Abstract  In wounds that are inflicted at least 60 min before death, histamine levels can increase up to 100%. This functional effect might have a morphometric counterpart. Mast cells play a crucial role in acute inflammatory reactions and in the healing process of wounds. Therefore, the density of these cells was immunohistochemically assessed in tissue from 20 healthy controls (Group 1), 20 vital skin lesions (Group 2) (age range: a few seconds to 1 h), and 20 postmortem lesions (Group 3). A piece of skin close to the vital lesion was also obtained from the homolateral part of the body (Group 4). Mast cell density was significantly higher at the level of the vital lesions (11.28±2.44) than elsewhere (healthy controls 7.66±1.27, postmortem lesions 4.13±1.46, skin close to the vital lesions 4.88±1.59). No differences were found between the values assessed in the skin samples close to the vital lesions and in those in the postmortem lesions. Therefore, mast cell richness in the vital lesions exhibited a proportional morphological correlation with previously detected histamine values in cutaneous vital lesions. These results suggest that the detection of mast cells with immunohistochemical techniques can lead to a high level of discrimination (based on statistical data) between antemortem and postmortem lesions. This method could also be used to ascertain the vitality of lesions.

Keywords  Immunohistochemistry · Mast cells · Postmortem lesions · Vital lesions

Introduction

In many cases, the forensic pathologist is not able to arrive at a diagnosis of the vital origin of some wounds. In forensic case work investigations, there are many situations where the vital or non-vital origin of the wound is able to be established using only macroscopical examination. Other circumstances require additional studies to obtain a more exact diagnosis of tissue vitality. Sensitive methodology must be employed so as to detect the numerous substances involved in the first steps of an inflammatory reaction [1]. During the past 50 years, the development of histochemistry, enzymology, and biochemistry, and the application of these studies to the diagnosis of wound vitality have allowed for a partial solution to the problem [1, 2, 3, 4, 5].

It is well known that histamine, an important vasoactive amine, participates in an acute inflammatory reaction [6]. Endogenous histamine is responsible for initiating vascular changes that involve vasodilatation and increased vascular permeability and other mechanisms are then required to maintain them [7].

In 1965 Fazekas and Virágos-Kis [8] observed that there was an increase in the free histamine content in marks caused by hanging. Their work encouraged a number of forensic pathologists to begin biochemical studies on the possible use of the histamine content in the skin to differentiate antemortem from postmortem wounds and to estimate lesion vitality [9, 10, 11]. In particular, Berg and Bonte demonstrated that the histamine levels in vital skin wounds inflicted at least 60 min before death could increase up to 100% [12].

We hypothesized that these biochemical events might also have a histologic correlation: a quantitative alteration in the number of histamine secreting cells, such as mast cells (MC) [13, 14], could also take place.

We therefore carried out an in situ immunohistochemical study to compare the density of MC in vital wounds that had been inflicted from a few seconds to 1 h before death (according to hospital staff), and in postmortem lesions.
Material and methods

Protocol

A total of 80 lesions were examined and 20 tissue samples were taken during surgical treatment of wounds from patients who gave written consent after having been informed of the purpose of the sampling. The procedures were carried out with strict adherence to Italian law and in accordance with the ethical guidelines of the Italian National Medical Council.

A protocol was designed and the biopsy samples were divided into four different groups as follows:

- Group 1 (n=20) Consensually donated biopsies of clinically healthy skin that had been excised at surgery. These samples were used as positive controls.
- Group 2 (n=20) Vital skin lesions (surgical wounds, lacerations and abrasions) from injuries that had been sustained before death. The age of the examined samples ranged from a few seconds to 1 h. Vital wounds were classified into four different subgroups according to the time elapsed between the moment of injury and the estimated moment of death (survival time) as follows: <5 min (n=9), <15 min (n=6), <30 min (n=3) and <60 min (n=2).
- Group 3 (n=20) Post-mortem lesions that had been obtained during routine autopsies.
- Group 4 (n=20) Control pieces of skin, close to the vital lesions, that had been obtained from the homolateral part of the body.

All the autopsies were performed at the Department of Anatomy, Histology, and Forensic Medicine, a section of Forensic Medicine at the University of Florence (Italy). The cadavers were routinely kept at +4°C until autopsy. The time between death and autopsy was 24 h.

Histochemistry and morphometry

After fixing in Bouin or Carnoy’s fluid [15], all of the specimens were dehydrated in ethanol, embedded in paraplast and five sec-

Fig. 1a–c  Mast cells after anti-tryptase immunohistochemistry. Bouin’s fixation, magnification ×100. a Positive control, b a vital lesion, c a post-mortem lesion