H130 Caspase 9 and Caspase 3 Immunohistochemical Reactivity Pattern in Skeletal and Cardiac Muscle at Different Times After Death: A New Tool for Postmortem Interval (PMI) Determination?

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Learning Overview: After attending this presentation, attendees will understand the importance of the identification of new markers that could be used as possible PMI indicators, especially in contexts in which the reliance on the classic thanatochronological triad—livor, rigor, and algor mortis—is not conclusive.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by highlighting the relationship between the activation pattern of caspases 9 and 3 on skeletal and cardiac muscle samples and PMI.

The determination of the amount of time elapsed from one's death to the recovery of the body—the so-called PMI—has always relied on the evaluation of three main consecutive thanatological phenomena: livor, rigor and algor mortis. Despite its remarkable value, several conditions exist, such as premature decomposition, postmortem scavenging, extreme environmental conditions, in which this classic triad shows some limits, thus proving not conclusive in terms of PMI estimation. Such a reflection thus led to the evaluation, with a semi-quantitative approach, of the immunohistochemical reactivity pattern of two apoptosis mediators, caspase 9 and caspase 3, on samples of skeletal and cardiac muscle obtained from sacrificed rats at different times after death.

For this purpose, 23 male C57BL/6J rats were sacrificed by means of chloroform inhalation, and samples of quadriceps femoris and left ventricular wall were collected at 0, 4, 8, 12, 24, and 72 hours after death. All samples were fixed in formalin, then embedded in paraffin blocks for light microscopy. Ten sections were prepared from each sample, nine of which assessed for the immunohistochemical reactivity to caspases 9 and 3, the remaining one stained with hematoxylin-eosin in order to study the tissue morphology.

Both in skeletal and cardiac muscle, a slight increase in caspase 9, but not caspase 3, immunoreactivity was detected 4 hours after death. Caspase 9 immunoreactivity kept increasing up to 12 hours after death; a slight caspase 3 immunoreactivity appeared 8 hours after death, and further increased after 12 hours, although lower than caspase 9 reactivity. Such a pattern is consistent with a primary activation of caspase 9, which in turn activates caspase 3, the "executioner caspase" responsible for the apoptotic degradation of the cell molecules. A general decrease of immunoreactivity—though the caspases 9 and 3 relative pattern was maintained—was observed at 24 hours, followed by the absence of caspase 3 and just a moderate caspase 9 immunoreactivity 72 hours after death. Such general decrease ran parallel with the morphologic changes the muscular tissue underwent, consisting of a gradual, increasing alteration of its architecture at 24 and 72 hours.

The present results indicate that both skeletal and cardiac muscular tissue maintain metabolic activity up until 72 hours after death. Within this lapse of time, there is a gradual variation of the pattern of caspases 9 and 3 immunoreactivity—a first increase up to 12 hours after death, when the muscular tissue's architecture is still maintained, followed by a decrease up to 72 hours, as the degradation processes go on—observed, thus making it possible to consider both apoptotic mediators useful immunohistochemical markers for PMI estimation.

PMI, Caspase 9, Caspase 3

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