Autolytic ultrastructural changes in rat and human hepatocytes

Radovan Karadžić*1, Goran Ilić2, Aleksandra Antović3, Lidija Kostić Banović4

Abstract: The estimation of the time since death is one of the most difficult problems in forensic pathology. Changes in the tissue structure provide another possible means by which the time of death can be estimated. The results of all the previous researches of post-mortem autolytic processes have not provided satisfactory results in establishing the criteria for determining the time of death based on autolytic ultrastructural changes. In the current paper, autolytic ultrastructural changes have been observed on experimental materials of Wistar rat liver tissue in different time intervals after death (2, 4, 6, 12 and 24 h) and in different temperature conditions (8°C, 18°C and 28°C), as well as on human cadaver livers (6, 12 and 24 h at the temperature of 18°C). This research represents the attempt to determine the dynamics and degree of ultrastructural changes in hepatocytes in the process of post-mortem autolysis with the aim to use the obtained results in everyday forensic practice for more precise determination of the time of death. By comparative analysis of the established changes between human and experimental material, it has been proven that the kind, degree and dynamics of morphological changes are directly dependent on not only the length of post-mortem time, but also, to a bigger extent, on the temperature of environment where autolysis is happening.

Key words: ultrastructural changes, autolysis, post mortem interval, hepatocytes

Autolysis represents the rotting of tissue without any vital reactions (without any signs of soreness). This phenomenon explained by Salkowski (1890) as an intracellular enzyme activity and named “auto-digestion.” Jacobi (1900) introduced the name “autolysis.” It is a nonbacterial post-mortem self-destruction (decomposition) of tissue by its own enzymes [1-3].

The estimation of the time since death is one of the most difficult problems in forensic pathology [4]. The estimation of accurate post-mortem interval (PMI) has been an unfinished quest of forensic pathologist world for over millennia [5]. The results of all the previous researches of post-mortem autolytic processes indicate the polymorph intra- and extra-cellular changes, not only on the level of one organ, but in relation to different organs as well.

Consequently, the regularities of post-mortem autolytic changes, which would serve, as criteria for safe and precise determination of the time of death, have not been defined yet [6, 7]. The comprehensiveness of professional literature in the area of thanatology connected to the determination of the time of death is in reverse correlation with the importance of this issue in everyday practical work [8]. Encouraged by the lack of similar researches in forensic medicine, we have undertaken this preliminary examination in order to determine the correlation between the time of death and autolytic ultrastructural changes in hepatocytes by using a morphological method.
Material and methods

Autolytic changes have been observed on an experimental tissue material of a rat and human liver by electron microscopy (EM) method. A hundred eight Wistar rats, 4 months old, weighting 250 g, were sacrificed by cervical dislocation. The analysis of the human material was performed on the human liver tissue of a 20 male and female cadavers, aged 30 to 35 years, of good health with the cause of death not connected to liver pathology.

With the aim to observe the changes of external temperature influence in relation to post-mortem time passage on the occurrence of autolytic changes, the analysis of ultrastructural features of rat hepatocytes was done in three experimental groups (3 male and female animals per group): I group - 8°C, II group - 18°C, III group - 28°C, in 6 time intervals between sacrifice and section (0 - controls, 2, 4, 6, 12 and 24 h after death) in the conditions of constant air humidity of 70%. The analysis of the human material was obtained during the routine autopsies, at room temperature (18°C), in three time groups (6, 12, and 24 h after death). Due to legal prohibition in our country to perform autopsy on bodies before 6 hours have elapsed since the time of death, changes in the human liver in time sub-groups 0, 2 and 4 were not analysed.

At each post-mortem time in all groups, liver tissue were excised from the frontal part of the right liver lobe, and fixed in cacodylate buffer with glutaraldehyde and post-fixed with 1% osmium tetrochloride. Each sample dehydrated through alcohol and acetone and embedded in Epon 812. Ultrathin sections were cut from the epoxy block using a diamond knife, followed by trimming and contrasting for EM research [9, 10]. The sections were examined and photographed under a transmission electron microscope of “JEM-100CX” (Jeol, Tokyo, Japan).

The experiment was carried out following the Regulation book on working with experimental animals of the Institute for Biomedical Research at the Medical Faculty University of Nis (Serbia), based on European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [11]. Research on the human cadavers was approved by the Internal Ethic Committee, and conducted at the Institute of Forensic Medicine of this Faculty.

Results

1. In relation to time passage, the following ultrastructural changes in rat hepatocytes were determined: Group I at 8°C temperature: mitochondrial swelling and slight granularity of chromatin (4h); initial homogenisation of mitochondrial matrix (6h); detaching of the inner and outer nuclear membrane, initial vacuolisation of sarcoplasm (12h); peripheral chromatim migration, significant mitochondrial cristae fragmentation, moderate vacuolisation of sarcoplasm, initial reduction of the number of glycogen granules (24h). Group II at 18°C temperature: mitochondrial swelling, lysis and fragmentation of mitochondrial cristae (2h); slight granularity and clumping of chromatin, crenation of karyolemma, reduced transparency of mitochondrial matrix (4h); peripheral migration of chromatin, partial lysis of the outer nuclear membrane, vacuolisation of sarcoplasm (6h); initial lysis of the inner nuclear membrane with occasional leakage of chromatin in sarcoplasm, amorphous deposits in mitochondrial matrix, prominent sarcoplasm vacuolarity, swelling and turbidness of Golgi apparatus, initial lysis of endoplasmic reticulum (ER) (12h); diffuse leakage of chromatin in sarcoplasm due to lysis of the inner nuclear membrane in the majority of rat hepatocytes, shape deformation with dense amorphous deposits in mitochondrial matrix, decrease in number of glycogen granules, diffuse moderate lysis of Golgi apparatus and ER membranes in the majority of hepatocytes (24h).

Group III at 28°C temperature: noticeable crenation and vacuolar detaching of nuclear membranes, granulation and clumping of chromatin, fragmentation of mitochondrial cristae, amorphous deposits in mitochondrial matrix, marked swelling of ER and Golgi apparatus membrane (2h); lysis of the outer nuclear membrane, dislocation of nucleolus, initial vacuolisation of sarcoplasm (4h), lysis of the inner nuclear membrane with consequent leakage of chromatin in sarcoplasm, significant loss of mitochondrial cristae, moderate vacuolisation of sarcoplasm, decrease in number of glycogen granules (6h); dense amorphous deposits in mitochondrial matrix, significant vacuolisation and turbidness of sarcoplasm, complete lysis of ER and Golgi apparatus membrane in the majority of hepatocytes (12h); lysis of the mitochondrial outer membrane in the majority of mitochondria, marked reduction of the number of glycogen granules (24).
Figure 1. Transmission electron micrograph of rat hepatocyte 6 h after death at 8°C temperature. Nucleus (Nu) with granularity of chromatin (Chr); moderate swollen mitochondria with initial homogenisation of mitochondrial matrix (Mt); regular shaped karyolemma (Klm) and endoplasmatic reticulum (Er); diffuse granular material representing glycogen (Gly). 8300 X.

Figure 2. Transmission electron micrograph of rat hepatocyte 12 h after death at 18°C temperature. Nucleus (Nu) with peripheral migration and occasional leakage of chromatin in sarcoplasm (arrowhead); arrows indicate lysis of the inner nuclear membrane; irregular shaped evidently swollen mitochondria with amorphous deposits in mitochondrial matrix (Mt); initial lysis of endoplasmatic reticulum (Er). 10000 X.
Figure 3. Transmission electron micrograph of rat hepatocyte 6 h after death at 28°C temperature. Nucleus (Nu) with lysis of the inner nuclear membrane and leakage of chromatin in sarcoplasm (arrowheads); dislocation of nucleolus (Nc), swelling of mitochondria with loss of cristae (Mt). 10000 X.

Figure 4. Transmission electron micrograph of human hepatocyte 12 h after death at 18°C temperature. Nucleus (Nu) with crenation and vacuolar detaching of nuclear membranes; peripheral migration of chromatin (arrows); swelling of mitochondria (Mt); slightly swollen but regular shaped endoplasmatic reticulum (Er). 13000 X.
2. In relation to time passage, the following ultrastructural changes in human hepatocytes were determined at 18°C temperature: slight mitochondrial swelling (6h); crenation and vacuolar detaching of nuclear membranes, dislocation of nucleolus, clumping and peripheral migration of chromatin, prominent swelling of mitochondria, fragmentation of mitochondrial cristae in the greater number of hepatocytes (12); partial lysis of the outer and intactness of the inner nuclear membrane, peripheral localisation of chromatin in the shape of an irregular homogenous ring of a snowflake form, significant lysis of mitochondrial cristae, clearly visible diffuse presence of dense glycogen granules, moderate vacuolisation of sarcoplasm, initial lysis of Golgi apparatus membrane and ER membranes (24h).

**Discussions**

Numerous methods have been proposed in the last 60 years for the determination of the time since death. However, none of the suggested methods has provided satisfactory results, which would be widely used in everyday forensic practice [7, 8]. The main reason lies in the fact that there are an extreme number of factors, which influence the post-mortal degradation of tissue in each concrete case [12].

It is known that after death or tissue/body removal, anoxic post-mortem effects (ischemia, glycolysis, and proteolysis) make changes in enzyme activity and ultrastructural cells [1, 2]. Autolytic process including changes in shape, size, electron density, and localisation of cell structures and generally causes gradual loss of highly arranged structural organisation of cells [13]. However, not only different types of tissues, but also different types of cells in a tissue or organ, as well as different organelles within one cell, can express different degree of sensitivity to autolytic process [14]. Scarpelli et al. established that the occurrence of autolysis is quick in tissues, which have a high concentration of autolytic enzymes, such as pancreas and gastric mucosis; it is moderate in the heart, liver, and kidney tissue, whereas it is slow in fibroblasts, which are poor in lysosomes and hydrolytic enzymes [3]. Similar results were obtained by other authors who have studied the autolytic tissue changes [13, 15-18].

In our research, simultaneously with observing changes in hepatocytes cell structure of experimental and human material in different temperature conditions in relation to the duration of PMI, a comparative analysis of the set changes between human and experimental material was conducted. The comparison of our research results with the results of other authors’ researches is objectively hindered by the fact that numerous experiments whose goal is to observe post-mortal ultrastructural changes are conducted under different conditions (a type of experimental animal, a type of examined tissue, applied methodology, conditions for conducting the study – temperature, post-mortem time interval and the like) [12, 18-20]. Nevertheless, it is doubtless that, subsequent to death, all tissues succumb to autolysis and observing those changes can be useful in determining the time of death, especially in combination with other methods [4, 12].

According to results in the present study, it has been established that ultrastructural changes of hepatocytes are conditioned by the duration of post-mortem time and temperature conditions of outer environment. The degree of ultrastructural changes of rat hepatocytes indicates the existence of significant differences between the group at temperature of 8°C and the one at 28°C, while ultrastructural changes in the group at 18°C represent the clearest mean value of these changes.

In the present study, significant nuclear morphological degeneration in the hepatocyte of the animal and human tissue was generally observed later than in the mitochondria, but earlier than in the organelles of sarcoplasm. The earliest and most prominent nuclear changes are clumping and condensation of chromatin along the nuclear membrane of hepatocyte. Karyolemma showed relatively delay in post-mortem change, so integrity of nucleus remains preserved in all examined groups. Initial post-mortem changes in mitochondrial structures of liver tissue are seen in mitochondria swelling because of increased membrane permeability as well as progressive fragmentation of cristae.

However, it is the fact that mitochondria of different cell types show differences in autolytic changes during the same time after death [21, 22]. Shape deformation and progressive reduction of the number of mitochondria as well as vacuolisation of sarcoplasm progressively grew with the increase of temperature and with the passage of PMI, which is in accordance with the results of other authors [19, 21]. Diffusely arranged glycogen granules in sarcoplasm became rougher, denser, and more transparent, with the vivid reduction of their number as the time elapsed. Other authors who studied
autolysis on the rat and the human liver tissue have published similar ultrastructural changes in nuclei, mitochondria, and sarcoplasm [18-20, 22].

Contrary to the results obtained from the animal experimental material, EM analysis of human hepatocytes did not show obvious autolytic changes in the first 6 hours of PMI at external temperature of 18°C. Having compared the results from the analysed animal and human material at temperature of 18°C it has been established that ultrastructural autolytic changes in the human material develop more slowly than the changes in the rat liver, by approximately 6 to 12 hours.

Conclusions

By EM analysis of the liver tissue sample on the human and experimental material, established ultrastructural changes indicate regularity dependant on post-mortem time and temperature. At the same temperature conditions (18°C), ultrastructural post-mortem autolytic changes in the rat liver manifest themselves faster in relation to the same ones in the human liver.

These regularities in ultrastructural changes in hepatocytes are determined within other parameters as possible indicators of more precise time of death in forensic practice. In addition, morphological changes stated in this paper and the dynamics of their occurrence could facilitate the evaluation of liver quality for the needs of transplantations as well as optimal time for liver preservation for the same needs.

References