Int J Leg Med (1993) 106:145–147

International Journal of
Legal Medicine

Cathepsin D as a vitality marker in human skin wounds

C. Hernández-Cueto[1], J. A. Lorente[1], I. Pedal[2], E. Villanueva[1], G. Zimmer[2], E. Girela[1], and E. Miltner[2]

[1]Department of Legal Medicine, University of Granada, Av. Madrid 11, E-18071 Granada, Spain
[2]Institute of Legal Medicine, University of Heidelberg, Vossstraße 2, D-69115 Heidelberg, Germany

Received August 2, 1991 / Received in revised form May 27, 1993

Summary. This paper shows the results obtained by studying the lysosomal enzyme Cathepsin D as a potential marker for the vitality of wounds in human specimens. We have analyzed 53 samples using enzymological and histological techniques. Our results show the ability of Cathepsin D to establish the vital origin of wounds inflicted 5 minutes or less before death, where the specific activity of cathepsin D reached 0.055 units at the wound edge and 0.01 units in their respective controls ($P < 0.001$). As previously demonstrated in an experimental series, Cathepsin D seems to be a very useful marker of high forensic interest in especially difficult cases. Further studies are in progress to check the influence of different factors such as drugs intake and clinical conditions on Cathepsin D activity.

Key words: Skin wounds – Vitality diagnosis – Histology – Cathepsin D – Forensic pathology

Zusammefassung. Das Manuskript zeigt Ergebnisse, wie sie durch Untersuchung des lysosomalen Enzyms Cathepsin D als potentiellen Marker für Vitalität in menschlichen Hautwunden erzielt wurden. Wir haben 53 Proben mit Hilfe enzymatischer und histologischer Techniken untersucht. Die Ergebnisse zeigen die Fähigkeit von Cathepsin D, den vitalen Ursprung von Wunden zu etablieren, selbst wenn sie 5 Minuten oder geringere Zeit vor dem Tod gesetzt wurden. In den letzteren erreichte die spezifische Aktivität von Cathepsin D 0,055 Einheiten an den Wundränden und 0,01 Einheiten in den entsprechenden Kontrollen ($P < 0{,}001$). Wie früher in experimentellen Untersuchungen gezeigt wurde, scheint Cathepsin D ein sehr nützlicher Marker von hoher forensischer Bedeutung besonders in schwierigen Fällen zu sein. Weitere Studien sind in der Bearbeitung, um den Einfluß verschiedener Faktoren, wie Medikamenteneinnahme und klinischer Zustand, auf die Cathepsin D-Aktivität zu überprüfen.

This work was supported by grants from the Ministry of Education and Science of Spain ("Acciones Integradas 1990") and from the Deutscher Akademischer Austauschdienst, FRG.
Correspondence to: C. Hernández-Cueto

Schlüsselwörter: Hautwunden – Diagnostik der Vitalität – Histologie – Cathpesin D – Forensische Pathologie

Introduction

Although essential for the correct resolution of legal problems, the establishment of a vital or postmortem origin of wounds is usually a particularly difficult issue in forensic pathology.

In the second century b.C., the physician Aulo Cornelio Celso [11] described the "Acute Inflammatory Reaction" as the way living tissues react to injuries. This reaction needs 3–6 hours to become macroscopically evident [2, 3, 6, 8], therefore it will not be observed in injuries occuring in moments close to death. It is well known that there is no exact border between life and death, and that different tissues and organs retain their vital functions for a period of time, depending on their ability to support anoxia [11].

In forensic casework there are many cases where the vital or non-vital origin can be established only by macroscopical examination, but there are also some cases in which additional studies are necessary to obtain a more exact diagnosis of vitality by employing more sensitive techniques that detect an increased activity of biochemical substances involved in the first steps of the inflammatory reaction [4, 6, 11, 13].

In recent years, studies conducted by Raekallio clearly demonstrated the usefulness – as vitality markers – of some enzyme-histochemical [6, 7, 8] and biochemical substances such as histamine and serotonin [5, 6, 7, 8]. The most recently described markers – ions and lysosomal enzymes [1, 2, 11, 14] – allow the forensic pathologist to establish the vital origin of wounds inflicted 5 minutes before death.

Cathepsin D (E.C. 3.4.23.5) is the main acid proteinase in most animal tissues. The high molecular weight substrate used by Cathepsin D is the main difference with other cathepsins. Cathalytic activity is optimal at a low pH, strongly suggesting the presence of carboxylic groups in this enzyme [9]. Lazarus et al. demon-

strated its activity in human skin and also established that cathepsin D is the main cathepsin in this tissue [10].

Cathepsin D has a particulary important function in early phases of acute inflammatory reaction. After tissue damage the enzyme is activiated to digest death cells [9] by the low pH induced by necrosis. Based on this role, we have determined its levels in different wounds to establish its usefulness as a biochemical marker for forensic science.

Materials and methods

We have studied a total of 38 incision wounds from 53 autopsies (46 men and 7 women) performed at the Institute of Legal Medicine of the University of Heidelberg (FRG). The cadavers were routinely kept at +4°C from the moment of death until the autopsies were performed. For each wound of vital origin (VW: injuries inflicted before death whose data was approximately known), a control piece of skin was obtained from the homolateral part of the body (PW: postmortem wound control: homolateral tissue).

We have also studied skin samples from 15 cadavers with no obvious macroscopical injuries as control samples (CS) from 2 homolateral areas of the body (CS_1 and CS_2). In order to study the normal distribution of Cathepsin D in human skin. All tissues were obtained by scalpel dissection before autopsy, and included, in the case of vital wounds, a 5–6 cm piece of skin without fat around the wound. Samples were immediately frozen at −30°C and divided into 2 aliquots, one for histological study in Heidelberg and the other for Cathepsin D determination in Granada.

Histological studies were performed using naphtol-D-chloracetate-sterase and a Leitz Orthoplan microscope.

Cathepsin D was quantified according to the method of Turk [16] slightly modified by Yamamoto [15], as described elsewhere [14]. A 1 g piece of tissue without subcutaneous fat was homogenized at 4°C in 2 ml buffered 1% NaCl, 2% butanol, 0.1% Triton X-100; 1 ml from this homogenate was incubated at 37°C with 0.5 ml 2.5% buffered hemoglobin (w/v) in 0.1 *M* sodium acetate pH 3.8 for 40 minutes and stopped by the addition of 1 ml buffered 5% trichloroacetic acid at 4°C for 10 minutes. After centrifugation (5000 rpm for 20 min) the supernatant was filtered through Whatmann No 4 filter paper. Cathepsin D activity was quantified in the supernatant by UV-spectrophotometry at 280 nm in a Shimadzu UV 160 spectrophotometer. Results were compared with those obtained with a calibrated curve of tyrosine activity as described by Turk et al. [16]. Cathepsin D specific activity (SA) was calculated in relation to the amount of proteins, determined according to the classical procedure of Lowry et al. [17].

Statistical studies were carried out using the Student *t*-test for paired samples [2, 11, 12, 14].

Results and discussion

Samples were obtained from corpses whose ages ranged from 14 to 82 years (mean values: 36.7 years). Traffic fatalities were the main cause of violent death (94%), followed by suicides (4 by hanging, 4 by drowning, and 1 railway accident). We have also included 4 non-violent causes of death: 1 case of pneumonia and 3 of acute myocardial infarction. For obvious reasons, pharmacological treatment and drugs intake that could interfere with Cathepsin D levels in moments previous to death, could not be evaluated in cases of violent death, although none of the victims included in this sample was known to be under medical treatment.

Table 1. Mean values and standard deviations in vital wounds (VW) and their respective homolateral zones (HZ) and in control samples (CS_1, CS_2)

Sample	*n*	Mean (SA/g)	SD
VW	38	0.017	0.024
HZ	38	0.009	0.010
CS_1	15	0.006	0.003
CS_2	15	0.006	0.005

Table 2. Mean values and standard deviations in vital wounds (VW) and their homolateral zones (HZ) for each group

Group	Sample	Mean (SA/g)	SD
1	VW	0.055	0.005
1	HZ	0.001	0.001
2	VW	0.005	0.001
2	HZ	0.006	0.002
3	VW	0.009	0.008
3	HZ	0.009	0.010
4	VW	0.009	0.010
4	HZ	0.008	0.010
5	VW	0.041	0.052
5	HZ	0.007	0.004

Mean Cathepsin D values for all vital wounds (VW), postmortem wound controls (PW) and 30 control samples (CS1 and CS2) from 15 different cadavers are shown in Table 1.

Vital wounds ($n = 38$) were divided into 5 groups, according to the time elapsed between the moment of injury and the estimated moment of death (survival time) as follows:

Group 1: 0–5 min.
Group 2: 6–15 min.
Group 3: 16–60 min.
Group 4: 1–48 hours.
Group 5: >48 hours.

Mean Cathepsin D values for these 5 groups are shown in Table 2 (Figure 1).

Statistical comparisons were made between every injury (VW) and its control (PW), as shown in Table 3.

It is well known that enzyme activity is influenced by different factors, but mainly depends on the anatomical region of the body [9, 10]. Therefore we have obtained a variation coefficient (VC) of 50% for CS_1 and 83% for CS_2. Among other facts that could influence Cathepsin D levels are the individual age, dermatological pathology, small injuries invisible to macroscopical examination, cause of death and agony interval. None of these factors have previously been studied nevertheless, circumstances such as cause of death or pharmacological treatment have no special influence in this study, since most of the fatalities (94%) were due to traffic accidents and victims had received no medical or surgical treatment.

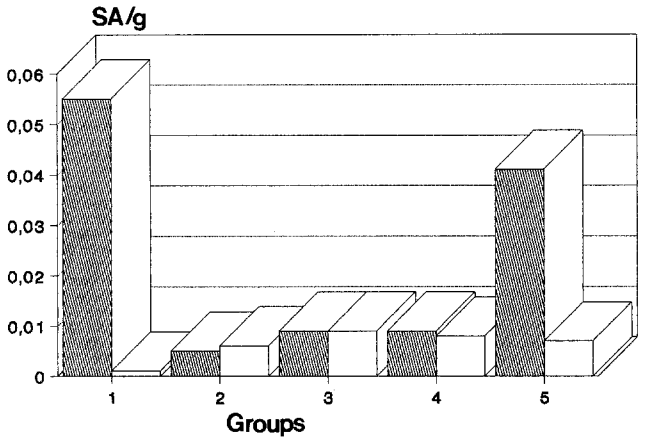

Fig. 1. Cathepsin D levels: comparisons between vital wounds (VW) and their homolateral zones without injury (HZ) in the different groups (1–5). (▨) VW; (□) HZ

Table 3. Statistical comparison of every injury with its own control

Group	F	n	Significance
1	3.082	13	$P < 0.001$
2	0.006	10	No
3	0.006	17	No
4	0.820	12	No
5	0.003	13	$P < 0.005$

The results obtained in this study for Cathepsin D agree with our previous experimental studies [2, 11, 14]. As shown in Table 2, Cathepsin D levels in groups 1 and 5 are significantly higher in vital wounds (VW) when compared to their respective controls (PW). Higher levels of Cathepsin D in group are due to early stages of cellular necrosis and tissue acidification that leads lysosomal activation and phagocytosis of the damaged cells. To our knowledge, there is no logical explanation to justify Cathepsin D increase in group 5, since this enzyme is supposed to play no role at this stage of healing.

The parallel histological study showed, as expected, that signs of vital reaction cannot be observed microscopically if the time elapsed between wounding and death is less than 30 mins.

To summarize, we can confirm that the quantification of Cathepsin D is very useful to establish the vital origin of wounds inflicted in moments close to death. Despite the large variability among individuals and even among different body regions from the same person, the results are informative when Cathepsin D levels quantified in wounds are compared to control regions from the same person (homolateral and non-wounded skin).

Studies to evaluate the exact influence of different factors on Cathepsin D levels in particular and in wound healing in general are at present being carried out.

Acknowledgement to Prof. Dr. med. Georg Schmidt for his kind collaboration.

References

1. Hernandez-Cueto C, Villanueva E, Luna A (1987) Differential diagnosis between vital and postmortem wounds: ions as markers. J Forensic Sci 3:14–23
2. Hernandez-Cueto C, Luna A, Lorente JA, Villanueva E (1987) Study of Cathepsin A, B and D activities in the skin wound edges. It's application to the differential diagnosis between vital and postmortem wounds. Forensic Sci Int 35:51–60
3. Berg S (1972) Die Alterbestimmung von Hautverletzungen. Z Rechtsmed 70:121–135
4. Fatteh A (1972) The role of histochemistry in forensic pathology. In: Wecht CH (ed) Legal medicine annual. Appelton Century Croft, New York, pp 27–46
5. Fazekas IGy, Viragos-Kis E (1965) Der Gehalt der Erhängungsturche am freien Histamin als vitale Reaktion. Dtsch Z Gerichtl Med 56:250–268
6. Raekallio J (1961) Histochemical studies in vital and postmortem skin wounds. Merkatorin Kirjapaino, Helsinki
7. Raekallio J (1965) Die Alterbestimmung mechanisch bedingter Hautwunden mit enzymhistochemischen Methoden. Max-Schmidt-Römhild Verlag, Stuttgart
8. Raekallio J (1970) Enzyme histochemistry of wound healing. Gustav Fischer, Stuttgart
9. Barret AJ (1972) Cathepsin D. In: Dingle JT (ed) Lysosomes: a laboratory handbook. North-Holland, Amsterdam, pp 123–124
10. Lazarus GS, Hatcher VB, Levine N (1975) Lysosomes and the skin. J Invest Dermatol 65:259–271
11. Villanueva E, Hernandez-Cueto C (1990) Lesions postmortales: Diagnóstico diferencial con las producidas en vida. In: Gisbert JA (ed) Medicina legal y Toxicologia. Salvat Editores, Barcelona, pp 254–261
12. Girela E, Hernandez-Cueto C, Lorente JA, Villanueva E (1989) Postmortem stability of some markers of intravital wounds. Forensic Sci Int 40:123–130
13. Lasarov I, Franzova S, Ruseva Y (1988) Veränderungen in der Menge der Prostaglandin F_{2a} bei Schnittwunden der Haut mit verschiedener Zeitdauer ihres Bestehens. Z Rechtsmed 99: 273–280
14. Lorente JA, Hernandez-Cueto C, Villanueva E (1987) Cathepsin D: a new marker of the vitality of the wounds. Comparative study with histamine and serotonin. Z Rechtsmed 98: 95–101
15. Yamamoto K, Katsuda N, Himeno M, Kato K (1979) Cathepsin D of rat spleen. Affinity, purification and properties of two types of Cathepsin D. Eur J Biochem 95:459–467
16. Turk V, Tamara L, Kregar I (1974) Cathepsin D, cathepsin E. Methods of enzymatic analysis, 3rd edn. Verlag Chemie, Weinheim
17. Lowry OH, Rossbrough NJ, Farr AL, Randall J (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275