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Studies on the Estimation of Postmortem Time : An Application of Interactive Image Analysis System*)

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ABSTRACT

On the supposition of the human violent death cases, the liver cell area and its nucleus area of the mice which were left out in the several experimental conditions (Temperature: 4°C, 20°C and 37°C; Period: 1, 2, 3, 4 and 7 days) were measured for estimating the postmortem time by the interactive image analysis system with computer management.

By using this system, it is possible to quantify the histological alteration and morphological changes in cells resulted from autolytic deterioration for the postmortem intervals. The ratio of the nucleus area to the cell area which was measured by this system could be wide application to the estimation of death in forensic sciences.

This system will offer the potential to the forensic pathologists for deciding the time of death.

INTRODUCTION

In forensic science, the estimation of the time of death is of prime importance. The estimation of the time of death has been examined throughly by using the physical and/or biological techniques[1], whereas its histological techniques are not yet known, to our knowledge.

In this paper, by using the interactive image analysis system recently advanced, the early cell changes as the postmortem interval progresses was examined histologically.

Especially, in forensic sciences, the availability for estimating the time of death by this instrument was presented. This system is sufficiently specific and sensitive to be of forensic science value on a very small samples.

MATERIALS AND METHOD

The interactive image analysis system (Kontron Co. W. Germany, IBAS I, Videoplan) is possible to form a image of some materials on the monitor television (CRT) electrically through the television cammera system from the light microscope (for example, initial magnification: 400), then by selecting some parameters, for example, perimeter, area, point, length, maximum diameter and angle etc, the materials are measured exactly (final magnification: 2400). These formed images are directly measured and calculated statistically at the same time.

Sixty C57BL/6N male mice approximately ten-weeks old, weighing 30–35 g, were obtained from the Japan CLEA laboratory, Japan. Five mice of each group were sacrificed by cervical dislocation, were held in the several experimental conditions (Temperature 4°C, 20°C and 37°C). In 1, 2, 3, 4 and 7 days after death, the livers were collected from these mice. These mice livers were demonstrated by the routine staining technique as formaldehyde fixation, parafine embedding and haematoxyline-eosine staining, then histomorphological alterations of

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mice liver cells for the postmortem intervals were studied extensively.

In this paper, the observation area of liver tissue was midzonal of liver lobule. In each group, more fifty liver cells and its nuclei in the liver midzonal were observed by the light microscope, then the areas of liver cells and its nuclei were measured directly by the interactive image analysis system. Control groups used the liver cells and its nuclei of mice sacrificed immediately by the same acute death.

Statistical significance of differences between the groups was determined by the Student's t-test.

RESULTS

The changes of the areas of mouse liver cell and its nucleus were shown in Table 1.

The areas of nuclei of mouse liver cell, in 4°C group, showed the maximum value (55.2 $\pm$16.7 μm^2) on 2nd day after death, thereafter the nucleus area was a tendency of decreasing with postmortem time. The area of nuclei decreased gradually from 4 days to seven days after death significantly.

The area of liver cells decreased once in one day after death and showed maximum value (350.3$\pm$104.9 μm^2) on 2nd day, thereafter the cell area was a tendency of decreasing with postmortem time. In histological examination of 4°C group, the liver cells and its nuclei have been stained clearly at the seventh day after death and showed only minor alteration, i. e., slight decrease in stainability.

In 20°C group compared to 4°C group, it was difficult to recognize that the area of nuclei in the course of postmortem time decreased gradually. The nucleus area was decrese sharply in four days after death. The area of liver cells decreased once in one day after death, in two days showed the maximum value (381.8$\pm$104.6 μm^2), thereafter there was a tendency of decreasing with postmortem time in cell area. In histological examination of 20°C group, from four days after death, the stainability was decreased and it was difficult to distinct nucleus and cytoplasm on the monitor television in this system, thereafter showed that hepatocyte cord arrangement became disruped and karyorrhexis, karyolysis in the liver cells. This cause of the histological alteration may be related to that the value of nucleus area on fourth day after death was more lower than the value of nucleus area on third day after death.

In 37°C group, the area of liver cell showed about 53 percents to control group on 2nd day after death, compared with 4°C and 20°C group, about 103% and 114% respectively, and a large alteration of ultrastructure in the cell was estimated. The area of liver cell showed

Table 1. Postmortem Changes of Mouse Liver Cell Area and its Nucleus Area by Interactive Image Analysis System

Temp.	DAYS	NUCLEUS AREA (μm^2)	CELL AREA (μm^2)
Control	0	53.4±10.9	334.6±52.1
4°C	1	55.1±13.3	295.6±63.1
	2	55.2±16.7	350.3±104.9
	3	41.9±13.4	321.8±104.7
	4	36.9±5.2*	245.6±48.9*
	7	36.5±8.6*	301.4±51.1
20°C	1	41.6±9.5*	241.0±44.1*
	2	50.0±12.4	381.8±104.6
	3	51.6±14.6	347.8±80.5
	4	21.7±8.3**	258.3±72.8*
37°C	1	50.6±13.0	402.8±97.1
	2	28.3±11.1**	255.7±79.5*

* and ** denote statistical significance at the $p<0.05$ and $p<0.01$, as compared to control group, respectively. Values are means±S. E. M.

the maximum value once as the same other two groups in one day after death, thereafter decreased sharply. In histological examination of 37°C group, the hepatocyte cords were destroyed very nearly in two days after death, and the liver cells were recognized weakly by this time, and it was impossible to recognize the distinction of the nucleus and the cytoplasm. In this experimental condition, it was impossible to quantify the area data of cell and nucleus by using this instrument system after 2 days in 37°C group and 4 days in 20°C group. Thereafter, the area of liver cell showed once time decreased in one day after death and in two days after death it showed the maximum values and there was a tendency of decreasing with postmortem time of the area of liver cell in 4°C and 20°C group. The area of the nucleus was decreased with postmortem time in three groups.

Fig. 1 showed the ratio of the area of its

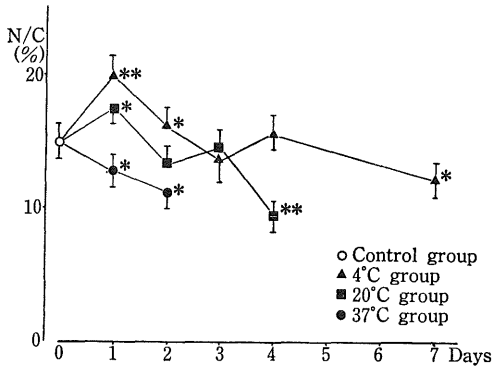

Fig. 1. Postmortem Changes on the Relation between Nucleus Area and Cell Area. N/C (%) shows the ratio of nucleus area to cell area in mouse livers. * and ** denote statistical significance at the $p<0.05$ and $p<0.01$, as compared to control group, respectively. Values are mean±S. E. M.

nuclei to the area of liver cells as shown in Table 1. The ratio of control group showed about fifteen percents. The ratio increased in one day in 4°C and 20°C group, thereafter the ratio decreased gradually. The change rate in one day after death may be resulted from the cause of the more variable value of the cell area than that of the nucleus area.

In the course of postmotem time, the change of the nuclei area may be estimated more intensive as compared with the change of the cells areas.

DISUSSION

When we were examining the samples as a forensic science subjects, the autolysis and putrefaction were often recognized in these time. Whereas these two phenomena would be complicated the estimation of the time of death, the changes by these actions were utilized in the estimation of the postmortem time, as the case may be a practical forensic science. In the experimental research of postmortem changes, we should treat the tissues in the experimental germ-free conditions and demonstrate the autolysis in distinction from the putrefaction which was discussed in paralleled and at the same time.

In this paper, we investigated the early postmortem changes in the mouse liver on the course of autolysis and putrefaction, as supposed practical violent death cases.

Practically there was a absence of quantitative data from the histological examination of postmortem changes. According to Marshall[2], the early postmortem changes were described only as decrease of a stainability and nucleolysis. Therefore, these histological findings did not show the quantitative changes of the cells in the several conditions and in the course of postmortem time as mentioned above.

Especially, in foresic medicine, the autolysis was given an explanation as the changes of stainability, cell swelling and granulolysis. From our results, this cell swelling was recognized in two days after death in 4°C and 20°C groups only, thereafter these cell areas were decreased conversely. Eventually the discrepancies as cell swelling seem to be due to the large decrease of the nuclei areas to the cell areas on fourth day after death in 20°C group and on second day in 37°C group, and relatively should be considered as the cell swelling in this exprement. In Fig. 1, the ratio of the nuclei areas to the cell areas in two groups except 37°C group tends to decrease from 2 days after death. In the ultrastucture alterations, Wyllie[3] reported that the early changes of autolysis are recognized to indicate the altered abnormal membrane permeability resulted from decreasing the osmorality in the cytoplasm, and this is comfirmed by the early disappearance of the membrane

ion-pumping activity. Then the change of cell showed the cytoplasmic vacuoles and the oedema resulted from the mitochondrial swelling. In this time, the stainability of cell whitishes in color clearly.

Furthermore according to these reports and our results in the mouse liver cell, the more earlier autolysis inclued the biochemical, ultrastructural alteration of the nuclei and cytoplasms.

In conclusion, the postmortem changes of the cells showed the cell swelling in about 24 hr after death, thereafter, to be more precise, the ratio of the nucleus area to the cell area decreased.

By using this system in this paper, we recognized that the ratio of the nuclei areas were more variable than the ratio of the cell areas.

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