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

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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

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 presumption an early occurrence of this collagen type in the granulation tissue of wounds. These findings were correlated to the appearance and
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