G60 The Contribution of Researching DNA Breaks to the Evaluation of Postmortem Delay

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After attending this presentation, attendees will understand the results of a study on intranuclear DNA breaks on skin in an animal model.

This presentation will impact the forensic community and/or humanity by demonstrating an initial approach to be followed by further study on human skin, in order to improve evaluation of PMD.

Introduction: Postmortem delay (PMD) evaluation remains a problem for the forensic pathologist. Although Professor KNIGHT recently resumed all the methods used in his last book, it still seemed worthwhile to test a pathological method as a first stage on an animal pattern.

Material and Methods: 2.1) Material: 30 adult male rats, sacrificed according to protocol. Cutaneous samples 2 mm by 2 mm from the inner thigh every 3 hours up the 24th hour, then every 6 hours until the 48th hour.

2.2) Methods: TUNEL technique: a commercially available DNA end labelling kit, the TUNEL method was standardised, validated and used for this purpose. This TUNEL technique (apoptag oncor) has shown itself to be the most reliable (reference) in a previous work.

2.3) Statistical analysis: For each PMD the average and the standard variation in the number of marked cells was calculated. The comparisons between the different PMDs were analysed with the Fischer test. The estimated PMD from the number of marked cells was based on linear regression. All the statistics were treated with the SAS software and the threshold of 5% significance was retained.

Results: The statistic analysis allowed the establishment of the following regression based on the number of marked cutaneous cells and according to their topography:

Delay = 32.648 – 1.114 x basal – 6.886 x intensity + 0.209 x superficial + 0.434 x intermediate.

Interpretation of Results: From the statistical results it appears that before the 18th hour there is an intranuclear break in the DNA fragments particularly in the superficial layers mainly constituted of mature keratinocytes, that after the 18th hour whatever the layer these apoptotic phenomena diminish. This reduction in breaks thus presents an interest in the evaluation of post mortem delay since all the layers behave practically in the same way with almost parallel kinetics, up to about the 48th hour.

Conclusion: In the next stage of this work true apoptotic variations will be verified by immuno-histochemistry (detecting the expression of bcl2, P53, caspases 3/9) or simple post mortem DNA breaks related to apoptosis. But having said this the TUNEL technique retains all its relevance in the evaluation of post-mortem delay. If it is really a case of apoptotic mechanisms, its evaluation in certain organs, taking into account an agonic phase such as brain death but with a heartbeat, the mechanisms leading to apoptosis, blocking some of the factors leading to it could be of interest in organ transplants. A second objective would be to study a human model by taking a sample from a skin fragment at the site where the corpse has been discovered, in order to study it very quickly.

PMD, DNA Breaks, Skin

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