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Features of expression of iNOS mRNA in postmortem brain

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Abstract. The purpose of this research was to use RNA as a subject of legal medicine. Tissue samples were obtained from postmortem human brains collected from cadavers, whose reasons of death were exactly determined. Total RNA was isolated by the standard method and used for reverse transcription-polymerase chain reaction analysis (RT-PCR). The subject of our special concern was NOS and its expression in various brain tissues during some pathological processes. In this study, we showed that postmortem brain tissue can yield good quality mRNA for the successful application of traditional molecular biology methods from postmortem brain tissue even with prolonged postmortem intervals (up to 7 days). The relationship between a level of inducible NOS expression and intensity of pathomorphologic development in organs and tissues of people who died from different reasons was detected.

Keywords: iNOS; RNA expression; RT-PCR

1. Introduction

Technological advances in molecular biology and genetics allowed scientists from different fields to use them for solving their own goals. DNA is successfully used as a subject of legal medicine as the individual stabile code of a person. Reversely, the regulation of mRNA gene expression represents an important and growing field in biological research. Messenger RNAs may elucidate features of processes, which took place before and on the moment of death. There are dozens of individual genes of interest which are all expressed in human tissues. As a subject of our investigation, we chose the NO^- synthesis system.

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The aim of the present study was to investigate the pattern of expression of inducible nitric oxide-synthase (iNOS) in various tissues of postmortem human brain.

2. Materials and methods

Tissue samples were obtained from postmortem human brains, lungs, hearts, livers and kidneys collected from cadavers, whose reasons of death were determined. Total RNA was isolated by guanidine isothiocyanate lysis and phenol:chloroform extraction by a method based on Chomczynski and Sacchi [1]. The RNA samples were used for reverse transcription-polymerase chain reaction analysis (RT-PCR). To normalize cDNA amounts used during the PCR amplification, a parallel amplification was performed using glyceraldehyde-3-phosphate dehydrogenases (G3PDH) specific primers (Clontech, USA). For amplification, $iNOS^{-}$ transcripts were used as specific primers.

3. Results

At first, we had to determine whether the high-molecular weight RNA could keep its stability or not in the main human organs, such as liver, lung, heart, kidney and brain after death (Fig. 1).

Our results showed that the high-molecular weight RNA appear to be rather stable in lung and heart, whereas the resistance of RNA within kidney and liver is slightly less. The brain tissues were the steadiest after the postmortem changes: we managed to isolate high-molecular weight RNA from the samples of brain tissues which were stored at 4 °C up to 7 days.

RNA, isolated from the postmortem brain, was found in good quantity and the samples taken from the brains 1, 2 or 3 days after death, were revealed to be not different from one another. Some degradation of RNA isolated from the brain has been found only after storing it 7 days following death. cDNA library, obtained by using reverse transcription of RNA, was isolated from the samples of the postmortem brains and displayed the content of a set of mRNAs, encoded by the "housekeeping genes". However, the amplification with primers for iNOS mRNA demonstrated a reply from only two brains and only some structures of brain, namely hippocampus and cerebellum in this case.

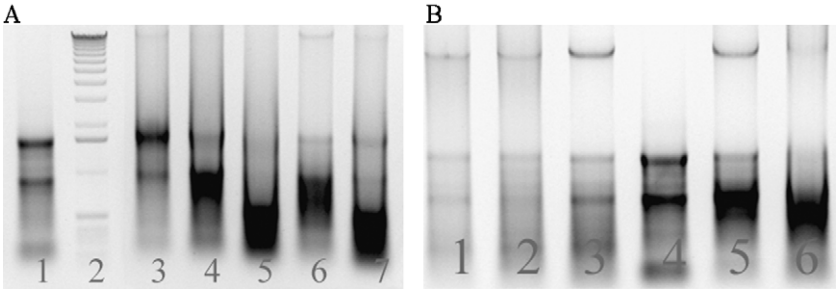

Fig. 1. Electrophoresis of RNA. (A) 1—Standard human RNA; 2—1 kb ladder; 3, 4, 5—liver (1, 2 and 3 days after death); 6, 7—kidney (1 and 2 days). (B) 1, 2—lung (1 and 3 days); 3—brain (5 days); 4—standard human RNA; 5, 6—heart muscle (1 and 3 days).

The reason for death of the first person was a drug overdose. But the second person died after a road accident without brain damage. Drugs and alcohol in the blood and urine of the last person were not detected.

4. Discussion

Our investigations for stability of RNA in the main organs corresponded to theoretical notions. It depended on nuclease activity in tissue. Nuclease content in a human brain was almost not detected. Therefore, DNA and RNA were the successfully isolated brain samples of various ages. In this study, we showed that postmortem brain tissue can yield good quality mRNA for the successful application of traditional molecular biology methods from postmortem brain tissue even with prolonged postmortem intervals (up to 7 days). Besides this, RNA from the other tissues potentially could be used but only at the first 1–3 days after death.

The amplitude of bands and the number of PCR cycles applied to get uniform bands for "housekeeping genes" were consistent with differences in the mRNA contents. A relationship was detected between level of iNOS expression and intensity of pathomorphologic development in tissues of people who died from different reasons. Besides that, RT-PCR analysis demonstrated a structure-specific picture. A matter of interest was that data obtained by different groups of scientists appeared very contradictory. Especially when they used commercial RNA which seemed to be a sum of RNAs, isolated from the same tissue of many bodies. For example, Park et al. [2], who used samples of commercial RNA, found the presence of iNOS mRNA within the different structures of the normal brain, taken after the death of a human.

No statistical evaluation of the data was performed due to the qualitative nature of this study, but we suppose that iNOS expression in some tissues could serve as a marker of toxic influence before the death of a human.

In conclusion, we showed that high-quality RNA, suitable for routine molecular analyses, could be obtained from postmortem brain tissue even with prolonged postmortem intervals. We would suggest further research of different human marker RNA for an ascertaining of the reason, in some cases, of a conjecturable time of death.

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