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Mutsumi Abe · Hisako Saitoh · Yayoi Sato
Kin-ichi Hamaguchi · Masahiro Kiuchi

Immunohistochemical study of the kidneys after severe muscular injury

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Abstract Severe muscular injury sometimes causes renal failure, and myoglobin in skeletal muscle is known to induce toxic free oxygen radicals in the kidneys. The relationship between the immunohistochemical expression of myoglobin and the scavenger copper/zinc superoxide dismutase (Cu/Zn-SOD) was investigated in kidneys taken from two autopsy groups, a group with tourniquet shock (n = 4), and a group with severely injured skeletal muscle (n = 18). Paraffin-embedded kidney sections were used for immunohistochemical staining by the avidin-biotin-complex (ABC) method using antibodies against myoglobin and Cu/Zn-SOD. Detection of the two antigens was analyzed qualitatively. In most cases of tourniquet shock in which the survival time was considered to be relatively long, myoglobin staining was positive and Cu/Zn-SOD was negative. Among the seven cases of severely injured skeletal muscle in which the survival period was considered to be relatively short, positive staining was detected immunohistochemically for both myoglobin and Cu/Zn-SOD. Moreover, in most of the cases in this group that showed acute tubular necrosis, immunohistochemical staining was negative for both markers, whereas positive staining was found for most of the cases in which the kidneys were revealed to be normal by HE staining. These findings suggest that when myoglobin enters the kidneys via the circulation, Cu/Zn-SOD reacts to eliminate free radicals, but is depleted by consumption in the long run, and that there might be a relationship between these histological findings and immunohistochemical expression.

Keywords Immunohistochemistry · Kidney · Myoglobin · Cu/Zn superoxide dismutase · Renal failure

M. Abe (✉) · H. Saitoh · Y. Sato · M. Kiuchi
Department of Legal Medicine, School of Medicine,
Chiba University, 1-8-1 Inohana, Chuo-ku,
Chiba City, Chiba, Japan
Tel.: +81-43-2262078, Fax: +81-43-2262005

K. Hamaguchi
Department of Clinical Laboratory, National Sakura Hospital,
2-36-2 Ebaradai, Sakura City, Chiba, Japan

Introduction

Renal failure in cases of severe muscular injury has presented a great challenge in both clinical and forensic fields. This is known as the crush syndrome and the first case was reported in 1941 [1]. Generally, the cause of renal failure is thought to be massive leakage of the contents of myocytes into the plasma due to rhabdomyolysis [2] and many subsequent studies have suggested that circular reperfusion to the ischemically damaged muscle tissue induces the production of free oxygen radicals [3, 4, 5, 6]. These free radicals somatically increase vascular permeability and attack the unsaturated free fatty acids in the phospholipid layer of the cell membrane, thereby leading to cell death and, ultimately, tissue necrosis [3, 7]. In the kidneys, the iron component of myoglobin also produces free radicals and leads to renal failure in the same way [8, 9]. On the other hand, there are some substances, known as scavengers, that have protective effects on free radicals. Superoxide dismutase (SOD) is the most common scavenger and catalyses the following reaction [10]:

$$2O_2^{\cdot} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (1).$$

A recent study reported that post-mortem changes do not influence the activity of SOD in myocardial tissue, therefore SOD was thought to be the best marker to estimate the internal reaction against free radicals [11, 12].

There are three major kinds of SOD, copper/zinc SOD (Cu/Zn-SOD), which is expressed throughout the cell, manganese SOD (Mn-SOD), which is located mainly in the mitochondrial matrix, and iron SOD (Fe-SOD), which is found in many bacteria [10, 13]. During renal failure in cases of severe muscular injury, it is thought that scavengers participate in some type of reaction that protects against free radicals produced by myoglobin. Cu/Zn-SOD was selected from among the three types of SOD for investigation in the present study.

The aim of this study was to investigate the distribution of myoglobin and Cu/Zn-SOD in the kidneys when muscle tissue is massively damaged and renal failure is suspected.

Table 1 Description of cases included in this study

Group	Case no.	Age (years)	Sex	Cause of death	Survival time	Time after death
A	1	27	Male	Tourniquet shock	1 day	2 days
	2	45	Male	Tourniquet shock	2 days	17 h
	3	23	Male	Tourniquet shock	Unknown	1.5 days
	4	26	Female	Tourniquet shock	Unknown	Unknown
B	5	16	Male	Traumatic shock	< 1 h	2 days
	6	16	Male	Traumatic shock	< 1 h	12 h
	7	44	Male	Traumatic shock	< 1 h	1.5 days
	8	40	Male	Traumatic shock	< 1 h	1.5 days
	9	84	Male	Traumatic shock	< 1 h	1.5 days
	10	42	Male	Traumatic shock	< 1 h	20 h
	11	49	Male	Brain injury	< 1 h	1.5 days
	12	53	Male	Traumatic shock	< 1 h	1.5 days
	13	61	Male	Traumatic shock	< 1 h	14 h
	14	63	Male	Traumatic shock	< 1 h	3.5 days
	15	60	Male	Traumatic shock	< 1 h	1.5 days
	16	39	Male	Brain injury	< 1 h	19 h
	17	23	Male	Compression	< 1 h	1.5 days
	18	55	Male	Compression	1 h	2.5 days
	19	64	Male	Traumatic shock	2 h	2 days
	20	80	Male	Traumatic shock	5 h	3 days
	21	29	Male	Traumatic shock	7.5 h	9 h
	22	16	Male	Brain injury	39 h	2 days
C	23	28	Male	Hypoxia	< 1 h	23 h
	24	13	Male	Acute cardiac failure	< 1 h	22 h
	25	26	Male	Acute cardiac failure	< 1 h	2 days
	26	31	Male	Exsanguination	18 h	23 h
	27	13	Female	Pneumonia	6.5 days	1.5 days
	28	34	Female	Acute pancreatic necrosis	Unknown	2.5 days
	29	63	Male	Hepatocellular cancer	Unknown	2 h

Materials and methods

We selected 29 autopsy cases carried out between 1972 and 1998 at the Department of Legal Medicine, School of Medicine, Chiba University and the National Sakura Hospital which were divided into the following three groups:

– Group A: Victims diagnosed with tourniquet shock (n = 4) which is known to cause renal failure after long-term muscle tissue ischemia and reperfusion due to the production of free radicals in the kidney by myoglobin from the necrotic muscle tissue.

– Group B: Victims where muscle tissue was severely injured (n = 18).

– Group C: Control subjects with uninjured or with minimal injuries to skeletal muscle (n = 7).

The descriptions for each case are shown in Table 1.

Both kidneys from each of the 29 autopsies were fixed in 10% formalin, embedded in paraffin, sliced into 2–4 μm-thick sections and stained with hematoxylin-eosin (HE). Immunostaining was performed using polyclonal rabbit antibodies against human skeletal muscle myoglobin (Biogenesis, New Fields, England) at a dilution of 1:150 and polyclonal sheep antibodies against Cu/Zn-SOD (Biogenesis) at a dilution of 1:100. Detection was carried out using the avidin-biotin-complex (ABC) method with a Vectastain elite ABC kit (Vector, Burlingame, Calif.) for rabbit antibodies to myoglobin and for sheep antibodies to Cu/Zn-SOD.

The sections that showed staining of tubular casts were graded as (+), non-specific background artifacts and intracellular granular Cu/Zn-SOD staining were excluded.

Results

The immunohistochemical staining reactions showed various patterns (Table 2) which are described for each group. There were no cases where negative reactions were found for myoglobin and positive reactions for Cu/Zn-SOD.

Group A (victims with tourniquet shock)

All cases showed acute tubular necrosis with interstitial edema in HE staining (Fig. 1a), and positive staining for myoglobin (Fig. 1b). However, Cu/Zn-SOD staining was negative (Fig. 1c) in all cases except case no.2.

Group B (victims with severely injured skeletal muscle)

In HE staining, 12 cases from this group showed acute tubular necrosis and 2 cases showed positive immunohistochemical staining for both myoglobin and Cu/Zn-SOD (Fig. 2a,b). The other six cases of this group showed normal kidney tissue, and five of these cases showed positive staining for both antigens. Unlike in group A, there was no case that was positive for myoglobin and negative for

Table 2 Results of immunostaining for myoglobin and Cu/Zn-SOD (*ATN* acute tubular necrosis)

Group	Case no.	ATN	Myoglobin	Cu/Zn-SOD
A	1	+	+	–
	2	+	+	–
	3	+	+	–
	4	+	+	+
B	5	+	–	–
	6	+	–	–
	7	+	–	–
	8	–	+	+
	9	–	+	+
	10	–	+	+
	11	+	–	–
	12	+	–	–
	13	+	–	–
	14	+	–	–
	15	+	–	–
	16	+	–	–
	17	–	–	–
	18	–	+	+
	19	+	+	+
	20	+	+	+
	21	–	+	+
	22	+	–	–
C	23	–	–	–
	24	–	–	–
	25	–	–	–
	26	–	–	–
	27	–	–	–
	28	–	–	–
	29	–	–	–

Cu/Zn-SOD. The survival period was greater than 1 h in four out of seven cases that were immunohistochemically positive for both myoglobin and Cu/Zn-SOD, and in the other three cases this period was even shorter. In the other 11 cases with negative immunohistochemical staining for both antigens, the survival period was less than 1 h, with the exception of case no.15. The relationship between HE and immunohistochemical staining reaction is shown in Table 2.

Group C (controls)

All control cases showed negative staining for both myoglobin and Cu/Zn-SOD.

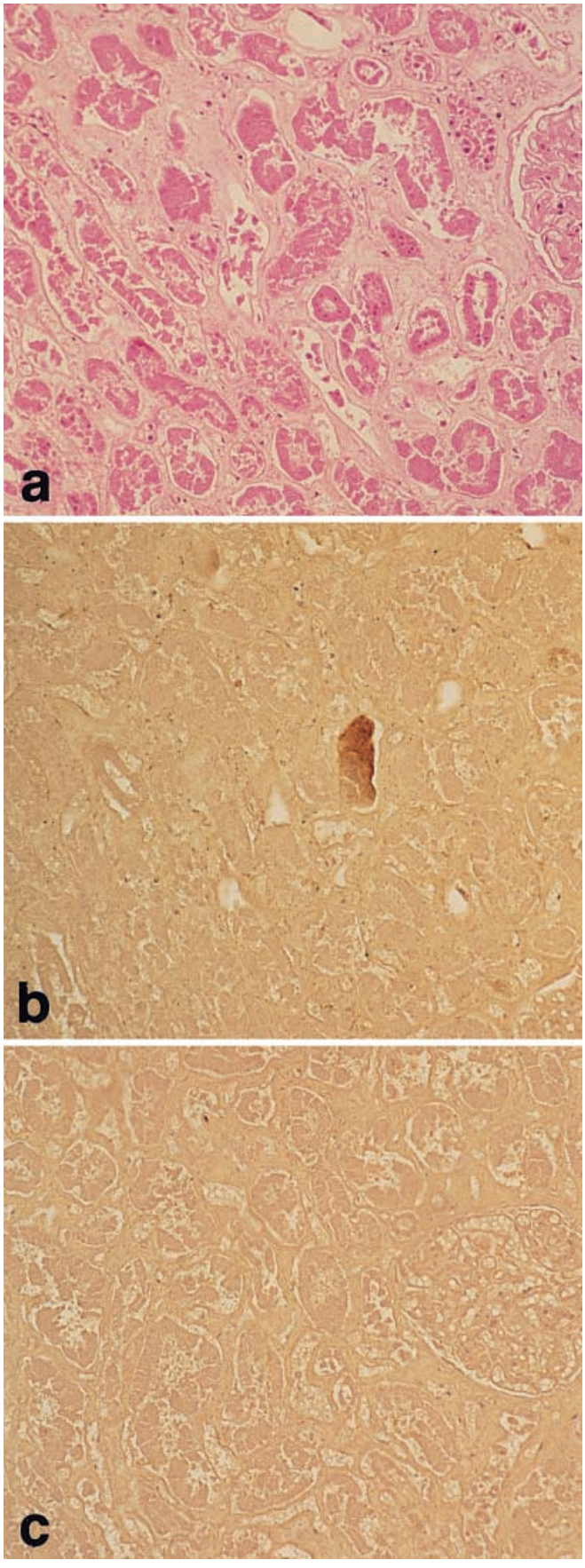

Fig. 1 Case (no. 2) of tourniquet shock showing **a** acute tubular necrosis with tubular necrosis and apparent interstitial edema (HE staining × 150); **b** positive myoglobin immunostaining around necrotic tubular epithelium forming tubular cast (ABC method × 150); **c** negative Cu/Zn-SOD staining (ABC method × 150)

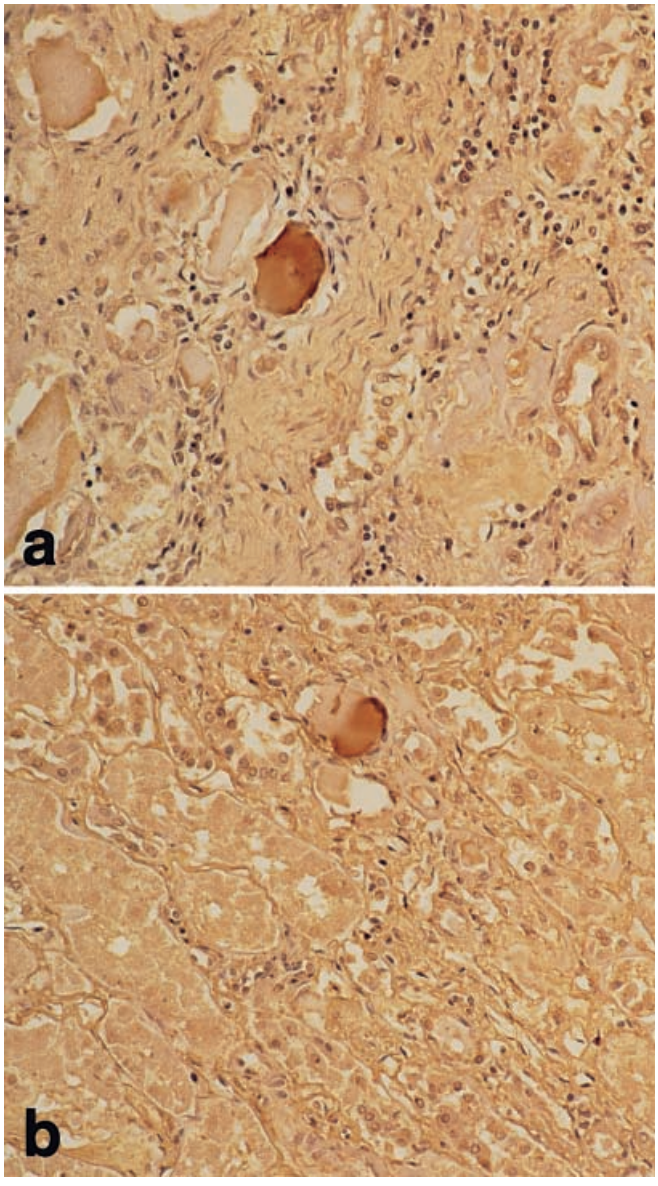

Fig. 2 Case (no. 19) of traumatic shock showing **a** myoglobin positive staining of kidney casts (ABC method × 250); **b** positive Cu/Zn-SOD staining similar to the myoglobin staining (ABC method × 250)

Discussion

Oxygen free radical toxicity has been discussed in many studies [14, 15]. Moreover, several reports have described the use of scavenger administration to treat various animals experimentally exposed to free radicals [3, 4, 16, 17, 18, 19].

In most of the present cases of tourniquet shock (group A), tubular necrosis could be seen by HE staining, and myoglobin was positive and Cu/Zn-SOD negative after immunohistochemical staining. The cases in group B, with severe muscular injury, were selected because in such cases, there was the potential for myoglobin released from injured muscle tissue to leach into the blood and accumulate in the kidneys. In cases of tourniquet shock the survival period was considered to be more than several hours but in group B, the survival period was somewhat shorter than in group A. Moreover, the survival period of the cases that were immunohistochemically positive for both antigens, was thought to be longer than that of the cases with immunohistochemically negative staining for both antigens. We can therefore hypothesize that when myoglobin from the injured skeletal muscle enters the bloodstream and reaches the kidneys, Cu/Zn-SOD reacts to eliminate free radicals, whereas, if there is a long period between injury and death, Cu/Zn-SOD is consumed and depleted. In immunohistochemically negative cases in which the survival period was short, skeletal muscle was severely injured, but there would not have been enough time for myoglobin to accumulate in the kidneys.

The results of this study suggest that the expression of myoglobin and Cu/Zn-SOD are dependent on the survival time. Moreover, 12 cases of group B showed tubular necrosis, but only 2 cases from this group expressed immunohistochemically positive staining. Among the other six cases in which the kidneys were revealed to be normal by HE staining, five cases showed positive immunohistochemical staining for both antigens, i.e., most of the immunohistochemically negative cases had tubular necrosis in the kidney. Therefore, in these cases the injuries were so severe that renal disorders occurred shortly after injury without any contribution from free oxygen radicals.

The results of this study confirmed that Cu/Zn-SOD acts as a scavenger to protect against free radical nephrotoxicity. There is a possibility that other scavengers act in such cases, so further investigations will be needed to confirm the protective effects of free radicals and the histological relationship in cases of severe muscular injury.

In many cases of renal failure, victims suffer from several types of physical violence simultaneously, and thus it can be difficult to determine which of these was the cause of death. The present results should therefore provide useful information that may help in determining the time to death and cause of death in such cases.

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