Int J Leg Med (1993) 105:329–332

International Journal of
Legal Medicine

Analysis of the immunohistochemical localization of collagen type III and V for the time-estimation of human skin wounds

P. Betz[1], A. Nerlich[2], J. Wilske[1], J. Tübel[1], R. Penning[1], and W. Eisenmenger[1]

Departments of Legal Medicine[1] and Pathology[2], University of Munich, Frauenlobstrasse 7a, W-8000 München 2, Germany

Received June 22, 1992 / Received in revised form November 30, 1992

Summary. Collagen type III and V were visualized immunohistochemically in 79 surgically treated human skin wounds with a wound age between 8 h and 2.5 months. Network-like structures positively staining for collagen type III and associated with fibroblastic cells in the wound area were first detectable in a 2.5-day-old skin lesion and occurred regularly in wounds more than 5 days old. Collagen type V appeared first in the wound area after about 3 days, slightly later than collagen type III, and was detectable regularly in wounds with a survival time of 6 days or more. The immunohistochemical detection of collagen type III or type V thus indicates a wound age of at least 2–3 days. The lack of a positive reaction in a sufficient number of specimens indicates a wound age of less than 6 days. Even though both collagen types could also be detected in older wounds (wound age 2.5 months), further information for the time-estimation of older skin wounds cannot be given due to the observation that the time period during which reparative processes can be observed depends on the extent of the wound area.

Key words: Collagen type III – Collagen type V – Wound age – Immunohistochemistry

Zusammenfassung. Es wurden insgesamt 79 chirurgisch versorgte menschliche Hautwunden untersucht und Kollagen Typ III und V immunhistochemisch dargestellt. Netzwerk-artige und Kollagen Typ III-positive, mit Fibroblasten des Wundgebietes assoziierte Strukturen waren erstmals nach 2,5 Tagen Überlebenszeit nachweisbar und traten ab einem Wundalter von mehr als 5 Tagen regelmäßig auf. Eine entsprechende Reaktion für Kollagen Typ V war erstmals nach 3 Tagen und damit etwas später als für Kollagen III zu beobachten. Regelmäßig war eine entsprechende Reaktion ab einem Wundalter von 6 Tagen feststellbar. Der positive immunhistochemische Nachweis von Kollagen Typ III bzw. Typ V belegt somit ein Wundalter von mindestens 2–3 bzw. 3 Tagen, das Fehlen positiver Reaktionen weist auf eine Überlebenszeit von weniger als ca. 6 Tagen hin. Beide Kollagen-Typen waren auch in älteren Wunden (Wundalter 2,5 Monate) nachweisbar, eine weitere Differenzierung des Alters von länger überlebten Verletzungen ist jedoch wegen der Abhängigkeit der längsten Nachweisbarkeit einer reaktiven Veränderung und der ursprünglichen Ausdehnung der Wundfläche nicht möglich.

Schlüsselwörter: Kollagen III – Kollagen V – Wundalter – Immunhistochemie

Introduction

The collagen family comprises a group of extracellular matrix proteins with various functional properties. Up to 14 different collagen subtypes have been identified and at least 6 are identifiable in skin. They can be subdivided into interstitial collagens (type I, III, V, and VI) and into specific basement membrane-collagens (type IV and VII) [11].

These collagen subtypes presumably fulfill major functions during reparative processes, especially during wound healing. On the basis of the diversity of function of these extracellular matrix proteins, time-dependent differences in the appearance of collagen subtypes in the wound area could exist which may be applicable for the estimation of the time since infliction in human skin wounds.

The appearance of the collagen types I, III, IV, and VII in the wound area has previously been investigated by immunohistochemistry [2, 3, 4, 6]. Studies dealing with the time-dependent localization of collagen type V or VI in human skin wounds have not yet been published.

The present study was performed to localize the interstitial collagen type V presuming an early occurrence of this collagen type in the granulation tissue of wounds. These findings were correlated to the appearance and

Correspondence to: P. Betz

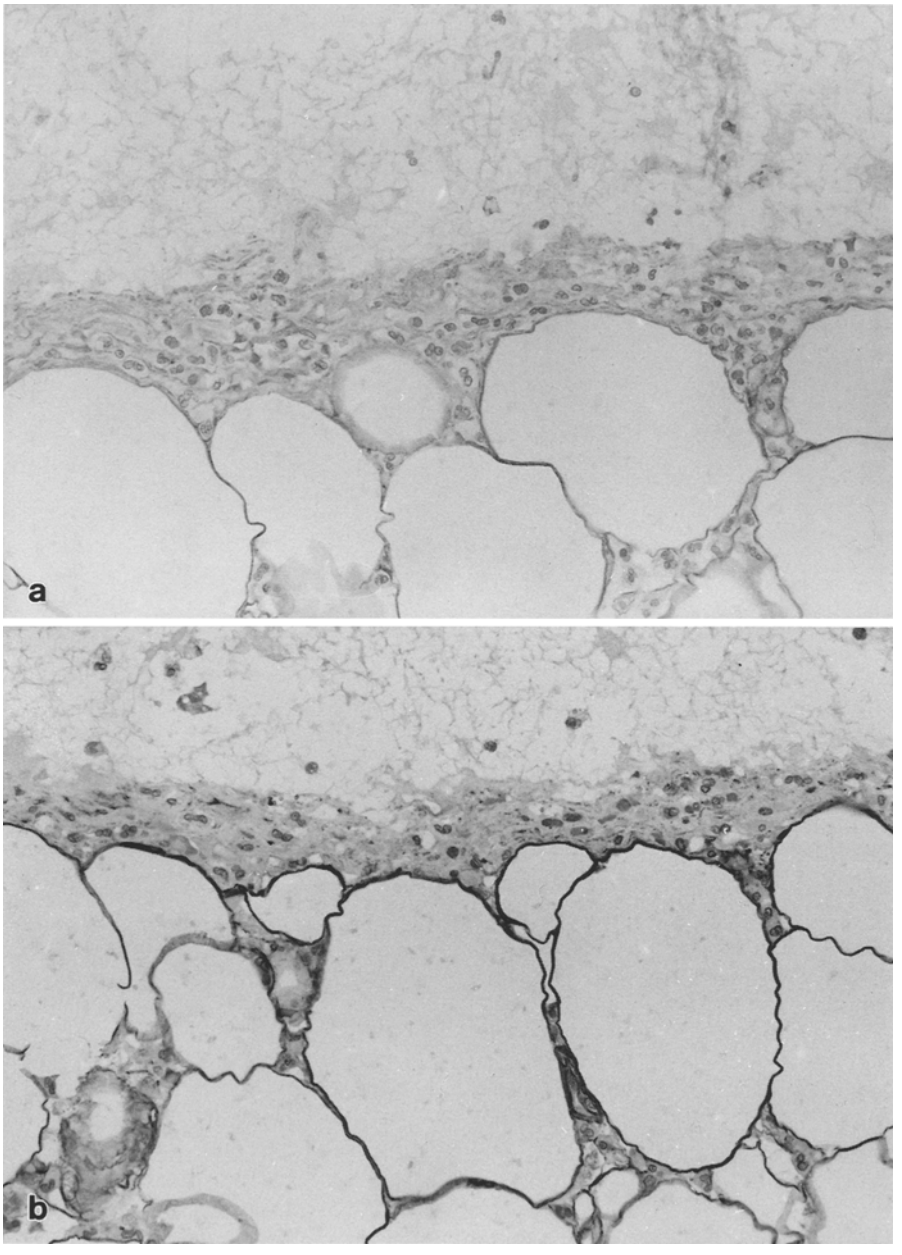

Fig. 1.a, b. Serial sections of a 4-day-old human skin wound (paraffin, ABC-method, × 380). **a** immunohistochemical localization of collagen type III in the subcutaneous layer adjacent to the wound area. **b** localization of type V collagen; slightly reduced amounts when compared with collagen type III

localization of collagen III – another interstitial collagen sub-type already investigated in previous studies [4, 6].

Materials and methods

A total of 79 human skin wounds (surgical wounds, stab wounds and lacerations after surgical treatment) with a wound age since infliction between 8 h and 2.5 months were investigated. The specimens were obtained and prepared as previously described [2, 3]. The immunohistochemical staining was performed using polyclonal antibodies directed against human collagen type III (kindly supplied by Dr. E. Schleicher, Institut für Diabetes-Forschung, Hospital Schwabing-Munich, Germany) and collagen type V (kindly supplied by Dr. R. Brenner, Max-Planck-Institut für Biochemie, Martinsried, Germany) according to the ABC-method [9]. The specificity of both antibodies had been tested before by ELISA showing monospecificity of each antibody. Non-traumatized skin from the same patients as well as sections without application of the primary antibody acted as controls. Furthermore, 15 wounds were produced postmortem in the same patients and also investigated.

Specimens were only regarded as "positive" if they showed distinct positively reacting ramifying or network-like structures associated with fibroblastic cells in the wound area.

Results

Normal skin

In undamaged skin a positive staining for both collagen types III and V was detected diffusely throughout the dermis with a pronounced reactivity in the papillary layer. Furthermore, a strongly positive reaction was observed near the basement membranes of the epidermal

layer, of skin appendages, of nerve muscle and fat cells as well as in blood vessel walls.

Postmortem lesions

In skin wounds inflicted after death no positive reactions for collagen type III or V could be observed in the "wound area" apart from the normally reacting structures. In some specimens, however, strongly reacting string-like structures due to damaged connective tissue fibers or fat cells could be detected. In these cases positively staining structures were not associated with fibroblastic cells and could therefore be excluded.

Skin wounds

A distinct positive staining for type III collagen in the form of network-like structures was not detectable in lesions less than 2.5 days old. In wounds aged more than 5 days, collagen type III could be observed and there was an increase in staining intensity with advancing wound age. In 6 out of 17 wounds (35%) aged between 2 and 5 days no definite positive reaction for this protein was found apart from the normally reacting structures acting as internal controls.

Collagen type V was first observed 3 days after wound infliction in the lesional area and therefore appeared somewhat later than collagen III. In serial sections of the same skin wound the staining intensity for collagen III exceeded that of collagen V due to the different time-dependent appearance in the wound area (Fig. 1). Collagen type V was detectable in all cases with a duration of 6 days or more. Negative results were obtained in only 4 out of 18 cases (22%) with a survival time between 3 and 6 days.

Both collagen types III and V were demonstrable in the oldest skin wound investigated (wound age: 2.5 months) and the reaction was limited to thin fibrillar structures in the scar tissue, but distinct positively reacting networks as found in wounds with shorter survival times, could not be observed.

Even though some wounds of older individuals showed a somewhat delayed appearance of reparative changes in comparison to younger ones, no relevant time-dependent differences in the appearance of the collagen types which would exceed the interindividual variability could be observed. In addition, the localization of the wound also seemed to be of less importance since no significant differences in the appearance of these proteins depending on wound localization could be found.

Discussion

Previous reports from this group have dealt with the time-dependent appearance of the collagen types I, III, IV, and VII in the wound area as demonstrated by immunohistochemistry [2–4, 6]. Studies dealing with the immunohistochemical localization of collagen type V or VI in human skin wounds have not been performed.

The collagen types III and V are presumed to fulfill different functions during wound healing. Type III collagen may provide the first basic structure for wound contraction to occur [5], whereas collagen type V may be involved in the migration of endothelial and pericytic cells during angiogenesis [8]. Furthermore, it has been suggested that type V collagen controls the diameter of type I fibers by hybrid-fibril-formation [1] which may also have a major impact on tissue repair.

In experimental studies collagen type III was found to appear in the wound area as early as 24–48 h after wound infliction [7, 10].

Eisenmenger et al. [6] investigated 24 human skin lesions using immunofluorescence and detected collagen type III in wounds with a duration of more than 2 days.

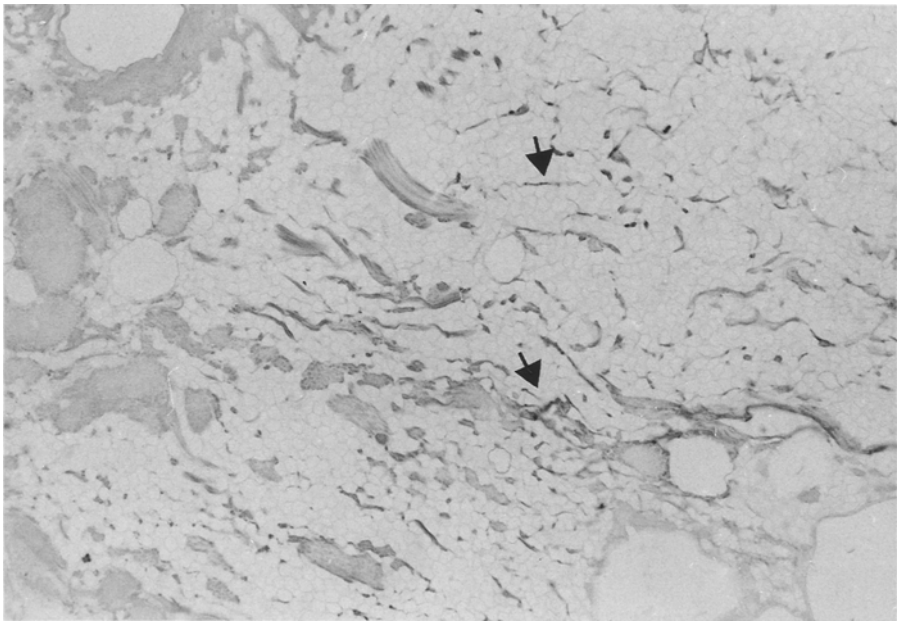

Fig. 2. 1.5-day-old skin wound: string-like structures positively staining for collagen type III *(arrows)* due to destroyed connective fibers in the wound area; no association with fibroblastic cells (paraffin, ABC-method, × 380)

This concurs with the results obtained for collagen type III in our series in the wound area of skin wounds with a duration of 2.5 days or more.

The time-dependent immunohistochemical appearance of collagen type V has only been investigated in experimental studies [12]. These authors reported the occurrence of collagen type V 7 days after implantation, but collagen type III and type IV were first detectable 30 days after implantation. The enormous variations in the occurrence of these various matrix components means that these results cannot be applied to any time-estimation of human skin wounds.

In our series the earliest distinct positive staining for type V collagen was detectable in the lesional area of a skin wound after 3 days, i.e. somewhat later than for collagen type III. In serial sections the staining intensity for collagen V was reduced in comparison to collagen III and can be explained by the delayed appearance of type V collagen.

Both collagen type III and V were detectable in the scar tissue of older skin wounds (wound age 2.5 months), especially in the form of thin fibrillar strands mainly associated with fibroblasts. Further information, however, useful for the time-estimation of older skin wounds cannot be obtained since the interval in which reparative changes can be observed depends on the extent of the former wound area.

For the evaluation of the immunohistochemical localization of collagen type III or V in human skin wounds it must be emphasized that only network-like structures positively staining for collagen type III or V which are associated with fibroblastic cells can be regarded as a vital reaction. String-like structures positive for these collagen types can also occur in the wound area of vital skin lesions and in postmortem wounds due to damaged connective tissue fibers of fat cells and must not be interpreted as a vital sign (Fig. 2).

Conclusion

Our results provide the following information for the time-estimation of human skin lesions by the immunohistochemical localization of type III and type V collagen:

1. Positive reactions for collagen type III indicate a wound age of at least 2–3 days and positive staining for collagen V in the wound area a survival time of 3 days or more.

2. Collagen type III and V occur regularly after 6 days or more and the lack of a positive reaction in a sufficient number of specimens indicates that a wound is aged less than 6 days.

3. Only network-like structures in the wound area showing distinct positive staining and associated with infiltrates of fibroblastic cells can be regarded as "positive".

4. Collagen types III and V are also detectable in older skin wounds (maximum wound age investigated: 2.5 months), but further information for a time-estimation of older wounds cannot be obtained.

Acknowledgements. This study was supported by grants from the "Deutsche Forschungsgemeinschaft" (grant Be 1555/1-1 and 1-2) and the "Friedrich-Baur-Stiftung", University of Munich, Germany.

References

1. Adachi E, Hayashi T (1986) In vitro formation of hybrid fibrils of type V collagen and type I collagen. Limited growth of type I collagen into thick fibrils by type V collagen. Connect Tissue Res 14:257–266
2. Betz P, Nerlich A, Wilske J, Tübel J, Wiest I, Penning R, Eisenmenger W (1992) The time-dependent rearrangement of the epithelial basement membrane – immunohistochemical localization of collagen type IV and VII. Int J Leg Med 105: 93–97
3. Betz P, Nerlich A, Wilske J, Tübel J, Penning R, Eisenmenger W (1992) The temporary pericellular expression of collagen type IV, laminin, and heparan sulfate proteoglycan in myofibroblasts of human skin wounds. Int J Leg Med 105:169–172
4. Betz P, Nerlich A, Wilske J, Wiest I, Penning R, Eisenmenger W (1992) Comparison of the Solophenyl-Red-polarization method and the immunohistochemical analysis for collagen type III. Int J Leg Med 105:27–29
5. Ehrlich HP (1988) Wound closure: evidence of cooperation between fibroblasts and collagen matrix. Eye 2:149–157
6. Eisenmenger W, Nerlich A, Glück G (1988) Die Bedeutung des Kollagens bei der Wundaltersbestimmung. Z Rechtsmed 100:79–100
7. Gay St, Viljanto J, Raekallio J, Penttinen R (1978) Collagen types in early phases of wound healing in children. Acta Chir Scand 144:205–211
8. Hering TM, Marchant RE, Anderson JM (1983) Type V collagen during granulation tissue development. Exp Mol Pathol 39:219–229
9. Hsu SM, Raine L, Fanger HC (1981) A comparative study of the peroxidase-anti peroxidase method and an avidin-biotin-complex method for studying polypeptide hormones with radio immunoassay antibodies. Am J Clin Pathol 75:734–739
10. Kurkinen M, Vaheri A, Roberts PJ, Stenman S (1980) Sequential appearance of fibronectin and collagen in experimental granulation tissue. Lab Invest 43:47–51
11. van der Rest M, Garrone R (1991) Collagen family of proteins. FASEB J 5:2814–2823
12. Vialle-Presles MJ, Hartmann DJ, Franc S, Herbage D (1989) Immunohistochemical study of the biological fate of a subcutaneous bovine collagen implant in rat. Histochemistry 91: 177–184