Burkitt HG, Daniels VG (1987) Functional histology. Churchill Livingstone, Edinburgh London Melbourne New York, p 141
21. Ojala K, Lempinen M, Hirvonen J (1969) A comparative study of the character and rapidity of the vital reaction in the incised wounds of human skin and subcutaneous adipose tissue. J Forensic Med 16:29–34
22. Berg S (1975) Vitale Reaktionen und Zeitschätzungen. In: Mueller B (ed) Gerichtliche Medizin. Springer, Berlin Heidelberg New York, pp 326–340