Histopathological Characteristics of Human Cardiac Tissues in Accidental Hypothermia Using Conventional Staining Techniques

Satoshi Furukawa*, Lisa Wingenfeld, Ikuo Sakaguchi, Tokiko Nakagawa,

Akari Takaya, Satomu Morita, Shigeru Yamasaki, Katsuji Nishi

The Department of Legal Medicine, Shiga University of Medical Science, Shiga, Japan

*31041220@belle.shiga-med.ac.jp

***Abstract*- We examined characteristics or cardiac cell damage in the myocardium obtained from individuals who died due to hypothermia or coldwater immersion. We could find four kinds of histological changes in the heart tissues obtained from hypothermia and coldwater immersion death. Although the red or orange colored cardiac cells, vacuolar cardiac cells and contraction band of the cardiac cells were observed in the heart from individuals with cardiac infarction, the frequency and intensity of the finding was remarkable in hypothermic death, and closely adhered cardiac cells were detected only in the myocardium from hypothermic or coldwater immersion death. We additionally discussed the mechanisms of development of these findings by comparing the results obtained in this examination with some reports in the literatures.**

Keywords- Hypothermic Death; Cardiac Cell Damages; Conventional Stain; Diagnosis of Hypothermic Death

I INTRODUCTION

Accidental hypothermia is defined as unintentional fall in core temperature under 35℃, and sever hypothermia is usually defined as under 28℃. Without through review of circumstances, the diagnosis of environmentally induced hypothermia is difficult at an autopsy, however hypothermia effects the cardiovascular, hematological, neurological, respiratory, renal, metabolic, and gastrointestinal systems [1].

In this study we examined histo-pathological phenomena of the cardiac muscle obtained from individuals who died due to accidental hypothermia or immersion in cold fresh water. We found several changes occurred in the cardiac tissues in them, and are able to say that Azan staining may give the easily detected characteristics in the cardiac tissues from individuals who died due to hypothermia and/or immersion in cold water as a routine staining compared with HE staining.

II MATERIAL AND METHODS

We performed 16 autopsies of hypothermic death at Department of Legal Medicine, Shiga University of Medical Science and Osaka Medical Examiner's Office during the last 4 years. The myocardium from 13 of 16 cases were examined in this study. Thirteen cases contain two old women who lived alone and died from hypothermia together with head injury after stumbling or fall from a stairs in their houses, and one child who died from abuse. The other 10 victims were found their death inside or around their houses and no remarkable injury was found on their body except the erythema on their knees in some cases. The eliminated three cases in which the victims showed prolonged postmortem interval were not suitable for the histological research. We also examined the myocardium from three cases as the comparative cases in which individuals died from drowning after immersion in a lake or canals in winter, that is, a young man was forced to go into a lake in winter, and in the last two cases young woman and man separately fell into canals with a motorcycle or a bicycle. In the last two cases, climbing traces were remained on the canal banks. Additionally we used the myocardium from traffic accidents cases with or without brain and/or cardiac injury, and cardiac infarction cases with bleeding and leukocyte infiltration as the control cases. Four parts of the heart, that is, crosscutting tissues of left and right ventricle and septum, and longitudinal section of septum from each individual were obtained and fixed in formalin. The sections with 3 μm thickness of paraffin embedded myocardium were prepared for HE and Azan staining. These staining were usually performed at our department for postmortem investigation. We used microscope apparatus from Nikon (EcliPSE 80i attaching HPxw4300 workstation) for making pictures of the cardiac tissues by HE and Azan, and from Keyence (BioRevo BZ-9000 attaching HPxw4300 workstation) for 3D analysis of extent of the orange colored cardiac cells in 25 serial sections by Azan stain.

III RESULTS

Four different kinds of histological findings were detected by Azan and/or HE stains in every cardiac tissue section from individuals who died from hypothermic and coldwater-immersion death, although intensity, extent and distribution of the each findings were different. Although these findings were also recognized in the cardiac tissues from other cause of death cases, intensive, remarkable and frequency of the findings varied in each cases and the finding in the hypothermic related death cases was conspicuous.

The first finding: the cardiac cells in hypothermic death cases remarkably adhered to each other. Although the area of adherence was not found on all fields of tissue sections, the mass of adhered cardiac cells was detected especially detected in the hypothermia death cases. The small amount of the cells also adhered in the tissues from coldwater

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immersion deaths, however the adherence of cardiac cells was not found in tissue sections from cardiac infarction and traffic accidental cases. The results stained by HE and Azan were shown in Fig. 1.

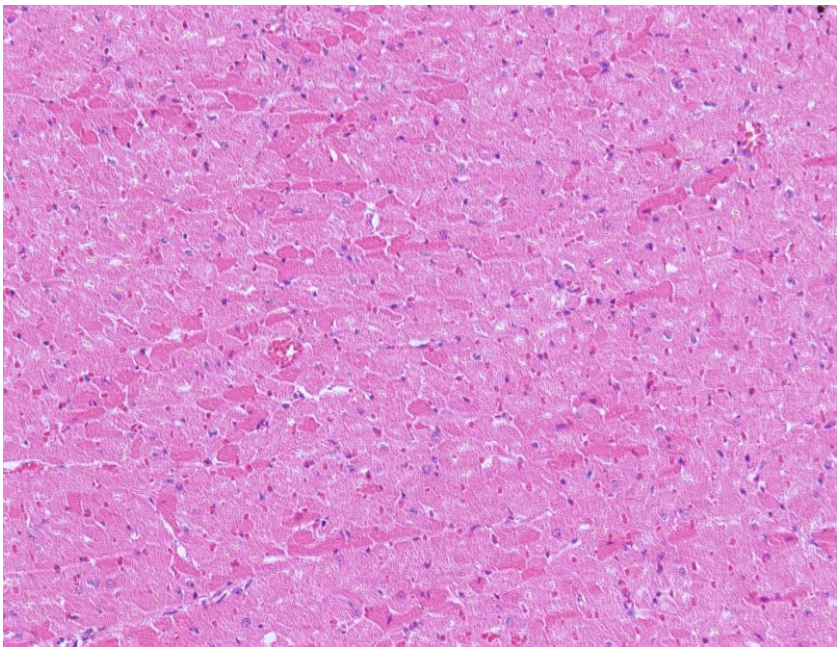

Fig. 1 The adhered cardiac cells in hypothermic death

The cardiac cells in hypothermic death cases remarkably adhered to each other and red colored cells are also recognized. The myocardial tissue was obtained from a 26 year-old female who died on a road covered with snow. HE stain ×100.

The second finding: the cells with clearly red color by HE and with intensive orange color by Azan were observed in cardiac tissues from all myocardium examined in this study. However frequency and intensive of the reddish or orange cells were high in hypothermia, middle in coldwater immersion death and low in cardiac infarction and traffic accidental cases. The results stained by Azan were shown in Fig. 2. Since the cells with orange color might be resemble in the position of each section to the cells with red by HE stain, the mirror sections were stained by HE and Azan method, respectively. The color changed cells by both staining were fit in the serial sections as shown in Fig. 3. Twenty-five serial sections of cardiac ventricle from one hypothermic death individual were stained by Azan method and orange colored cardiac cells in selected area were photographed. When 25 sheets of pictures were stacked up in a computer, the orange colored cells showed larger size than the expected size, as shown in Fig. 4.

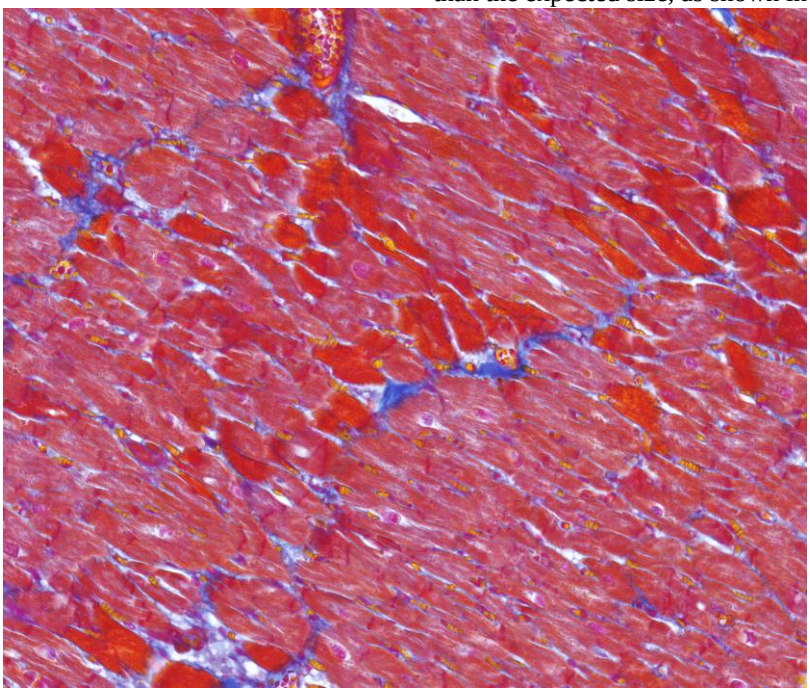

Fig. 2 The cardiac cells changed into orange color by Azan stain

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The cells with intensive orange color by Azan were observed. The myocardial tissue was obtained from a 29 year-old female who was found hear death from cold-water immersion in a canal.

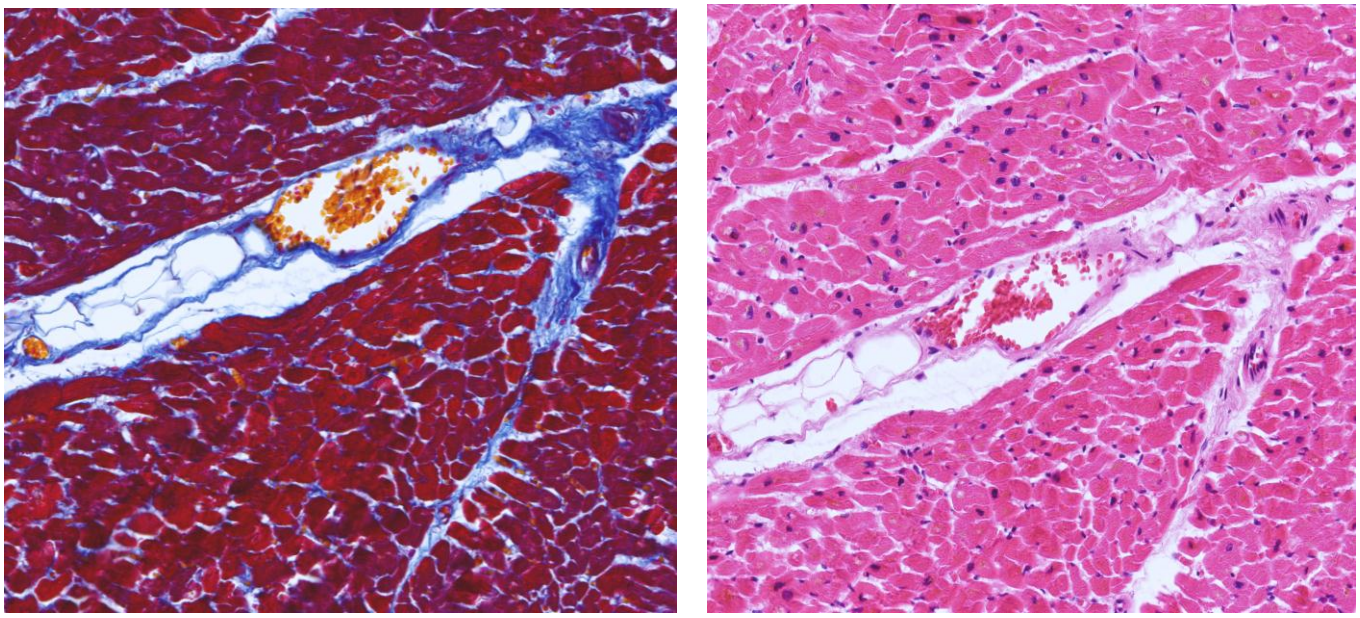

Fig. 3 Mirror Staining by HE and Azan

The color changed cells by HE (left side) and Azan (right side) staining were fit in the serial sections. The myocardial tissue was obtained from a 75 year-old female with dementia who was fond on a road in wither.

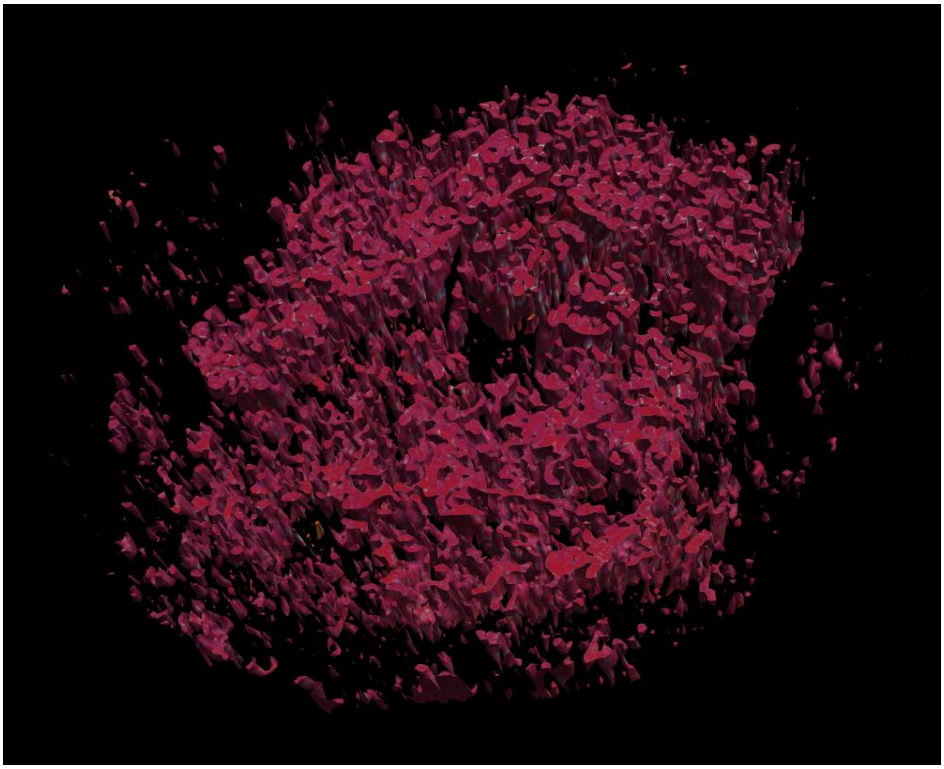

Fig. 4 3D image of orange colored cells by Azan stain

When 25 sheets of pictures were stacked up in a computer, orange colored cells showed larger size than the expected size. The tissue was obtained from the same individual in Fig. 1. Azan staining

The third finding: the gathering of vacuolar cells, colliquative myocytolysis, was observed mainly in the papillary and left ventricle muscles of the heart from hypothermic death individuals, as shown in Fig. 5. The same finding was observed in the tissues from traffic accident death without cardiac injury, in which victim died due to blood loss from cranial base fracture and no blood was recognized in the heart cavity. The cardiac cells with weak staining by HE and Azan were recognized around the gathering of vacuolar cells.

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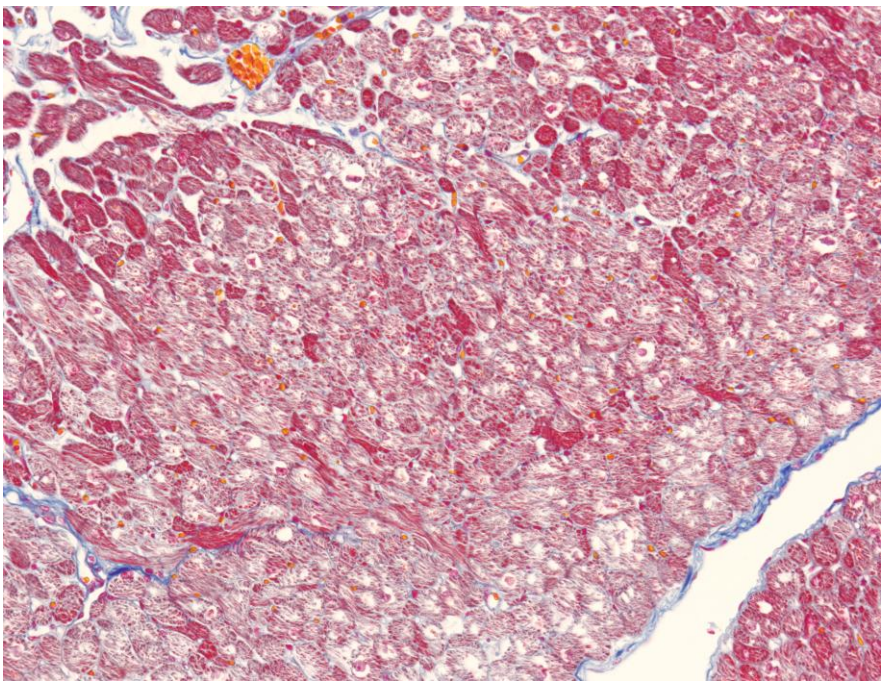

Fig. 5 The gathering of vacuolar cardiac cells

The gathering of vacuolar cells, colliquative myocytolysis, was observed mainly in the papillary muscles. The nucleuses were not observed in the cells. The myocardial tissue was obtained from the same individual in Fig. 2. Azan stain ×100

The fourth finding: the contraction band necrosis was detected in all sections from the septum of the hearts in hypothermic death and frequency of the contraction band necrosis was high in ventricular septum, middle in left ventricle and low in right ventricle. The color in contraction band necrosis detected as clear red by HE and intensive orange by Azan. The staining result in the septum was shown in Fig. 6. In the traffic accidental cases with cardiac injury, a large quantity of contraction bands and orange or red colored cells were detected around the injury, however gathering of the adhered cells was not detected. In the traffic accidental cases without cardiac injury in which victims died due to blood loss, the contraction band and the gathering of vacuolar cells were remarkably detected but no adhered cells was observed. Although in the cardiac infarction cases, intensive contraction bands, orange colored by Azan stain and vacuolar cells were recognized around the bleeding field, no adhered cells were observed.

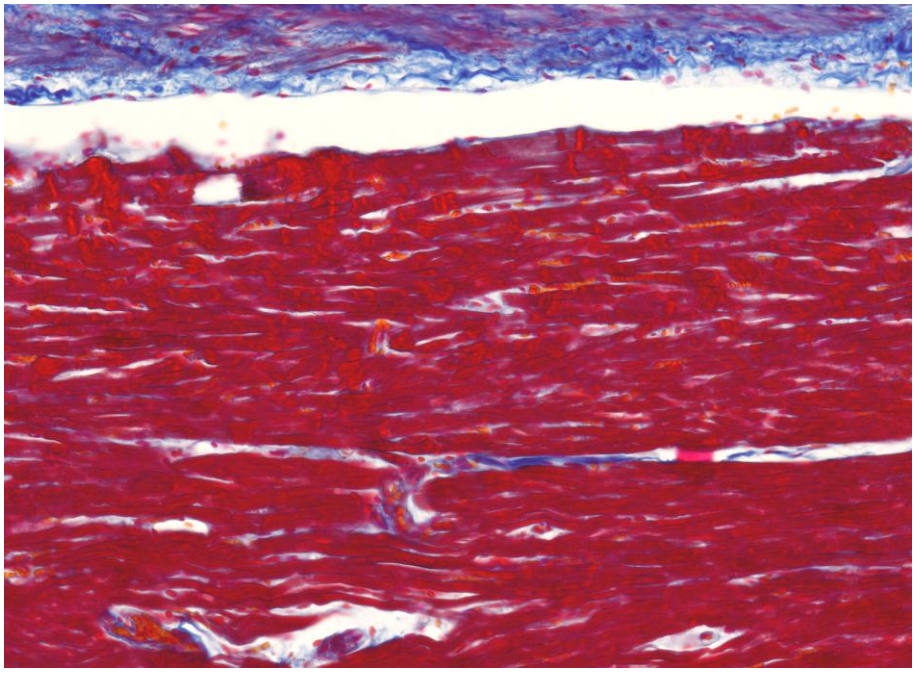

Fig. 6 Contraction necrosis

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Large amount of the contraction band necrosis with intensive orange color was detected in a section from the septum of the hearts. The myocardial tissue was obtained from a 20 year-old male who died due to coldwater immersion. Azan stain ×100

Although the findings described above were detectable by both staining methods, the Azan staining showed clear and remarkable results compared with those by HE stain.

IV DISCUSSION

In this examination, we could find four kinds of histological characteristic changes in the myocardium obtained from hypothermia and coldwater immersion death, they are: 1) the cardiac cells remarkably closed adherence to each other; 2) the number of red or orange colored cardiac cells by HE or Azan stain was frequent than that of control cases; 3) the cardiac cells with severe vacuolar, colliquative myocytolysis, were identified in the papillary and/or left ventricle muscles; 4) the contraction bands were recognized in all sections from the septum of the hearts from hypothermic and coldwater immersion death. The pathological findings detected in this study are similar to those in the cardiac infarction cases [2], excepting adhered cardiac cells. Baroldi et al summarized the results of histopathological and clinincal imaging studies to assess myocardial necrosis in humans [2].

They proposed that the myocardial cell may irreversibly arrest in: (1) relaxation in which mild eosinophilia, increased length of sarcomeres and elongated of nuclei are observed as the early histological pattern within 30 minutes before polymorphonuclear leukocytic infiltration; (2) contraction in which the myocytes stop in contraction or better in hypercontarction, where hypercontracted myocardial cells break down forming hypereosinophlic transverse bands constitutesd by hypercontracted, extremely short sarcomeres with highly thickened Z lines within 10 minutes; (3) after progressive failure in which a disappearance of myofibrils with increasing myocardial cell vacuolization, edema and small mitochondria without any reaction such as macrophages or inflammatory element.

In the mirror imaging sections orange and red colored cells stained by Azan and HE respectively were fit and coexist showing that hypoxic cells are able to detect similarly by both staining. To detection four findings observed in this study, Azan staining may be more useful than HE stain. Azan stain could indicate clear orange changing and contraction bands occurred in cardiac cells, since Azan staining solution contains four dyes such as Orange G, Azocarmine G, Aniline and Aniline blue, and each dye is able to react to own reactive site, on the other hand HE stain contains only eosin dye to stain cytosol of the cells, Twenty five-serial sections stained by Azan revealed that the broadening of hypoxic cardiac cells was large. This finding may result from cooling of the heart in the hypothermia, on the other hand hypoxia occurs only in the flowing area of obstructed blood vessels in the cardiac infarction cases.

Although the contraction band necrosis was also detected in the sections from cardiac infarction death, traffic accident death with or without heart injury and coldwater immersion death, the frequency is relatively lower compared with hypothermic death. The adhering of the cells may be specific finding in the hypothermic and coldwater immersion death, since this finding could not detect in control cases containing cardiac infarction and traffic accidental cases examine in this study. It is well known that hypothermia affects cytological, physiological and biochemical function of the heart. The phenomena of close adherence of cardiac cells in hypothermic death cases were firstly reported by Matsuo et al [3], which was confirmed by this examination. Concerning to close adherence of the cardiac cells, there are some studies in the literatures. Frank and Langer [4] studied on rabbit myocardial interstitium and reported that the extracellular space contains abundant ground substance (23%) distributed in a homogeneous mat throughout the space and within the T tubules and the remainder of the space contained 59% blood vessels, 6% empty space, 4% collagen, and 7% connective tissue cells. Tveita et al [5] described that in hypothermic hearts at 15-13℃ capillary volume fraction was significantly decreased, from 97 ± 20 in control to 70 ± 25 μl and the cytosolic volume increased from 25 ± 11 in control to 43 ± 8 μl. Cold–induced diuresis caused by suppression of ADH and shunting of blood volume centrally leads to further depletion of intravascular volume [3]. It seems to be indicated that over contraction of cardiac cells may induce adherence of the cells. Commonly, as cardiac cell contraction is in part an active energetic process, hypothermia might be expected to reduce the cardiac contractile state. However, Shutt and Howlett [6] described that paradoxical experiments with experimental animals such as guinea pig, rabbit, canine and ferret, have shown an increase in contractility and the gain of excitation-contraction coupling with cooling. They [6] also described that many studies using experimental animals had shown that cooling increases action potential duration, and slowed the rate of Na/Ca exchange in cardiac cells. Hypothermia also increases the open probability of cardiac sarcoplasmic reticulum Ca2+ release channels, and reduces the activity of sarcoplasmic reticulum Ca2+ ATPase but increases sarcoplasmic reticulum Ca2+ load in cardiac cells [6].

Cooling a body from normal temperature to low temperature has long been known to result in a large inotropic response in the mammalian and human heart [7]. The inotropic response to cooling has been attributed to a number of different mechanisms including an increasing in calcium influx during the action potential, an increasing in the subsequent release of calcium triggered by the action potential, and a change in the sensitivity of the myofilaments to activator calcium [8]. Since the adherence of the cardiac cells did not berecognized in the individuals from other death cases such as cardiac infarction, traffic accident and prolonged suffocation, this phenomenon will be useful to diagnose the hypothermic death and cold immersion death.

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Studies concerning directly to the second and third findings are few. However, the involving between hypothermia and hypoxia was studied by many physiologists who studied on cardiac metabolism of hibernating mammals [9, 10, 11]. It was presumed that eosinophilic cells stained by HE result from hypoxic changes of cardiac cells, since the red or orange color cells were detected around the infarction sit in the cardiac infarction cases in this study. It was well known that reducing of body temperature indicates utilization of low energy. Hypothermia shifts the oxy-hemoglobin-dissociation curve to the left, resulting in decreased oxygen release from hemoglobin into the tissue at a lower partial pressure of oxygen [12]. In case of severe O2 limitation, most excitable cells of mammals cannot continue to meet the energy demands of active ion-transporting systems, leading to rapid exhaustion of fermentable substrate, catastrophic membrane failure and cell death.

Although in poikilonthermal and/or hibernation animals hypoxia-induced membrane destabilization is either slow to develop or does not occur at all as a result of adaptive decreases in membrane permeability that dramatically reduce the energetic costs of ion-balancing ATPase [13], the hypothermic-induced mismatch between ATP supply and demand immediately preceding the forced hypo-metabolism of cold-sensitive animals containing human being is thought to occur as a result of metabolic imbalances caused by the differential effects of temperature on the rates of independent ATP supply and ATP demand pathways [13]. The second and third findings may result from mild exhausting energy and the third one shows cell death situation caused by absolute exhausting of ATP.

The cardiac conduction system is cold-sensitive, leading to symptoms like bradycardia, prolonged PR intervals, widening of the QRS-complex, and prolongation of the QT intervals resulting in the typical J wave in hypothermic situation [14]. As described above, hypothermia increases the gain of excitation-contraction coupling. The increasing of the gain of this phenomenon may cause hyper contraction of cardiac cells and contraction band necrosis may occur in cardiac tissues. The gain of exciting-contraction coupling may cause the alteration of the conduction system.

All findings detected in the cardiac tissues obtained from individuals who died due to hypothermia and/or cold immersion may be explained by the cell reactions resulting from imbalance of ATP supply and ATP demand pathways.

The Na+/K+ pump is largely inhibited by the cold either as a result of the direct thermodynamic effects of decreased ATP production [15] or of the progressive development of a hypothermia-induced mismatch between ATP supply and ATP demand pathways [13]. Deep hypothermia in non-hibernating mammals leads to marked disturbance in cellular ion homeostasis that may be further influenced in the cold by alterations in membrane fluidity [10]. Wang and Zhou [16] proposed the idea that intracellular Ca overload take place in cardiac cells of non-hibernating mammals during deep hypothermia. Exhausting of ATP in other situations such as hyperthermia caused by drug side effect or heat stroke may cause similar physiological changes in the cardiac cells, since the gain of excitation-contraction coupling was also detected in methamphetamine addiction cases [17], and the increasing of Ca2+ release from the sarcoplasmic reticulum of skeletal muscles was observed in hyperthermia [18].Further studies concerning to signal pathway in the cardiac cells affected hypothermic stress was necessary by using immunohistochemical technique, since in this study we firstly examined the morphological changes in hypothermic death by using conventional staining techniques.

V CONCLUSION

We could find four kinds of histological changes in the heart tissues obtained from hypothermia and coldwater immersion death, they are: 1) the cardiac cells remarkably closed adherence to each other; 2) the number of red or orange colored cardiac cells by HE or Azan stain was frequent; 3) the cardiac cells with severe vacuolar, colliquative myocytolysis, were identified in the papillary and left ventricle muscles 4) the contraction bands in the cardiac cells were recognized.

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