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Original Investigation

Changes of MDA and SOD in Brain Tissue after Secondary Brain Injury with Seawater Immersion in Rats

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ABSTRACT

AIM: To investigate the variation and significance of malondialdehyde (MDA) and superoxide dismutase (SOD) in brain tissue after secondary brain injury (SBI) with seawater immersion in rats.

MATERIAL and METHODS: We randomly divided 163 male Sprague Dawley rats into 4 groups, as normal (Group A), SBI (Group B), SBI with physiological saline immersion (Group C) and SBI with seawater immersion (Group D) groups. The animal model of ischemic SBI with seawater immersion was established based on the Marmarou's model of diffuse brain injury. The water content, and the MDA and SOD contents of brain tissue were detected at 1, 3, 6, 12, 24 and 48 hours after the injury.

RESULTS: Compared to group A, there were significant changes of various indicators in group D after injury at 1 hour after injury ($P < 0.05$). The water content and MDA contents in brain tissue were persistently elevated and significantly higher than that in groups B and C at each time phase ($P < 0.05$). The SOD content showed a persistent decline and was significantly lower than that in groups B and C at each time phase ($P < 0.05$). The SOD content was negatively correlated with the MDA content with a correlation coefficient of -0.992 ($P < 0.01$).

CONCLUSION: The SBI with seawater immersion is faster and more serious than the simple SBI.

KEYWORDS: Secondary brain injury, Seawater immersion, Malondialdehyde, Superoxide dismutase

INTRODUCTION

Researches on traumatic craniocerebral injury, a disorder with a high incidence of morbidity and mortality, have positive social and clinical value. In 1978, Miller, a neurosurgeon in University of Edinburgh, UK, first put forward the concept of secondary brain injury (SBI). After primary brain injury, the abnormal changes of blood pressure, body temperature, intracranial pressure, cerebral blood flow and cerebral perfusion pressure could cause secondary brain injury to aggravate the primary brain injury and traumatic brain edema, resulting in increased mortality rate and disability rate and decreased quality of life in patients with brain injury (15).

Clinical studies have shown that SBI can happen in only a few hours to a few days after the primary injury (18). On the way to the hospital, about 37% of patients with severe craniocerebral injury were complicated by hypoxemia events and more than one-third of patients in pre-hospital emergency had experienced at least one of the SBI-induced factors, with an increased death rate (7). Even in the intensive care unit, 25% of patients with severe brain injury showed SBI-induced factors, such as hypotension (5). Patients with severe brain injury were found to have a change in intracranial pressure. Through the regulation and control of the intracranial perfusion pressure, it was found that maintaining effective oxygen content in brain

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tissue can reduce the mortality rate (17, 19). During production of SBI based on the fluid percussion model of traumatic brain injury, it was found that those animals with SBI seemed to have a prolonged injury time in hippocampal CA1 cells within 24 h compared to the single hydraulic injury group (2).

Oceans account for 71% of the Earth's total surface area. The rich marine resources draw many countries' interest, bringing constant conflicts. With the addition of the worsening environment and frequent natural disasters (such as tsunami), craniocerebral injuries occurring in offshore operations, naval battles or with shipwrecks are becoming common. The delayed treatment at a specialized center could easily induce SBI on the basis of the first brain injury. At the same time, the wounded are vulnerable to being continuously exposed to seawater. A lot of SBI studies (2) are based on a stable indoor environment. There are few open studies combined with seawater immersion, except for those skin injuries caused by marine organisms and seawater drowning injuries (10, 11). Based on this knowledge, we wanted to determine whether brain edema would further aggravate the SBI after seawater immersion and how this could be changed? Can the mortality of the wounded be changed?

We established an animal model of SBI with seawater immersion and observed the variations of malondialdehyde (MDA) and superoxide dismutase (SOD) to explore the influence of seawater immersion on SBI.

MATERIAL and METHODS

Experimental Animals and Groups

One hundred and sixty three clean-level Sprague Dawley (SD) rats (about 14 weeks) weighing 350±30 g were fed in the Department of Comparative Medicine in Fuzhou General Hospital,, at a constant temperature of 23°C and a humidity of 55-60%. The SD rats were randomly divided into 4 groups as normal group (Group A, n=5), SBI group (Group B, n=32), SBI with physiological saline immersion group (Group C, n=33) and SBI with seawater immersion group (Group D, n=93). Groups B, C and D were divided into six sub-groups according to six time phases at 1 hour (h), 3h, 6h, 12h, 24h and 48h. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Fuzhou General Hospital, Xiamen University.

Acquisition and Physicochemical Properties of Seawater

Experimental seawater was taken from particular water along the southeast coast, specifically a coastal beach in Changle City, Fujian Province. The seawater was taken about 60 m far from the beach, at a depth of about 80 cm and 40 cm away from the surface of the water at about 15:00 in one sunny afternoon after the falling tide. The physicochemical properties of the seawater were as follows: water temperature 19.5°C, pH value 7.5, proportion 1.025, osmotic pressure 803.3 mmol/Kg, K^+ 9.4 mmol/L, Na^+ 427 mmol/L, chloride 484 mmol/L, Ca^{2+} 12.67 mmol/L, and Mg^{2+} 16.25 mmol/L.

Establishment of Animal Models

Marmarou et al., (14) established an animal model for diffuse brain injury. Bilateral common carotid arteries were rapidly separated and ligated for 30 minutes to establish the animal model of ischemic SBI. In group C and D, on the basis of animal model of SBI, the dental drill was used to open a cranial window at the right side of the mid-sagittal line in the rat's head, between the coronal and lambdoid sutures. Part of the skull was taken out by a curved forceps to form a bone window about 0.7×0.5 cm^2. The dura was opened to expose the cortex of the right hemisphere (Figure 1) and the cortical surface was covered with cotton sheets. An infusion catheter with openings at both sides and with a diameter of about 0.3 cm was used for irrigation of the cortex. The middle part of the skin incision was sutured to form a subcutaneous tunnel which was open at both ends in brain surface. The seawater/physiological saline was continued to be brought from the tunnel entrance to the contused cerebral cortex in order to imitate a continuous immersion in seawater (Figure 2).

Figure 1: Part of skull was taken off and the dura was opened to expose the right hemisphere cortex.

Figure 2: The seawater was continued to be brought from the tunnel entrance through the cerebral cortex containing contusion parts in order to imitate a continuous immersion in seawater.

Sample and Tissue Treatment

Animals were anesthetized and the chest was rapidly opened after intervention for 1h, 3h, 6h, 12h, 24h and 48h in accordance with the animal grouping. Physiological saline (100 ml) was perfused for 5 minutes to replace the blood. The needle was on the left ventricle and the right atrium was opened until the liver became white. Then, 250 ml of 4% phosphate buffered formaldehyde was slowly perfused and fixed until the liver became hard and the tail was stiff. Then, the rats were quickly decapitated and their brains were took out. The brain tissues from frontal pole to occipital pole were divided into five equal portions as a, b, c, d, e. They were kept in the freezer at -20°C. They could be taken and thawed at room temperature when necessary.

Water Content

About 300±50 mg cortex was taken and dried with filter paper. After weighting, it was kept at 80°C for 24 h, the dry brain tissue was weighted and we calculated its water content with the Elliott formula (wet and dry weight formula) as follows: Water content in brain tissue (%) = (brain wet weight - brain dry weight) / brain wet weight ×100%.

MDA and SOD Determination

The brain tissue was thawed at room temperature and dried with filter paper. About 200 ± 50 mg brain tissue was taken and washed with precooled normal saline (NS). It was converted to 100g / L of brain homogenates in a homogenizer filled with 9 times the mass of precooled NS. The homogenates were centrifuged at low temperature for 15 minutes with a speed of 3500 r/min. Proper amount of supernatant were given tissue protein quantification, MDA and SOD determination with biuret method, thiobarbituric acid method and xanthine oxidase method according to the specifications of SOD and MDA kit (Nanjing Jiancheng Bio-Technology Co., Ltd.).

Statistical Analysis

The numerical data were tested by χ^2 inspections with SPSS13.0 software. The measurement data were presented as (x±s) and compared with one-way analysis of variance (ANOVA). $P < 0.05$ was presented as statistical significance.

RESULTS

Mortality Rates

Before normal disposal, 5 rats in group A were survived, but 62 were died in the group B, C, and D. Rats in group B and C were usually died 30 min~24 h after the injury. Some rats in group D also died 24 h after the injury. The mortality rate of group D was significantly higher than groups B and C ($P < 0.05$) (Table I).

Changes of Water Content, MDA and SOD Contents

The water contents in brain tissue in groups B and C were increased and then decreased, tending to be stable. The peak appeared 12 h after the injury. The change of MDA content was parallel with the change of water content in brain tissue and the peak appeared 24 h after the injury. The change of SOD content was, as opposed to MDA, firstly decreased and then increased and the valley bottom appeared 24 h after the injury. There were no significant differences in each index at each time phase point between groups B and C ($P > 0.05$), but significant differences in the changes within 24 h after the injury in each group ($P < 0.05$). The water content and MDA content in brain tissue in group D were persistently increased, but SOD content was persistently decreased, with significant differences in the change within 48 h after the injury ($P < 0.05$). Compared to group B and C, the water content and MDA content in brain tissue at every time phase point were all significantly increased, but SOD content was significantly decreased ($P < 0.05$) (Table II). The SOD content was negatively correlated to MDA content in group D with a correlation coefficient of -0.992 ($P < 0.01$). Compared to group A, each index in group B and C appeared significant difference 3h after the injury ($P > 0.05$) but group D appeared significant difference 1 h after the injury ($P < 0.05$).

DISCUSSION

The study aims to establish an animal model of SBI with seawater immersion to observe the variation of water content, MDA and SOD contents in the brain tissue and to explore the influence of seawater immersion on SBI injury. The result showed that the mortality in SBI with seawater immersion group was obviously higher than other groups. The changes in different indices appeared 1 h after the injury in SBI with seawater immersion group, and appeared 3 h after the injury in SBI and SBI with physiological saline immersion groups. Our findings indicates that on the basis of serious cerebral ischemia and hypoxia after SBI with seawater immersion, seawater aggravated cerebral edema and developed more quick and lasting injury, which significantly increases mortality.

The essential of SBI is a pathophysiological change of brain tissue caused by very low cerebral perfusion pressure due to cerebral ischemia and hypoxia. The injury mechanism is complex. Many scholars and neurosurgeons investigated many aspects of molecular and cellular basis, as well as prevention methods, of SBI (1, 6, 8, 9, 16). The radical reaction enhancement after brain injury was considered as one of the most common contributing factors of SBI.

Free radicals have strong oxidation capacities with chemical activities, which could make the cell membrane peroxided and damages the cellular structure. The mechanism of free radicals on the brain tissue injury are peroxidation of lipid-rich cell membrane of the brain neurons and production of large number of lipid peroxides to change the cellular morphology

Table I: Comparison of the Mortality Rate Among Different Groups

Group	Number	Mortality	Mortality rate (%)
B	32	8	25.00
C	33	9	27.27
D	93	45	48.39▲

Versus group A, B, C: ▲P < 0.05.

Table II: Changes of Water, MDA and SOD Content in the Rat Brain Tissue Among Different Groups ($\overline{X}$±S)

Index	Group	n	Time after injury (hours)					
			1	3	6	12	24	48
Brain water content (%)	A	5	78.02±0.51					
	B	24	78.71±1.12	81.22±0.44#	82.03±0.31#	83.88±0.38#	82.26±0.52#	82.10±1.08
	C	24	78.55±1.06	80.68±0.63#	81.45±0.69#	83.57±1.15#	81.83±0.84#	81.47±0.93
	D	48	79.50±0.91▲	83.15±0.47▲#	84.10±0.48▲#	85.94±0.80▲#	87.14±0.66▲#	88.38±0.93▲#
MDA content (nmol/mg)	A	5	3.90±0.58					
	B	24	4.36±0.77	6.16±0.34#	7.67±0.99#	10.21±0.96#	13.85±1.35#	12.69±0.97
	C	24	4.23±0.68	6.10±0.31#	7.43±0.94#	10.16±1.08#	13.62±1.04#	12.55±0.84
	D	48	5.50±0.56▲	9.53±0.47▲#	10.91±0.70▲#	13.56±0.92▲#	15.33±0.89▲#	16.57±1.16▲#
SOD content (U/mg protein)	A	5	325.95±20.54					
	B	24	314.70±22.34	260.73±21.26#	217.78±19.47#	163.58±20.20#	119.67±19.24#	123.71±20.35
	C	24	328.68±22.06	275.31±22.02#	223.86±16.57#	173.9±18.00#	121.61±17.09#	124.22±21.27
	D	48	266.35±24.85▲	212.09±22.29▲#	185.93±15.09▲#	145.66±18.38▲#	96.29±14.05▲#	72.60±19.85▲#

Versus group A, B, C: ▲P <0.05; Versus the before time of the same group: #P <0.05.

and functions. Free radicals could attack sulphydryl enzymes, resulting in the inhibition and inactivation of enzyme activity. They could produce arachidonic acid substances, including prostaglandin D2, prostaglandin E2, prostaglandins I2 and leukotriene, etc. They could also make dysfunction on nerve cells and glial cells by damaging the cell membrane. So, the main mechanism of injury was lipid peroxidation.

MDA is the product of lipid peroxidation by the oxygen free radicals and poly-unsaturated fatty acids on biofilm. It could make intramolecular and intermolecular cross-linking with proteins to play a toxic effect on the cells. Since the change of MDA content is corresponding with the lipid peroxidation process, the in vivo determination of MDA content may reflect the severity of membrane damage, which could be used as an index reflecting the levels of free radicals and oxidative stress.

SOD is an important in vivo antioxidant enzyme. It dispro-portionates O_2^- into O_2 and H_2O_2, the latter of which could be turned into H_2O secondarily with catalase. SOD played a vital role on the balance of oxidation and anti-oxidation, which could protect cells from damage. The level of SOD may reflect the ability of scavenging free radicals. Our results suggested that SOD content was negatively correlated with MDA content. Therefore, the detection of MDA and SOD could speculate the metabolism of free radicals.

The seawater had characteristics of low temperature, alkali, high permeability, high sodium, potassium, calcium, magnesium and other electrolytes. When the wound was immersed into the hypertonic seawater, increased vascular leakage, cell dehydration, exacerbated microcirculation and energy metabolic disorder in injury tissue appeared. This declined the activities of Na^+-K^+-ATPase and enhanced the metabolism of Na^+-Ca^{2+} exchange system. The increased internal flow of extracellular Ca^{2+} and release of Ca^{2+} in mitochondria and sarcoplasmic reticulum could activate the nitric oxide (NO) synthase to accelerate the production and release of NO. NO and oxygen free radicals could form peroxynitrite and be further decomposed into hydroxyl radicals, which could generate peroxidation with membrane lipid to damage cytoplasmic membrane and increase the generation of MDA. Alkaline water also makes cells more susceptible to disintegration to release membrane phospholipids, which could attend active oxidation reaction. It was confirmed by the cell culture experiments in vitro (13) that the immersion of high concentrations of potassium ions in astrocytes could activate Cl^-/HCO^{3-} and Na^+/H^+ exchanges through cell membranes. Thereafter, the astrocytes took more NaCl, with a lot of water into the astrocytes. The swollen astrocytes could not only increase the intracranial pressure, but also release excitatory amino acids and other harmful neurotransmitters to aggravate the neuronal damage. In addition, the overload of Ca^{2+} in nerve cells was considered to be the final common pathway of cerebral edema and death of nerve cells after brain injury. Ca^{2+} overload could damage the oxidative phosphorylation in mitochondria, which caused insufficient ATP synthesis and damaged cellular structure and functions. Thereafter, the membrane lipid peroxidation and oxygen radicals are formed to activate the protein kinase II and accelerate the autodigestion of membrane system, resulting in intracellular and extracellular ion homeostasis and blood-brain barrier damage, aggravating cerebral edema. In addition, after the brain injury combined with seawater immersion, the inflammatory reaction could also promote the occurrence of cerebral edema.

IL-1β and TNF-α are the inflammatory mediators studied more recently for their close relations with central nervous system damage (3, 4). According to Kamm et al., (12) craniocerebral trauma combined with seawater immersion significantly increases IL-1β and IL-10 in injured brain tissue within 24 h after the injury. The specific cytokine antagonists could reduce the ischemic brain damage subsequent to craniocerebral trauma and get a good prognosis (20).

CONCLUSION

MDA increases as a result of enhanced lipid peroxidation after the SBI with seawater immersion. The increase of MDA leads to a sharp decline of SOD activity, resulting in decrease in body and local oxidation resistance. The free radicals could not be effectively removed. Cerebral edema develops faster and more seriously after craniocerebral injury and seawater immersion. Therefore, patients with seawater immersion should be treated as soon as possible. Determination of MDA and SOD in the brain tissue could indirectly reflect the metabolism of oxygen free radicals in the brain to explore the new treatment approach of SBI with seawater immersion.

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