Int J Legal Med (1999) 112 : 249–252

ORIGINAL ARTICLE

T. Kondo · T. Ohshima · W. Eisenmenger

Immunohistochemical and morphometrical study on the temporal expression of interleukin-1α (IL-1α) in human skin wounds for forensic wound age determination

Received: 24 August 1998 / Received in revised form: 21 October 1998

Abstract An immunohistochemical and morphometrical study on the temporal expression of interleukin-1α (IL-1α) was performed on 40 human skin wounds with different wound ages. Immunohistochemically, polymorphonuclear neutrophils mainly showed positive reactions for IL-1α in wounds aged between 4 h and 1 day, but with increasing wound age, neutrophils were no longer present, and macrophages and fibroblasts were positively stained. Morphometrically, the ratio of the number of IL-1α-positive infiltrating cells to the total number of infiltrating cells was evaluated. A considerable increase in the IL-1α-positive cell ratio was observed in wound specimens aged 4 h to 1 day, and the maximum ratio was 46.5% in a 7 h-old wound. The mean value of the IL-1α-positive ratio in 10 wound specimens with different wound ages between 4 h and 1 day was 32.8 ± 9.7%. In most cases the ratio of IL-1α-positive cells gradually decreased in wounds aged between 1.5 and 21 days to less than 30%, and the mean value was 17.5 ± 7.2% (n = 27). These results suggest that ratios of IL-1α-positive cells considerably exceeding 30%, indicate a postinfliction interval of 1 day or less.

Key words Forensic pathology · Wound age determination · Immunohistochemistry · Morphometrical analysis · Interleukin-1α (IL-1α)

This study was presented at the 3rd World Congress on Inflammation (Tokyo, November 1997)

T. Kondo · T. Ohshima (✉)
Department of Legal Medicine,
Kanazawa University Faculty of Medicine, School of Medicine,
Takara-machi 13-1, Kanazawa 920-8640, Japan
Fax +81-76-234-4234

W. Eisenmenger
Department of Legal Medicine, University of Munich,
Frauenlobstrasse 7a, D-80337 München, Germany

Introduction

There have been many studies on the forensic aspects of wound age determination [1–8]. At present, the immunohistochemical technique is mainly used for wound age determination. In particular, Betz et al. [9–12] examined extracellular matrix components (e.g., fibronectin, collagen fibers, etc.) and showed that they could be used as markers for wound age determination. Dreßler et al. [13] demonstrated the availability of intercellular adhesion molecule-1 (ICAM-1). Recently, morphometrical analysis in addition to an immunohistochemical or histochemical method was performed for a more accurate and objective estimation of wound age [14–16].

The present authors have performed experimental studies on the temporal dynamics of interleukin-1α (IL-1α) in the healing process of mouse skin wounds [17, 18]. The results of the animal experiments suggested that IL-1α could be a possible marker for wound age determination. In the present study, the authors re-examined whether IL-1α is a useful marker for wound age determination with human skin wounds in forensic autopsies.

Materials and methods

Materials

A total of 40 human skin wounds (8 stab wounds, 4 incised wounds, 25 surgical wounds and 3 lacerations) with different wound ages from a few minutes to 21 days were removed at autopsy (Department of Legal Medicine, University of Munich). The 40 wound specimens were obtained from 28 males and 12 females, the individual ages ranged from 8 to 75 years (mean age: 40.6 years) and the postmortem interval was less than 3 days in each case. None of the subjects had suffered from severe malnutrition, malignant diseases or metabolic disorders, and no substances such as cytostatic agents or glucocorticoids, which may influence wound healing, were administered during medical treatment. Unwounded skin from the same individuals was also taken as a control.

Immunohistochemical procedure

Specimens of wounded and unwounded skin were fixed in 4% formaldehyde solution with phosphate-buffered saline (PBS; pH

7.2), embedded in paraffin and sectioned at a thickness of 4–6 μm. Polyclonal rabbit antibody against human IL-1α (Genzyme, USA) was used as the primary antibody, and the alkaline phosphatase-anti-alkaline phosphatase (APAAP) method was employed to visualize the localization of IL-1α.

Morphometrical analysis

In each section, 10 microscopic fields (magnification × 200) were randomly selected, and the ratio of the number of IL-1α-positive infiltrating cells (neutrophils, macrophages and fibroblasts) to the total number of infiltrating cells was calculated in each microscopic field. The average ratio of the 10 selected microscopic fields was evaluated in each wound specimen.

Statistical analysis

Statistical significance was determined by the T-test with $P < 0.05$ considered significant.

Results

Immunohistochemistry

In unwounded skin specimens, only epidermal cells and sweat glands were positively immunostained with anti-IL-1α antibody.

In three wound specimens with wound ages of less than 30 min, no infiltrating cells were observed. Many polymorphonuclear neutrophils were observed first in a 4 h-old wound and the cytoplasm of the neutrophils was positively immunostained with anti-IL-1α antibody (Fig. 1). Furthermore, neutrophils showed a positive immunoreaction in wound specimens aged 5 h–1 day. With an increase in wound age, the IL-1α positive neutrophils had almost disappeared from the wound sites, and phagocytic macrophages and fibroblasts with positive reactions for IL-1α were observed (Fig. 2).

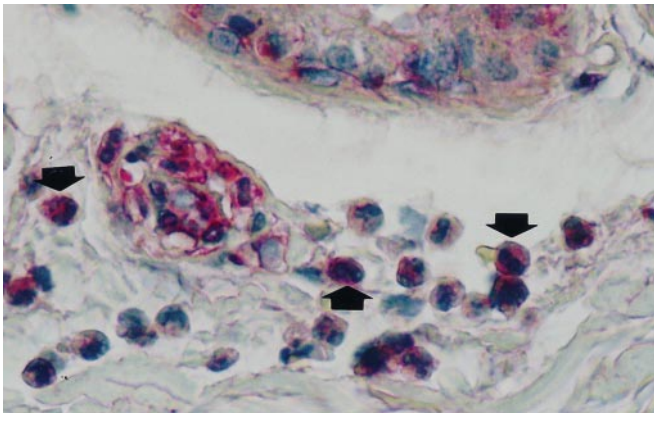

Fig. 1 A 4-hour-old skin wound with infiltration of neutrophils (arrows) showing a positive reaction (red in color) for IL-1α (APAAP, × 400)

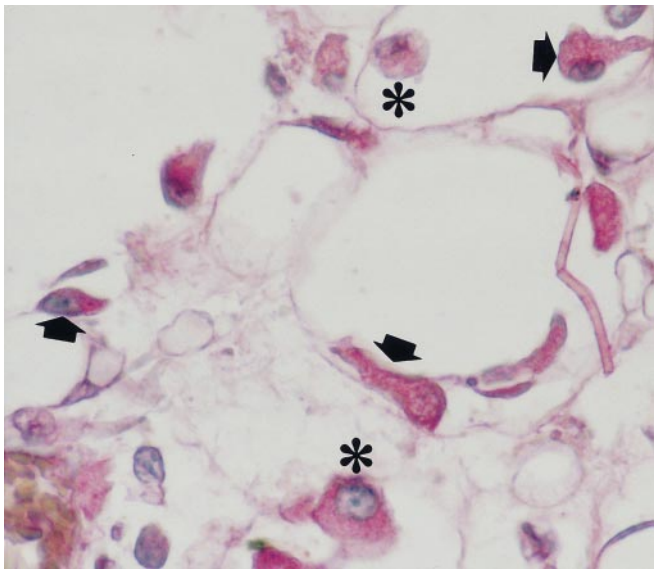

Fig. 2 In this 2-week-old wound, fibroblasts (arrows) as well as macrophages (asterisks) are immunostained with anti-IL-1α antibody (APAAP, × 400)

Morphometrical analysis

Figure 3 demonstrates the distribution of the ratios of IL-1α-positive infiltrating cells in relation to wound age. A considerable increase in the IL-1α-positive cell ratio was observed in wound specimens aged 4 h–1 day, and the maximum ratio was 46.5% in a 7 h-old wound. Thereafter, in the wound specimens with wound ages ranging from 1.5 to 21 days, the IL-1α-positive cell ratio gradually decreased with an increase in wound age. The mean positive ratio in the 10 wound specimens with the wound ages between 4 h and 1 day was 32.8 ± 9.7%. In most of the 27 wound specimens with wound ages between 1.5 and 21 days (except for a 4-day-old wound specimen), the

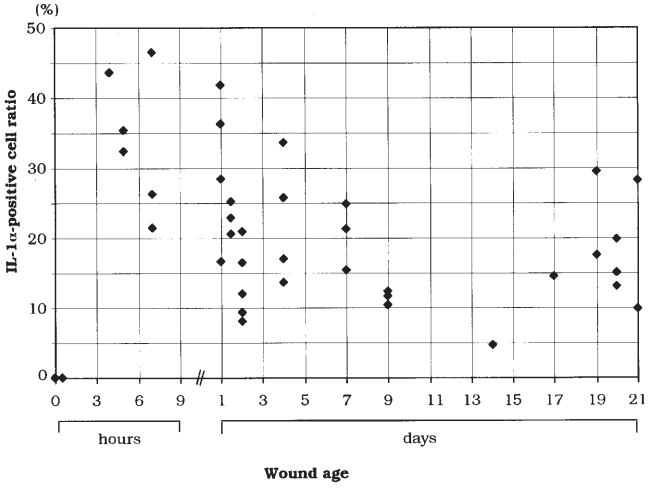

Fig. 3 Ratio of IL-1α-positive infiltrating cells in relation to the wound age

IL-1α-positive ratios were less than 30%, and the mean value was 17.5 ± 7.2%. Statistically, a significant difference in the mean ratio of IL-1α-positive cells was observed between wound specimens with wound ages ranging from 4 h to 1 day and those aged over 1.5 days ($P < 0.05$).

Relevant differences in the IL-1α positive ratio in relation to sex, individual ages and wound type, which should be taken into consideration for wound age determination, were not found.

Discussion

IL-1 is a biological polypeptide produced by various kinds of cells such as macrophages, neutrophils, fibroblasts and astrocytes [19]. There are two different genes for IL-1 proteins (IL-1α and IL-1β) [20, 21], although there is no difference in the biological functions. IL-1 has different biological functions by autocrine or paracrine such as activation of neutrophils and fibroblasts, production of acute phase proteins, induction of other cytokines (e.g., interleukin-6 and tumor necrosis factor-α), and regulation of glucocorticoid hormone production [22]. In particular, IL-1 is induced in the early inflammatory phase after invasive stimuli including trauma and bacterial infection and initiates inflammatory reactions. Therefore, IL-1 is known as a *pro*inflammatory cytokine or *alarm* cytokine [23].

As shown in immunohistochemical studies [24, 25], in normal human skin specimens, IL-1α was observed in epidermal cells and sweat glands. It appears that IL-1α is distributed in the epidermis in the normal physiological conditions as well as in the invasive conditions, since the skin is always under stimulation from the external environment. This speculation can be supported by the fact that the messenger RNA of cytokines including IL-1α is detected in unwounded skin specimens by the reverse transcription-polymerase chain reaction (RT-PCR) method [26, 27]. In the wounded skin specimens, epidermal cells and sweat glands were also positively immunostained. Therefore, it is considered that IL-1α-positive epidermal cells and sweat glands can provide no useful information for wound age determination and infiltrating cells such as neutrophils, macrophages and fibroblasts should be morphometrically analyzed.

The wound healing process is a well-controlled biological phenomenon that consists of inflammatory, proliferative and maturation phases. According to previous studies [17, 28], IL-1 is closely involved in the inflammatory phase, which precedes the proliferative and maturation phases. From medico-legal aspects, the results of a previous study [17] suggested that IL-1α might be a useful marker for forensic wound determination. However, this experimental study was not sufficient to confirm the usefulness of IL-1α as a wound age marker in forensic autopsies, since the results were obtained in animal experiments performed under strictly controlled conditions. According to previous studies [7–12, 14–16], human skin wound specimens with a post-mortem interval of less than 3 days were used to minimize unspecific background staining. In this study, wound specimens with a post-mortem interval of less than 3 days were thus examined.

The immunohistochemical localization of IL-1α in this study showed no differences concerning the kinds of positively stained cells between this study and the previous experimental studies using mice [17, 18]. The temporal course of the IL-1α positive ratio in this study was also similar to that of the IL-1α protein level in a previous experimental study [17]. The results obtained from the present study are closely correlated to patho-physiological concepts of wound healing, since high expression of IL-1α, as a *pro*inflammatory cytokine or *alarm* cytokine can be expected in wound specimens with a wound age of less than 1 day, whereas the IL-1α positive ratio decreased with increasing postinfliction intervals.

From the view point of forensic pathology, the immunohistochemical detection of IL-1α in infiltrating cells is useful for wound age determination in human skin wounds. In six out of ten wound specimens aged between 4 h and 1 day, IL-1α-positive ratios were greater than 30% (mean: 32.8%). However, in most of the 27 wound specimens with wound ages between 1.5 and 21 days, the ratios were less than 30%(mean: 17.5%). These results suggest that IL-1α can be a useful marker for wound age determination and that IL-1α-positive ratios, considerably exceeding a ratio of 30%, indicate a wound age of 1 day or less. Moreover, it is considered that the combined evaluation of positive cell types and positive ratios may become more useful for wound age determination.

Acknowledgements This study was financially supported by a Grant-in-Aid for Scientific Research (T.O.; 08457146) from the Ministry of Education, Science, Sports and Culture of Japan.

References

1. Raekallio J (1972) Determination of the age of wounds by histochemical and biochemical methods. Forensic Sci Int 1:3–16
2. Laiho K (1998) Myeloperoxidase activity in skin lesions. I. Influence of the loss of blood, depth of excoriations and thickness of the skin. Int J Legal Med 111:6–9
3. Laiho K (1998) Myeloperoxidase activity in skin lesions. II. Influence of alcohol and some medicines. Int J Legal Med 111: 10–12
4. Eisenmenger W, Nerlich A, Glück G (1988) Die Bedeutung des Kollagens bei Wundaltersbestimmung. Z Rechtsmed 100: 79–100
5. Oehmichen M (1990) Die Wundheilung. Springer, Berlin Heidelberg New York, pp 5–67
6. Oehmichen M, Cröpelin A (1995) Temporal course of intravital and postmortem proliferation of epidermal cells after injury – an immunohistochemical study using bromodeoxyuridine in rats. Int J Legal Med 107:257–262
7. Betz P (1994) Histological and enzyme histochemical parameters for the age estimation of human skin wounds. Int J Legal Med 107:60–68
8. Betz P, Nerlich A, Wilske J, Tübel J, Penning R, Eisenmenger W (1993) The time-dependent localization of Ki-67 antigen-positive cells in human skin wounds. Int J Legal Med 106:35–40

9. Betz P, Nerlich A, Wilske J, Tübel J, Wiest I, Penning R, Eisenmenger W (1992) Immunohistochemical localization of fibronectin as a tool for the age determination of human skin wounds. Int J Legal Med 105:21–26
10. Betz P, Nerlich A, Wilske J, Tübel J, Wiest I, Penning R, Eisenmenger W (1992) The time-dependent rearrangement of epithelial basement membrane in human skin wounds – immunohistochemical localization of collagen IV and VII. Int J Legal Med 105:93–97
11. Betz P, Nerlich A, Wilske J, Tübel J, Penning R, Eisenmenger W (1993) Analysis of the immunohistochemical localization of collagen type III and V for the time-estimation of human skin wounds. Int J Legal Med 105:329–332
12. Betz P, Nerlich A, Wilske J, Tübel J, Penning R, Eisenmenger W (1993) Immunohistochemical localization collagen types I and VI in human skin wounds. Int J Legal Med 106:31–34
13. Dreßler J, Bachmann L, Kasper M, Hauck JG, Müller E (1997) Time dependence of the expression of ICAM (CD-54) in human skin wounds. Int J Legal Med 110:299–304
14. Betz P, Nerlich A, Tilbel J, Wiest I, Hausmann R (1997) Detection of cell death in human skin wounds of various ages by an in situ end labeling of nuclear DNA fragments. Int J Legal Med 110:240–243
15. Betz P, Eisenmenger W (1996) Morphometrical analysis of hemosiderin deposits in relation to wound age. Int J Legal Med 108:262–264
16. Hausmann R, Nerlich A, Betz P (1998) The time-related expression of p53 protein in human skin wounds – a quantitative immunohistochemical analysis. Int J Legal Med 111:169–172
17. Kondo T, Ohshima T (1996) The dynamics of inflammatory cytokines in the healing process mouse skin wound: a preliminary study for possible wound age determination. Int J Legal Med 108:231–236
18. Kondo T, Ohshima T (1996) Experimental study on the estimation of skin wound age after injury by immunostaining interleukin 1α, collagen type I and fibronectin. In: Oehmichen M, Kirchner H (eds) Rechtsmedizinische Forschungsergebnisse, Band 13, The wound healing process – Forensic pathological aspects. Schmidt-Römhild, Lübeck, pp 123–132
19. Oppenheim JJ, Kovacs EJ, Matsushima K, Durum SK (1986) There is more than one interleukin-1. Immunol Today 7:45–56
20. Auron PE, Webb AC, Rosenwasser LJ, Mucci SF, Rich A, Wolff SM, Dinarello CA (1984) Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. Proc Natl Acad Sci USA 81:7907–7911
21. Lomedico PT, Gubler U, Hellmann CP, Dukovich M, Giri JG, Pan YCE, Collier K, Semionow R, Chua AO, Mizel SB (1984) Cloning and expression of murine interleukin-1 cDNA in *Escherichia coli*. Nature 312:458–462
22. Dinarello CA (1994) Interleukin-1. In: Thomson A (ed) The cytokine handbook, 2nd edn. Academic Press, London, pp 31–56
23. Baumann H, Gauldie J (1994) The acute phase response. Immunol Today 15:74–80
24. Anttila HSI, Reitamo S, Erkko P, Miettinen A, Didierjean L, Saurat JH (1990) Membrane and cytosolic interleukin-1 alpha and beta in normal human epidermal cells: variability of epitope exposure in immunohistochemistry. J Invest Dermatol 93: 31–38
25. Reitamo S, Anttila HSI, Didierjean L, Saurat JH (1990) Immunohistochemical identification of interleukin 1α and β in human eccrine sweat-gland apparatus. Br J Dermatol 122:315–323
26. Ohshima T, Sato Y (1998) Time-dependent expression of interleukin-10 (IL-10) mRNA during the early phase of skin wound healing as possible indicator of wound vitality. Int J Legal Med 111:251–255
27. Sato Y, Kondo T, Minamino T, Ohshima T (1997) Gene expression of inflammatory cytokines during mouse skin wound healing. Inflamm Res 46:s220
28. Ford HR, Hoffman RA, Wing EJ, Magee DM, McIntyre L, Simmons R (1989) Characterization of wound cytokines in the sponge matrix model. Arch Surg 124:1422–1428