G78 Quantitative Measurement of Ribonucleic Acid Degradation as a Possible Indicator of Postmortem Interval

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After attending this presentation the participant will understand that RNA degradation is time-dependent, can be quantified by reverse transcription and polymerase chain reaction and may serve as indicator of postmortem interval.

Ribonucleic acid (RNA) research is a major topic in molecular biology and medicine. RNA is less stable than DNA in vivo and in vitro and therefore is believed to undergo rapid postmortem degradation. This may be the reason why RNA analysis did not obtain significant attention in forensic science up to now. However, due to the nucleic acid structure small amounts of mRNA can be amplified by polymerase chain reaction after synthesis of complementary DNA (cDNA) by reverse transcription (RT-PCR). The poly-A-structure at the 3'end of most mRNAs allows exact imaging of the mRNA pattern by oligo(dT)-primed reverse transcription including differences in fragment size due to degradation. Primers designed for sequences near the 5'-end of the mRNA should provide weaker amplification results than primers located near the 3'end in degraded samples because the average mRNA size is expected to be smaller after RNA degradation so that less full-size transcripts will be generated during reverse transcription.

The objective of this study was to investigate whether quantitative measurement of RNA degradation could be helpful in determining the time of death in bodies. Blood taken from healthy volunteers was stored under various conditions and for variable time periods. Blood from bodies with exactly known time of death was taken from the femoral vein. After counting leucocytes RNA and DNA were extracted using standardized protocols. For estimation of the degree of degradation the 260/280 nm UV absorption ratio and the ratio of 28S to 18S ribosomal RNA of the RNA samples were determined. To assess the integrity of messenger RNA competitive RT-PCR using an synthetic competitor mRNA, one-step duplex RT-PCR with simultaneous amplification of a short and long fragment of the same mRNA (ß-actin) , multiplex RTPCR with amplification of four fragments located between the 3' and 5'end of the mRNA (fatty acid synthetase, FAS) and comparative RT-PCR of house-keeping genes (glycerin aldehyde-3-phosphate dehydrogenase) were performed. The amplification products were visualized with agarose gels or automated capillary electrophoresis. For quantification the staining intensity in ethidium-bromide stained agarose gels or the peak area in electropherogramms generated with laser-induced fluorescent capillary electrophoresis were calculated.

The results show, that RNA degradation is gradually increasing up to 5-6 days postmortem depending on the ambient temperature. The in vitro as well as the postmortem assays demonstrate that the in vitro/ postmortem time interval can be estimated by quantitative measurement of RNA degradation during the first week within a range of 1-2 days. Multiplex RT-PCR of the FAS-mRNA provided the most consistent results because the degradation of long (>4kb) mRNAs is measured showing a significant decrease of the amount of 5'-sequences that require full-size transcripts for detection relative to 3'-sequences which are close to the origin of reverse transcription.

Beside the forensic implications this study has high relevance for clinical and experimental RNA research because the exact time-course of postmortem or in-vitro RNA degradation is largely unknown. In forensic pathology quantitation of RNA degradation seems to close the gap between early postmortem interval (< 24 h) and the beginning of putrefaction. Further evaluation studies are currently performed with autopsy cases to enhance the significance of statistical calculations.

RNA Degradation, RT-PCR, Postmortem Interval

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