H81 From Oxidative Stress to Proteins: Postmortem Interval Estimation Based on Biochemical Parameters

Sara C. Zapico, PhD, International Committee of the Red Cross, 19 Avenue de la Paix, Geneva 1202, SWITZERLAND; Paula Núñez, PhD, Departamento de Biología Funcional. Fisiología, Facultad de Medicina. Universidad de Oviedo, C/ Julián Claveria s/n, Oviedo, Asturias 33006, SPAIN; M. Ángeles Villaronga, PhD, Hospital Universitario Central de Asturias, Edificio FINBA Lab ORL-IUOPA. Avda Roma s/n, Oviedo, Asturias 33011, SPAIN; Sofía T. Menéndez, PhD, King's College London, Randall Division. 2nd Floor New Hunt's House. Guy', London SE1 1UL, UNITED KINGDOM; Douglas H. Ubelaker, PhD, Smithsonian Institution, Dept of Anthropology, NMNH, MRC 112, Washington, DC 20560; and Juana M. García-Pedrero, PhD, Hospital Universitario Central de Asturias, Edificio FINBA Lab ORL - IUOPA, Avda Roma s/n, Oviedo, Asturias 33011, SPAIN*

After attending this presentation, attendees will be able to consider the possibility of using biochemical parameters related to cell death to study the early postmortem interval.

This presentation will impact the forensic science community by introducing novel quantitative indicators of postmortem interval in the first hours of death as a more accurate determination of this parameter.

Estimation of postmortem interval is one of the challenges in forensic sciences. Currently, it is analyzed using different approaches, including both physical and thanatochemistry methods; however, there are few reports studying this parameter from the point of view of cellular biology.

Decomposition is triggered by a process called autolysis, which induces destructive changes in tissues and cells involving cell death; however, the cell nucleus remains intact until four days after death, thus allowing/making feasible the application of cellular biology methods for time-since-death estimation. Nevertheless, current analyses based on this perspective have so far been focused on measurements of messengerRNA (mRNA) stability using human housekeeping genes.

The goal of this research was to study early postmortem interval (between two and eight hours) by applying various molecular and cellular biology approaches: (1) oxidative stress production; (2) mRNA expression analysis of cell death proteins; and, (3) expression of key proteins in the mitochondrial electron transport chain, such as cytochrome c.

Four adult male Wistar rats were euthanized at the same time with intra-peritoneal injection of 0.15ml/kg xylazine. Immediately after death, the rat bodies were placed in the laboratory under controlled conditions of temperature and humidity; 20mg of gastrocnemius muscle were biopsied from each rat at different time points (zero, two, four, six, and eight hours) after death. Each sample was divided in two halves that were placed into two independent sterile tubes containing standard lysis buffer for oxidative stress and protein expression analysis; the second half was preserved in a specific lysis buffer for posterior RNA extraction. Thus, a total of 20 muscle samples were obtained during the eight-hour period for each determination.

After tissue sample homogenization in lysis buffer, supernatants were subsequently divided in two to be used for both oxidative stress and protein expression analyses. The recovered supernatants for protein expression were quantified using a fluorometric approach. Protein quantification demonstrated variability between subjects and times, ranging concentrations between 0.41mg/ml-23.18mg/ml. Standard Western blot protocol was used to study cytochrome c expression in these samples, and its quantification was developed through imaging software.

Copyright 2017 by the AAFS. Unless stated otherwise, noncommercial *photocopying* of editorial published in this periodical is permitted by AAFS. Permission to reprint, publish, or otherwise reproduce such material in any form other than photocopying must be obtained by AAFS.

The analysis of oxidative stress was conducted by specific fluorometric assay, analyzing all types of free radicals in the sample. The results obtained were expressed as Relative Fluorescent Units (RFUs).

RNA samples were extracted using a silica-based methodology. Quantification of RNA was performed using a fluorometric assay. Variability on RNA concentrations among the 20 muscle samples ranged between 1ng/µl-25ng/µl, reaching the minimal RNA concentration required for mRNA expression analysis by Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR). Hence, reverse transcription was the next step, followed by RT-qPCR to analyze mRNA levels of FasL (gene implicated in death cell signaling and inflammation) and PTEN (inhibitor of PI3K/Akt pathway, which regulates cell proliferation). The analysis of relative gene expression data was calculated using the $2^{-\Delta\Delta CT}$ method.

The results of protein and mRNA expression were similar. There was a time-dependent increase in the mRNA levels of FasL and PTEN as well as cytochrome c protein expression until 6 hours after death. Though, at 8 hours, the levels decreased, probably due to the degradation of mRNA and proteins as a consequence of the progress in the autolysis process. Using a regression analysis in the first 6 hours after death, a strong positive linear correlation was found between the mRNA and protein expression of these genes and the time-since-death. These results are in agreement with the initial hypothesis, since these proteins are implicated in cell death and inflammatory signaling pathways. In contrast, oxidative stress showed more variability, with an initial increase from 0-4 hours, a decrease at six hours, and a higher increase at eight hours, which can be correlated with the summit of autolysis process.

The findings from this research provide a novel quantitative tool for estimating early postmortem interval based on biochemical parameters. Future research may be able to expand on these results, searching for other cell death markers and extending time-since-death estimates.

Postmortem Interval, Cell Death Proteins, Oxidative Stress

Copyright 2017 by the AAFS. Unless stated otherwise, noncommercial *photocopying* of editorial published in this periodical is permitted by AAFS. Permission to reprint, publish, or otherwise reproduce such material in any form other than photocopying must be obtained by AAFS.