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Hepatic Cell Membrane Changes of Rats in the Early Postmortem Period

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To investigate the postmortem changes in hepatic cell membrane, the rats were sacrificed with cervical dislocation and kept in an incubator at 25℃, 70% of humidity for 12 hours. The biochemical experiments in postmortem were done at 2, 4, 8 and 12 hours. The degree of rigor mortis and algor mortis were increased with the time during 12 hours. The contents of hepatic malondialdehyde were rapidly increased at 2 hours, and gradually decreased afterward. In histological findings, after 8 hours, the clotted blood was seen in central vein and sinusoids, and especially portal veins were dilated although the structure of hepatic lobules was preserved well. Furthermore, both in the histochemical and enzymatic examinations, membrane bounding alkaline phosphatase activities were gradually decreased with the time. In conclusion, the activity of membrane bounding alkaline phosphatase was linearly decreased with time in the early postmortem period and so it might be referred to the possibility for the estimation of death time in the early postmortem period.

Key Words: Postmortem, Hepatic cell membrane, Membrane-bounding alkaline phosphatase, Rat

INTRODUCTION

In recent years, life span of human has been getting longer than before, because of remarkable advance of medical science. But, nowdays we are suffering from all kind of accidents. When people die because of accidents, i.e., traffic, homicidal, suicidal, etc. When people die because of such as a accident, forensic medicine is involved to identify the causes of death. Histopathological examination through autopsy, postmortem change such as algor mortis, livor mortis[3] and rigor mortis[1] have been used to figure out time and causes of death. Furthermore, with such findings, a wealth of data on biochemical material in the blood after death is available. Some of these indices remain relatively stable during the early postmortem period while the other show varing degrees of change[8]. One of the most detailed reviews of postmortem blood chemistry was carried out by Coe in 1974[4]. But it seems to be difficult to get blood specimen in corpse for biochemical assay whereas the specimen can be easily collected from a tissue in somatic dead body. However, the site of biochemical monitoring in other tissue of corpse except fluid and muscle remains unclear yet. Furthermore it may be significant to observe biochemical changes together with morphological changes in a tissue of postmortem. Therefore, in this study we attempted to do the biochemical and morphological examination associated with cell membrane in liver tissue of dead body because alteration of cell membrane can be responsible for cellular changes in liver tissue of corpse. Malondialdehyde[6] content and membrane bounding alkaline phosphatase[9] are well known as a marker to identify the damage of a cell membrane. For this reason, it is very meaningful to compare morphological observation with the enzyme activity in order to determine postmortem changes. In this study, livers of rats during 12 hours after death were observed on the morphological side and the changes of alkaline phosphatase enzyme on basis of enzyme activity determination and histochemical technique.

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MATERIALS AND METHODS

1. Animals and treatment

Male Sprague-Dawley rats weighing 200±10 g purchased from Korean Animal Center have been adapted for one

Table 1. Changes of rigor mortis in rats

Time (hours)	0 (control)	0.5	1	2	4	8	12
Body Temp.(℃)	36.12±0.10	34.02±0.08	32.07±0.05	30.10±0.03	28.00±0.06	26.01±0.11	25.00±0.00
Rigor Mortis	−	+	++	++	+++	+++	+++

−: Rigor Mortis appeared negative, +: Rigor Mortis appeared weak
++: Rigor Mortis appeared moderate, +++: Rigor Mortis appeared strong

week at an animal laboratory in Keimyung University. The water and food were provided at libitum. The animals was sacrificed by the dislocation of cervical vertebrae[3] and then left at 25℃, 70% humidity in COD incubator, during 12 hours. With time, the body temperature and rigor mortis were observed, while the livers from 5 rats were respectively taken at 0.5, 1, 2, 4, 8, 12 hours and then frozen at -75℃ in ultra-low temperature freezer.

2. The preparation of hepatic tissue extract for enzyme assay and determination of enzyme activity

The liver of rats was rapidly removed from the freezer, thawing to 4℃ and homogenized in ice-cold 0.25 M sucrose. Homogenate (20%, v/v) in 0.25 M sucrose solution were centrifuged at 10,000×g for 30 min at 4℃. The postmitochondral fractions were again spum at 105,000×g for 60 min, and the cytosolic and homogenate fractions were used for determination of alkaline phosphatase (ALP) activity by the method of Bessey-Lowry[2]. And the membrane-bounding ALP activity was calculated by subtracting the enzyme activity in cytosol from that in homogenate.

3. Determination of malondialdehyde (MDA) content

Hepatic MDA content was determinated by the method of Ohkawa et al[6].

4. The preparation of liver tissue for light micrographs

A part of each liver tissue isolated from the animals was fixed in 10% neutral buffered formalin, made on paraffin embedded tissue and hematoxylin-eosin stain.

5. Histochemistry for alkaline phosphatase (ALP) with liver preparations

Isolated liver was rapidly frozen in liquid nitrogen and made frozen section (10 μm). The sectioned liver tissue was reacted with dimethylformamide, naphtol, AS-BI phosphate and fast red violet B at 25℃ for 30 min in a media and then made hematoxylin-eosin stain.

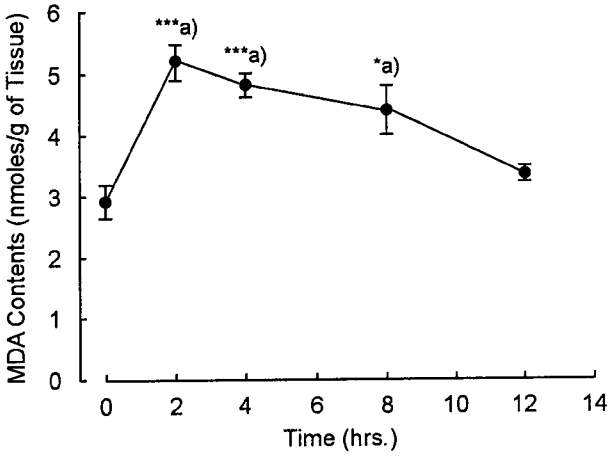

Fig. 1. Changes in hepatic MDA contents of postmorterm rats. Each value represents the mean±S.E. of 6 rats. a); Significantly different from the initial value (the value on sacrificed time).

6. Statistics

Experimental value were expressed as means±S.E. for 6 rats. Statistical significance were determined using unpaired Student's t-test, and probabilities of less than 5% ($P<0.05$) were considered significant[10].

RESULTS

1. Changes of body temperature and rigor mortis in postmortem

Table 1. shows the change of postmortem body temperature and rigo mortis. The body temperature, which had a basal of 36℃ revealed a gradual decline with the final one of 25℃, at 12 hours and with time the carcass showed the gradually enhanced rigor mortis with the completion of rigor mortis at 12 hours.

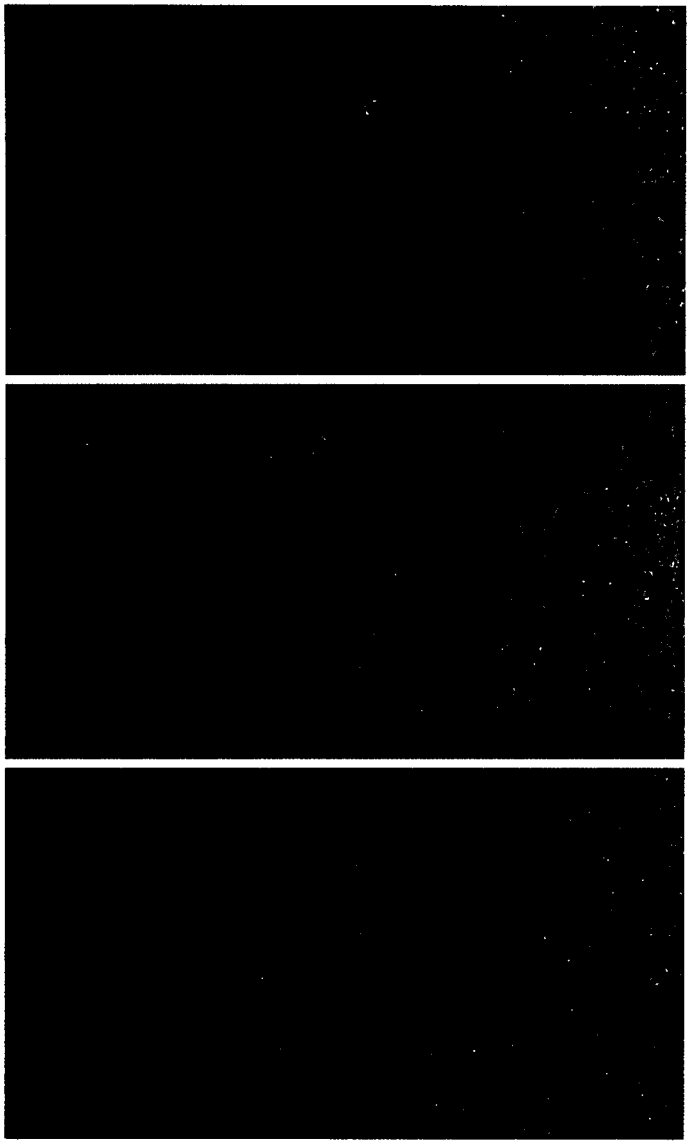

Fig. 2. Micrographs of liver tissue after somatic death in rats. Hematoxylin-eosin, *×100 a) control, b) postmortem 8 hr., c) postmortem 12 hr.*: The structure of hepatic lobules was preserved well in all the groups. C: central vein, P: portal vein.

2. Changes in hepatic malondialdehyde (MDA) in postmortem rats

As shown in Fig. 1. basal level of hepatic MDA (2.92± 0.28) rapidly increased, reaching a plateu at levels (5.19± 0.30) 1.8 fold at 2 hours, after that time, revealed a gradual decline and at 12 hours reached near basal line.

3. Micrographs of liver tissue at postmortem

As shown in Fig. 2, the structure of hepatic lobules showed no difference both in basal specimen (isolated at

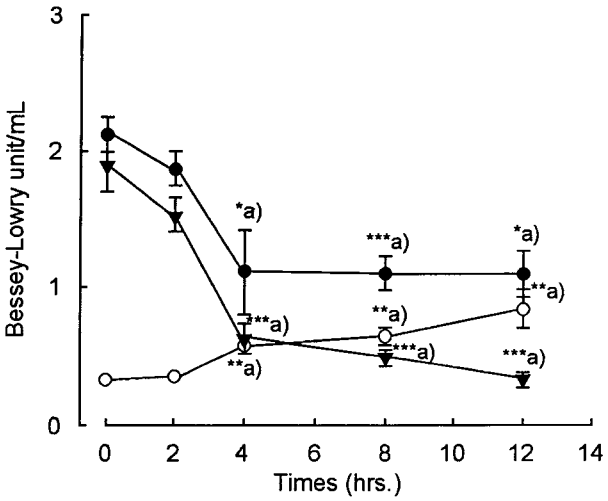

Fig. 3. Changes in hepatic alkaline phosphatase (ALP) activities in postmortem rats. Each value represents the mean±S.E. of 6 rats.
a); Significantly different from the initial value (*; $P<0.05$, **; $P<0.01$, ***; $P<0.001$).
●-●; homogenate, ▼-▼; membrane bounding, ○-○; cytosol

sacrificed time) and that done at 12 hours in hepatic lobule structure. That is, the structure of hepatic lobules was preserved well with time, but the portal vein in somewhat dilated and the clotted blood was seen both in the sinosoids and central veins at 8 hours and 12 hours.

4. Changes in ALP activities in liver of postmortem rats

Fig. 3. showed the changes of ALP activities in livers of postmortem. Total ALP (in homogenate) and membrane bounding enzyme activities were altogether gradually declined throughout experimental course. In the detailed description, total activity which had a basal level of 2.12±0.13 had a 47% greater decline ($P<0.001$) at 4 hours, after that time, with the continued same levels until 12 hours and membrane bounding enzyme, which showed a basal level of 1.90±0.2 had a 75% greater decline ($P<0.01$) at 8 hour, 83% greater ($P<0.01$) at 12 hour. On the contrary, cytosolic ALP activities showed a gradual increase throughout experimental course, that is, cytosolic ALP activities, which had a basal level of 0.32±0.02 showed a 81% greater rise ($P<0.01$) at 4 hr, 100% greater rise ($P<0.01$) at 8 hours and 162% greater increase ($P<0.01$) at 12 hours.

5. ALP activity in histochemical examination

In histochemical findings as shown in Fig. 4, a red color

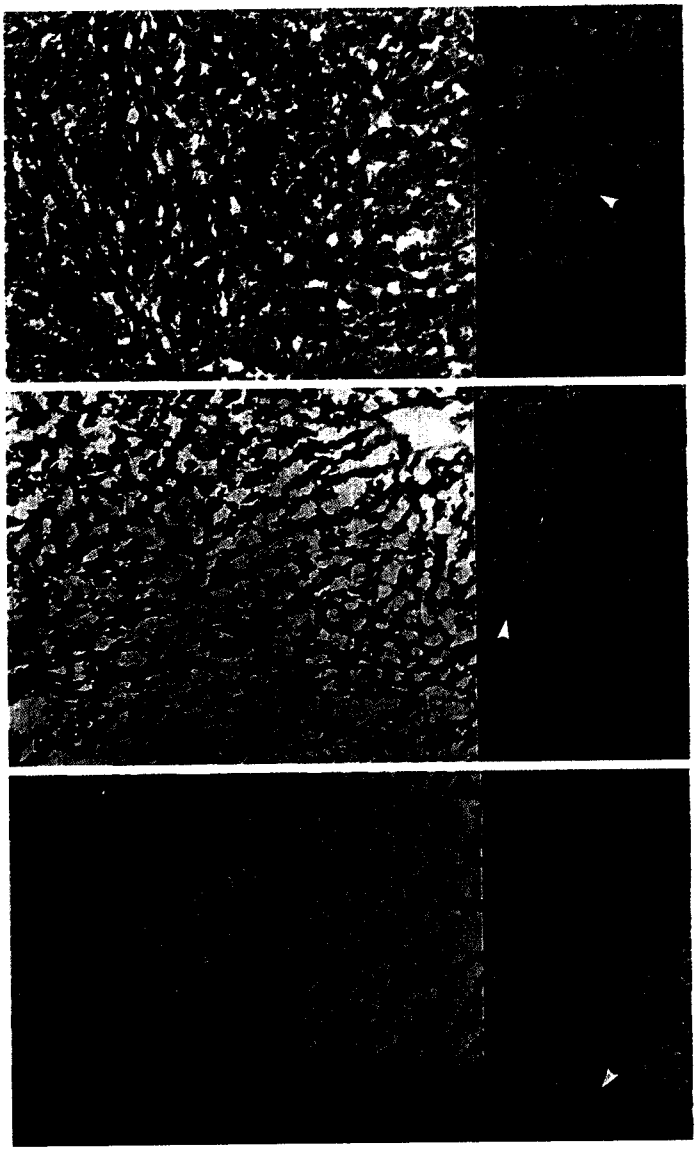

Fig. 4. Histochemical photographs of alkaline phosphatase activities in liver tissue after somatic death in rats. Hematoxylin, *left)×100, right)×200 a) control*: Showing the activity of ALP, a red color was clearly shown in whole tissue *(left)*, and the activity was found in the cytoplasm as well as in the plasma membrane (arrow head) of hepatocytes *(right). b) postmortem 8hr.*: The activity of ALP was markedly decreased compared to the control *(left)*, and the activity was found only in the plasma membrane (arrow head) of hepatocytes *(right). c) postmortem 12 hr.*: The tissue was colorless in an absence of ALP activity *(left)*, nevertheless the activity (arrow head) was limitedly found in the plasma membrane of a few hepatocytes *(right)*.

indicating ALP in basal specimen was clearly shown in whole tissue (a; left), and the enzyme was found in the cytoplasm as well as the plasma membrane (arrow head) of hepatocyte (a; right). At 8 hours postmortem, ALP was markedly decreased compared with the basal specimen (b; left) an the enzyme was found only in the plasma membrane (arrow head) of hepatocyte (b; right). At postmortem 12 hours, the tissue was colorless in an absence of ALP (c; left), nevertheless the enzyme (arrow head) was limitedly found in the plasma membrane of a few hepatocytes (c; right).

DISCUSSION

It is well known that with time, decline of body temperature[5], livor mortis[3] and rigor mortis[1] occur in a body after somatic death. It is also demonstrated in this study that the degree of algor mortis and rigor mortis were increased in rats during 12 hours after the animals were sacrificed by the dislocation of cervical vertebrae. Since the biochemical changes occur as well as changes of death in the early postmortem period[7], biochemical assay was done in liver tissue. Especially it is meaningful to observe the changes of cell in a tissue in the early postmortem period and furthermore focus on the cell membrane changes which can be occured at the early stage of cell injury. Therefore we determined the hepatic malondialdehyde (MDA)[6] contents known as the marker of cell membrane damage in the present experimental condition. The hepatic MDA contents remarkably increased at 2 hours and after that showed a gradual decline with similar with initial value. The MDA data obtained in this experiment indicates that with time an alteration of hepatocyte membrane will ensure dead body. To make an observation on the changes of cell membrane, histopathological study was done in this experimental conditions. At 8 hours and 12 hours after death, portal veins were characterized to be dilated, the clotted blood was seen in all veins and sinusoids, although the structure of hepatic lobules were preserved well with the unchanged state. It suggested that such a morphological change might be associated with alteration of cell membrane.

Furthermore, to clarify whether the postmortem could cause an alteration of cell membrane, a membrane bounding enzyme activity, ALP known as membrane bounding marker enzyme[9] and the histochemical technique for ALP were altogether demonstrated in this study. In both the histochemical and enzyme activity examination, membrane bounding ALP were gradually decreased with time. These results suggest that with time during 12 hours the hepatocyte was characterized to be the alteration of cell membrane,

and it could be an indicator of death time.

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