PCR-typing of DNA extracted from epidermal particles won by scratching

Sanchez-Hanke, M.[1] , Püschel, K.[1], Augustin, C.[1], Wiegand, P.[2], Brinkmann, B.[2]

[1] Institute for Legal Medicine, University of Hamburg, Butenfeld 34, D-22529 Hamburg

[2] Institute for Legal Medicine, Westfälische Wilhelms-University, von Esmarch-Straße 86, D-48149 Münster

INTRODUCTION

In medical emergency examinations and therapy minimal excoriations by violent attacks or abuse are not important, neither to the physican nor to the patient. But such lesions can be essential for the coroner or forensic pathologist concerning the reconstruction of the course of events. Sometimes, the aggressor's skin particles can be found under the victims fingernails. The conventional serological means of typing scratched epidermal particles are limited. The aim of this study was the application of PCR-based DNA-typing methods to this question.

MATERIALS AND METHODS

The epidermal particles were obtained by two experimental series. In the first experiments four volunteers were scratched by four other volunteers. The epidermal particles attached to the fingernails of the „victims" were preserved.
Subsequently to positive results of this pilot study, different scratches were produced at cadavers in a second experimental series. A special mechanical apparatus was developed for this experiment. On one side of the machine's balance arm a plastic fingernail was fixed. Different weights were applied to regulate force intensity. After every experiment the fingernail was cleaned with a fiberglas plate and preserved.
In both series, epidermal DNA material was isolated using the CHELEX-method and quantified by ACES (Advanced Chemiluminescence Enhancement System; Gibco-BRL, UK; Waye et al.1989). The STR loci HUMACTBP2 (SE 33), HUMTH01 (TC 11) and HUMVWFA31 (VWA) were amplified by PCR according to Wiegand et al.(1993). Alleles were resolved by non-denaturing PAA-gelelectrophoresis according to Wiegand et al.(1993) and visualized by silver staining according to Budowle (1992).

RESULTS

In the first phase of the experiments the skin wounds remained superficial and disappeared within 24 hours. The indices and middle fingers of our victims produced the longest and deepest excoriations so that usually epidermal residues being attached to the fingernails could be seen microscopally. When the attacks were carried out at the neck or the upper arm region, this observation could be made regulary. On the contrary, when the scratch was carried out at forearm positive results could not be obtained.
The serological results of the applied STR systems differed. The findings with the systems TC 11 and SE 33 were coincident; the VWA system showed fewer positive results than the other two systems.
In the second experimental phase the different scratching series exhibited different histological alterations. Only the stratum corneum was affected when applying 200-400g scratching force. At intensities between 400-800g the deeper epidermal layers were affected by the lesion but the basal membrane was always intact. At intensities of 800-1400g the epidermis, in some cases even the dermis lying directly underneath the basement membrane, was destroyed. Intensities above 1400g regulary produced massive morphological alterations, leading to a destruction of almost the complete skin.
The histological results in this second phase were accompanied by the following serological findings: Positive results were regularly obtained by high intensity scratches with all three PCR-systems. Occasionally negative PCR-typing results were found by middle or low force intensities. Sometimes DNA contamination occured in low force intensities. The relationship between the employed force and the quantity of DNA was confirmed by statistical analysis with the Pearson's-correlation test.

DISCUSSION

The possibility to discover epidermal particles under the fingernails used for scratching as reported by Wiegand et al. 1993 could be confirmed in the first experimental phase. The described soft asservation of scratched particles with a small fiberglas plate proved to be an effective method which enabled us to obtain sufficient DNA material for an analysis.
The negative results for the forearm area correlates to the skin's high resistance in this area. The soft skin structure of the neck and upper arm which are quive similar lead to a high percentage of positive and similar results.

In the second phase different force intensities were applied to the upper arms of deceased persons (6-8 hours after death). The subsequent histological findings showed a significant correlation to the acquired amount of DNA.

The DNA quantity gained with forces above 1200g remained constant. This finding can be explained by the micromorphological structure of the dermis which contains few cells and a comparably higher amount of soft tissue which does not contribute to the DNA quantity. In contrast to the dermis, the deep epidermal skin layers contain numerous melanocytes (which led to high amounts of extracted DNA). Subsequently the correlation curve between low scratching intensities and the amount of DNA attached to the fingernails increases logarithmically and remained constant at higher force intensities.

CONCLUSIONS

1) PCR-typing of scratched epidermal particles was sucessfull in about 70% of the cases.

2) A correlation between the quantity of DNA and the employed scratch force could be shown.

3) Force intensities over 1200 g led to levelling of the produced amount of DNA.

4) The interpretation of stain analysis performed by PCR-based VNTR-systems has to be done very carefully with respect to contaminations and conclusions concerning the reconstruction of the kind and timing of contact between the victim and the perpetretor.

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