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The Study of Proton Loss Level in Models of Coronary Heart Disease Deficiencies in an Experimental Animals

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Objectives: The objective, in this study is to create a pathological model of coronary heart disease in pneumonia, based on the new theory of membrane-redox potentials three-state line system-dependent -full 9 stepped cycle of proton conductance inside the human and animal body, to study the activity of respiratory dehydrogenase enzymes at the ischemic site, and the proton entry state formed by bicarbonate in the 8th stage of full chain for Traditional Medicine. **Methods:** The experimental model used in this study was a pathological model of myocardial ischemia as developed by Kogan and Ambaga in healthy white Wistar rats approximately weighing 200-250 ± 20g. **Results:** On days 3-21 of the experiment, we have observed that the proton entry formed bicarbonate decreased by 9.8-13.9% both within the red blood cell membrane surface and outside the red blood cell membrane during whole red blood cell lysis. Moreover, the level of proton depletion was high, but the unstained or cell respiration released area increased by 10.25–20.34% ($p \leq 0.05$). **Conclusions:** In the 8th stage of the full 9 stepped cycle closed chain of protons, the proton entry formed bicarbonate decreased in the red blood cell membrane surface, and ischemic standing nodule electron-proton leak and dehydrogenase enzyme activity was decreased significantly.

Keywords: Necrosis Factor, Dehydrogenase Inhibitors, Proton-Translocation

Introduction

The World Health Organization (WHO) first adopted the Strategy of Traditional Medicine (TM) in 2002, which consists of international standards of evidence-based health care. Currently "Strategy of TM" (2014-2023), and "The West Pacific Regional Strategy of TM" (2011-2020) has been updated and is being implemented internationally [1, 2]. It is, thus, valuable to study TM products and their mechanisms at the cellular and molecular level, develop appropriate methods, provide researchers with new information on TM raw materials and herbs, and study the pharmacology of traditional recipes [1-4].

In recent years, the incidence of cardiovascular disease, including myocardial infarction, has become younger and a leading cause of death [5]. Therefore, it is useful to study the herbal medicine that is suitable in the prevention of cardiovascular disease and develop a basic research methodology for the development of new herbal medicine for coronary heart disease [6, 7]. Ambaga et al. has developed a new theory, named "The membrane-redox potentials three-state line system dependent-full 9 stepped cycle of proton conductance". According to this theory, the pathogenesis of metabolic disorders, cell proliferation, there is a loss of electrons and protons, and the state of proton entry in the bicarbonate forms in the 8th phase of the full 9 stepped cycle of proton conductance [8-11]. In detail, there are three state of the membrane-redox potential: alfa state with high oxidation potential, beta state with high reduction potential and gamma state with low redox potential. This line system existed between donators of protons and electrons as food substrates and acceptors of the protons and electrons as oxygen in all cells, thus there is a strong interconnection in the transferring of electrons and protons, the creation of proton gradient, ATP, and NADH. In the research conducted by Ariuntsetseg et al. it has been demonstrated that lactate dehydrogenase (LDH) was increased by 46.37% - 66.9% in brain tissue homogeneity in the cerebral ischemia model. When antischemin, a biologically active preparation containing *Astragalus membranaceus*, *Scutellaria baicalensis georgi* as well as *Ginkgo biloba* extracts was applied to the model, LDH activity was decreased nearly to the healthy animal model, and the activity of the another enzyme, succinate dehydrogenase in the cerebral homogenate was increased 10.23% - 22.5% compared to the non-treated control group in cerebral ischemia, which in turn indicates the inhibition of the loss of electrons and protons in the cellular respiratory chain. Further, Pavlov et al. also described that in hypoxia vital organs such as brain and heart, electron transmitting succinate dehydrogenase activity decreases and lactate dehydrogenase activity increases due to in the lack of oxygen and ATP, electron-proton flow slows down and glycolysis increases in metabolism [12]. The authors analyzed intra-cellular succinate dehydrogenase and lactate dehydrogenase histochemistry in cerebral ischemic reperfusion, and as a result they found that it was activated in 2, 3 and 5 layers of neurons and CA1, CA2, CA3 and CA4 of the hippocampus as a result of the completion of the electronic transmission of the inner layer of mitochondria.

Up to now, there is no study using the theory of membrane-redox potentials which has been developed by Ambaga et al. as determinant of metabolic enzymes in ischemic model animals. Therefore, the objective in this study was to create a pathological model of coronary heart disease in pneumonia, based on the new theory of membrane-redox potentials three-state line system-dependent –full 9 stepped cycle of proton conductance inside the human or animal body. Moreover, we have aimed to study the activity of respiratory dehydrogenase enzymes at the ischemic site, especially at the red blood cell membrane surface and outside the red blood cell membrane during whole red blood cell lysis and determine proton flow losses and the activity of the dehydrogenase enzyme in animal models. The objective, therefore, in this study is to create a pathological model of coronary heart disease in pneumonia based on the new theory of membrane-redox potentials three-state line system-dependent –full 9 stepped cycle of proton conductance inside the human or animal body and to study the activity of respiratory dehydrogenase enzymes at the ischemic site and the proton entry state formed bicarbonate in the 8th stage of full chain for Traditional Medical.

Materials and Methods

Study design

All animlas were divided randomly into healthy, non-treated controls and an experimental group of acute infarction induced by coronary artery occlusion. We developed an acute infarction model in rat and measured the cell damage of the cardiac muscle, its destruction, and energy supply as well as the electron and

proton flow lose in the state of the proton input in bicarbonate form in the 8th phase of the full 9 stepped cycle of proton on the 3rd, 7th, 14th, and 21st days of the experiment by the nitro blue tetrazole staining method that is widely used to determine the activity of dehydrogenase. Blue staining indicates the normal redox potential as well as the transfer of the cell's electron and proton, while non-stained areas indicates necrosis of the cell. Further, we also have measured free proton content that is released from the erithrocyte membrane by the PHS-3D-01 pH-meter.

Adult male Wistar rats, weighing approximately 200 to 250 ± 20 gram, were used for the experiment. They were divided randomly into 2 groups (6-8 animals in each group): control-non treatment group, and the experimental-acute infarction group induced by coronary artery occlusion. All animals were supplied with standard food during the experiment with access to water. Experimental procedures were conducted according to the regulations of the Animal Ethical Committee. We did coronary artery occlusion induced myocardial ischemia according to the references [11-13]. The experiment was conducted in the Innovation Research, Bio-modeling Laboratory of the New Medicine Medical University, ELISA Laboratory of Hulj-Borjigon hospital".

Procedures

Ketamine hydrochloride was used for anesthesia and the experimental animal body temperature was kept between 36.7-37 °C. With help of laryngoscopy, a polyethylene tube was introduced to the trachea, and attached to the ventilator with 112-114/min, 1.8-2.0 cc volume. To perform a thoracotomy at the left side, rats were relocated on the right side and the skin was incised. The ventral serrated muscle of the thorax and the intercostal muscles are transected. The thorax is incised in the third intercostal space. The opening is widened with a rib retractor. The lung is displaced to help visualize the left auricle, at whose tip the left anterior descending artery takes course toward the apex of the heart. Before occlusion, it was essential to carefully open the pericardium. To examine the area where the cells have lost their respiration or the dead area of the heart muscle tissue [11,14] the free proton content of red blood cells was determined [15,16] by the gravimetric method.

Statistical analysis

The original data do not follow the bell curve so we carried out log transformation to make it "normal" and used the log transformed data for our analysis. The main effects of time, treatment type and their interaction were determined using a mixed two-way ANOVA with a Greenhouse-Geiser adjustment for lack of sphericity. A critical p-value of < 0.05 was used. The repeated measurements within subjects were then compared to the previous time interval using the paired t-tests. The control and study groups' differences at each time interval were tested using the one-way ANOVA. A Bonferroni-type correction was applied to all t-test results resulting in a significance level set at $p < 0.017$ ($p= 0.05/3$). SPSS version 24 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Ethical statement

The animal study was carried out following the approval to conduct the experiments obtained from the Ethical Committee of the Mongolian National University of Medical Sciences (Protocol No.2019/3-07). All efforts were made to minimize the number of animals used and their suffering. .

Results

Research models of infarction and myocardial ischemia are essential to investigate the acute and chronic pathobiological and pathophysiological processes in myocardial ischemia and to develop and optimize future treatment. The method is the permanent ligation of the left anterior descending artery (LAD). Here the LAD is ligated with one single stitch, forming an ischemia that can be seen almost immediately. By closing the LAD, no further blood flow is permitted in that area. This model was chosen because it is convenient for pathobiological and pathophysiological as well as immunobiological studies on cardiac infarction.

While the development of genetically targeted animals (mice, rats, and rabbits) resulted in an explosion of studies dissecting cell biological mechanisms and molecular pathways, large animal models are considered closer to the clinical situation for translational studies to test safety and effectiveness. In experimental models of MI, understanding the time course of the cellular and molecular events is critical for optimal study design. Longer coronary occlusion times have distinct effects

on cardiac repair by extending infarct size and by influencing susceptible noncardiomyocyte populations, such as endothelial cells, fibroblasts, pericytes, and immune cells. Most studies characterizing responses to myocardial injury, have so far been performed on healthy young animals.

Results of the study of the state of the proton input in the bicarbonate form in the 8th stage of the full 9 stepped cycle of proton conductance

Ambaga et al. determined, in the 8th phase of the full 9 stepped cycle of proton flow, protons enter the membrane environment of red blood cells (RBCs) in the form of bicarbonate or proton acceptor-HCO3--molecule, where they combine with free protons to form carbonate-acid, or the regularity of proton donor formation, which suggests that such reactions take place alternately within the membrane environment of the RBCs, binding oxygen to hemoglobin and separating it. In this study, on the 3rd, 7th, 14th, and 21st days of the experiment, the loss of ATP supply, electrons, and proton fluxes in the experimental animals was determined by the state of the proton input in bicarbonate form in the 8th phase of the full 9 stepped cycle of proton (Table 1).

Table 1. Free proton content in red blood cells membrane environment (ph meter).

Days of experiment	Control[a,b] (n=6)	Experimental[c] (n=8)	*p-value
	Mean ± SD	Mean ± SD	
3rd	7.05 ± 0.02	7.48 ± 0.08	0.068
7th	6.17 ± 0.19	6.44 ± 0.154	0.052
14th	6.27 ± 0.08	6.74 ± 0.11	0.072
21st	6.23 ± 0.10	6.47 ±0.02	0.068

Two-way mixed ANOVA results: Interaction of time and treatment F(1.928, 336.59) = 23.165, p < 0.001; Main effect of time F (1.928, 336.59) = 335.31, p < 0.041; Main effect of treatment F(1,186) = 0.785, p = 0.696; *Independent t-test control vs. experiment; Paired t-test: [a]day7th vs. 14th, p = 0.040; [b]day14th vs. 21st, p = 0.051; [c]day7th vs. 21st, p = 0.031.

As can be seen from the table above, the proton entry in the bicarbonate form, comparing the 3-day test with the other days, cell membrane environment in the red blood was 7.48 (7.48 ± 0.077, p < 0.05) on the third day of the test. Then it has been decreased by 6.44 or 13.9% (6.44 ± 0.153, p < 0.05) on the seventh day, 6.74 or 9.8% (6.74 ± 0.105, p < 0.05) on the 14th day, and 6.47 or 13.5% (6. 47 ± 0.02, p < 0.05) on the 21st day. But the outer red blood cell membrane environment on the third day of the test was 7.44 (7.44 ± 0.076, p < 0.05), and decreased by 6.52 or 12.3% (6.52 ± 0.09, p < 0.05) on the seventh day, 6.74 or 9.4% (6.74 ± 0.029, p < 0.05) on the 14th day, 6.55 or 11.9% (6.55 ± 0.004, p < 0.05) on the 21st day, respectively. Moreover, red blood cell lysis was 7.34 (7.34 ± 0.053, p < 0.05) on the third day of the test, decreased by 6.52 or 11.1% (6.52 ± 0.007, p < 0.05) on the seventh day, 6.64 or 9.5% (6.64 ± 0.009, p < 0.05) on the 14th day, 6.44 or 12.2% (6.44 ± 0.030, p < 0.05) on the 21st day (Table 1).

Table 2. Free proton content in red blood cell membrane environment (0.2% H2O Semi lysis).

Days of experiment	Control[a b,c] (n=6)	Experimental[d,e] (n=8)	*p-value
	Mean ± SD	**Mean ± SD**	
3rd	6.81 ± 0.20	7.44 ± 0.08	0.086
7th	6.24 ± 0.04	6.52 ± 0.10	0.039
14th	6.29 ± 0.04	6.74 ± 0.03	0.046
21st	6.32 ± 0.09	6.55 ± 0.04	0.029

Two-way mixed ANOVA results: Interaction of time and treatment F(1.927, 336.59) = 23.165, p < 0.001; Main effect of time F (1.928, 336.59) = 335.31, p < 0.041; Main effect of treatment F(1,186) = 0.785, p = 0.696; *Independent t-test control vs. experiment; Paired t-test: [a]day7th vs. 14th, p = 0.041; [b]day7th vs. 21st, p = 0.045; [c]day7th vs. 14th, p = 0.011; [d]day7th vs. 21st, p = 0.031; [e]day7th vs. 14st, p = 0.024.

Determinations were made in the 8th phase of the 9-stage closed circuit of the proton-by-proton entry in the bicarbonate form. Compared in the red blood cell membrane environment, it was 6.74 (6.74 ± 0.105, p < 0.05) on the 14th day of the test,

decreased by 6.47 or 4% (6.47 ± 0.02, $p < 0.05$) on the 21st day. In the outer red blood cell membrane environment, it was 6.74 (6.74 ± 0.029, $p < 0.05$) on the 14th day, decreased by 6.55 or 2.81% (6.55 ± 0.004, $p < 0.05$) on the 21st day. Red blood cell lysis was 6.64 (6.64 ± 0.009, $p < 0.05$) on the 14th day, decreased by 6.44 or 3.01% (6.44 ± 0.030, $p < 0.05$) on the 21st day (Table 1).

Table 3. Free proton content in red blood cells lysis (complete lysis).

Days of experiment	Control[a,b] (n=6)	Experimental[c,d] (n=8)	p-value
	Mean ± SD	Mean ± SD	
3rd	7.16 ± 0.08	7.34 ± 0.05	0.250
7th	6.34 ± 0.07	6.52 ± 0.01	0.018
14th	6.44 ± 0.05	6.64 ± 0.01	0.055
21st	6.28 ± 0.07	6.44 ± 0.03	0.000

Two-way mixed ANOVA results: Interaction of time and treatment $F(1.927, 336.59) = 23.165$, $p < 0.001$; Main effect of time $F(1.928, 336.59) = 335.31$, $p < 0.041$; Main effect of treatment $F(1,186) = 0.785$, $p = 0.696$; *Independent t-test control vs. experiment; Paired t-test: [a]day7th vs. 14th, $p = 0.041$; [b]day7th vs. 21st, $p = 0.045$; [c]day7th vs. 14th, $p = 0.011$; [d]day7th vs. 21st, $p = 0.031$; [e]day7th vs. 14st, $p = 0.024$.

Examine the area where the cells have lost their respiration or the dead area of the heart muscle

When we created a pathology model of coronary heart disease deficiency in white rats, there were three zones included: a healthy zone that maintains the flow of electrons and protons, energy supply, and high molecular weight ATP in the area of necrosis; a death zone that completely lost the flow of electrons and protons; and an intermediate zone located between these two zones (Table 2).

Table 4. The dead area of the heart muscle.

Days of experiment	Control[a,b,c] (n=6)	Experimental[d,e,f] (n=8)	p-value
	Mean +-SD	Mean +-SD	
1st	180.2 ± 11.04	128.6 ± 2.62	0.089
3rd	175.9 ± 12.36	123.9 ± 11.47	0.001
7th	177.3 ± 9.28	130.9 ± 10.43	0.034
14th	182.4 ± 7.51	126.9 ± 9.50	0.000
21st	190.8 ± 11.43	121.3 ± 8.32	0.069

Two-way mixed ANOVA results: Interaction of time and treatment $F(1.919, 338.59) = 23.135$, $p < 0.024$; Main effect of time $F(1.928, 338.57) = 345.31$, $p < 0.007$; Main effect of treatment $F(1,186) = 0.766$, $p = 0.066$; *Independent t-test control vs. experiment; Paired t-test: [a]day3rd vs. 7th, $p = 0.010$; [b]day7th vs. 21st, $p = 0.015$; [c]day3rd vs. 14th, $p = 0.021$; [d]day3rd vs. 7th, $p = 0.020$; [e]day3rd vs. 14th, $p = 0.020$, [f]day3rd vs. 21st, $p = 0.020$.

To obtain the ratio between these zones, the gravimetric method was used to separate the stained and unstained areas using nitro blue tetrazolium solution and the ratio was measured on an analytical balance. In the case of coronary heart disease deficiency, electron transport decreased with increasing trial day while the weight of the unstrained area increased statistically by 10.25–20.34% ($p < 0.05$) on days 3-21 of the first day of the experimental group of animals compared to the other days (Table 2).

The study of the size of the heart cavity in models of coronary heart disease deficiencies in experimental animals

On the 1st, 3rd, 7th, 14th, and 21st days of the experiment, the heart tissue was cross-sectioned and the area of the heart cavity was measured with a fine-grained line. A sharp increase of 37.59% to 85.03% ($p \leq 0.001$) in the control group compared with the experimental group indicates the dilatation of the left ventricle and thinning of the heart wall due to myocardial necrosis (Table 3).

Table 5. The size of the heart cavity (mm^2).

Days of experiment	Control[a,b,c,d,e] (n=6)	Experimental[f,g,h,i] (n=8)	p-value
	Mean ± SD	Mean ± SD	
1st	2.66 ± 0.44	1.66 ± 0.66	0.000
3rd	2.45 ± 0.38	5.83 ± 0.94	0.002
7th	3.36 ± 0.31	9.33 ± 1.90	0.001
14th	3.68 ± 0.43	19.0 ± 4.17	0.003
21st	3.86 ± 029	25.8 ± 4.44	0.000

Two-way mixed ANOVA results: Interaction of time and treatment $F(1.968, 377.69) = 24.195$, $p < 0.031$; Main effect of time $F(1.319, 347.69) = 345.31$, $p < 0.001$; Main effect of treatment $F(1,146) = 0.612$, $p = 0.061$; *Independent t-test control vs. experiment; Paired t-test: [a]day1st vs. 3rd, $p = 0.010$; [b]day1st vs. 3, $p = 0.045$; [c]day1st vs. 7th, $p = 0.051$; [d]day3rd vs. 14th, $p = 0.001$; [e]day7th vs. 21st, $p = 0.002$; [f]day1st vs. 7th, $p = 0.045$; [g]day1st vs. 3th, $p = 0.036$; [h]day3th vs. 21st, $p = 0.005$; [i]day14th vs. 21st, $p = 0.001$.

Discussion

Cardiovascular diseases (CVDs) are the number one cause of death globally [3, 4]. Ischemia occurs when blood flow to the myocardium is reduced [17-19]. Ischemia of prolonged duration induces myocardial infarction (MI), and MI is a common cause of heart failure [20]. Coronary heart disease affects the heart due to the detrimental effects of acute MI and ischemia-reperfusion injury (IR). During acute myocardial ischemia, the lack of oxygen switches the cell metabolism to anaerobic respiration, with lactate accumulation, ATP depletion, Na+ and Ca^{2}+ overload, and inhibition of myocardial contractile function. All of these processes can independently induce cardiomyocyte death of the acutely ischemic myocardium. MI model has permanent left anterior descending (LAD) artery ligation for 24 hours and IR model has LAD ligation for 30 min followed by reperfusion for 24 hours. In conclusion, the myocardial damage in MI is mainly due to ischemic necrosis with accompanying inflammation while apoptosis is the main mechanism of cell death in IR in addition to limited ischemic necrosis [21]. In the report by Kumar et al. it has been mentioned that apoptosis of the coronary endothelial cells leads to the endothelial dysfunction as well as the apoptosis of cardiac myocytes. Here, ion homeostasis change plays a key factor in ischemic environment. Especially, outer and intracellular accumulation of the proton has been shown to induce apoptosis in many cell types including coronary cells. Further, the regulation of this proton accumulation can be dependent on Na+/H+ system and bicarbonate transport system [22].

A medical system is based on well-established texts that are composed by a complete system of theory and practice, evolved parallel to biomedicine. In biomedicine, there are a few major theories which had their own diagnosis and treatment. For example, Ayurveda, a well acknowledged system of medicine in India, has more than thousand-year history. According to this theory, each person has own unique combination of bioenergetic states (*dosha*). There are three states of the *doshas* and each represents distinct physiologic processes: *vata* (principle of movement), *pitta* (principle of transformation), and *kapha* (principle of structure). Each individual's health is influenced by the innate proportion of these three states. Moreover, in traditional Chinese medicine, the concept of *Yin* and *Yang* serves as the foundation for diagnosing and treating illnesses. According to yin-yang theory, yin is associated with lower parts of the body while yang is associated with the upper part of the body. All phenomena are composed of these two opposites but mutually interconnected forces.

Ambaga et al. demonstrated the first time that the whole body's membrane structure, which existed in three basic states as a fluid alpha state, a solid beta state, and an intermediate gamma state of excitable cells which could play the role of biological substance, leading to the appearance of the abstract theory of "RLung, Mkhris, and Badgan-membrane structure". Further, this theory has been enriched and perfected over the last few years, and expanded into a new theory called the "The membrane-redox potentials three-state line system dependent-full 9 stepped cycle of proton conductance" [8, 15, 22]. The basic principle of this theory is that the closed-loop of the proton flow, which sustains human and mammalian life, flows continuously in 9 stages, with oxygen in the nose, food in the mouth, and free proton in the form of bicarbonate within the red blood cell membrane. It was discovered that 160 kg of ATP, 360 ml of metabolic water, and carbon dioxide are produced through a 9-step closed circuit to keep the body alive [15, 22]. The concentration of free protons, carbon dioxide, carbonates, and bicarbonates in the blood-serum medium formed on the "The membrane-redox potentials three-state line system dependent-full 9 stepped cycle of proton conductance" is calculated by the formula $pH = HCO_3/CO_2$,

and the free proton is directly related to the carbon dioxide content [22]. In the 8th phase of this "The membrane-redox potentials three-state line system dependent-full 9 stepped cycle of proton conductance", free protons and metabolic water pass through the plasma membrane of red blood cells through the aquaporin protein duct, and diffuse oxygen and 14 trillion cells of carbon dioxide from the alveoli into these cells. As shown in the figure below by Jacobs-Stewart Cycle as first discovered in red blood cells, the nature of the reaction is that in the presence of the enzyme carbonic anhydrase (CA) inside and outside cells such as red blood cells, the proton interacts with the plasma membrane anion-carrying protein to enter the cell [22].

Acid is defined as a proton donor, a hydrogen ion (H+) is a proton because it results when an electron is lost from a hydrogen atom, which consists of a proton end electron. Therefore, a molecule that releases hydrogen ions (H+) is acid. A base is defined as a proton acceptor, and any substance that accepts hydrogen ion (H+) is a base. Many bases function as proton acceptors by releasing hydroxide ions (OH-) when they dissociate. The hydroxide ion (OH-) is a proton acceptor that combines with H+ to form water (Seeley) [23].

By our knowledge, this is the first study investigating the acute infarction model using membrane-redox potentials three-state line system dependent-full 9 stepped cycle theory. In our previous study, we had reported the biological significances of proton release in erythrocyte membranes as well as some effects of the antischemin preparations on cerebral ischemia model through the 9-full stepped cycle theory. In coronary heart disease deficiency in experimental animals, in the 8th stage of the "The membrane-redox potentials three-state line system dependent-full 9 stepped cycle of proton conductance", bicarbonate proton input decreases within the red blood cell membrane environment due to a decrease in donor substrates at the beginning of the three-state membrane-redox potential. This indicates that the cellular environment has shifted to the acidic side. This is due to the supply of electrons, proton donors, electrons, and proton acceptors through the second compartment of the coronary arteries to the heart muscle during ischemia, as well as the efficient use of ATP and CF generated by this electron and proton flow. One is closely related but has the opposite direction "The membrane-redox potential takes place on a three-state line, the other on the 4th compartment-5 main membrane-5 main activity, the balance between the two phenomena is disturbed and the acceptor and donor in the coronary heart disease deficiency can lead to myocardial infarction, which can lead to necrosis of the heart muscle and trigger a heart attack [24].

There are several methods for determining the activity of dehydrogenase in biological medicine, the most widely used in heart attack and ischemia is using nitro blue tetrazole solution to determine the ratio of stained and unstained areas [25, 26]. If the heart tissue is not stained with nitro blue tetrazole, it indicates dehydrogenase inactivity or cell death [27]. This substance oxidizes itself when it does not react with any substance, and the main substrates for its interaction are the enzymes oxidase and dehydrogenase, which play an important role in the cellular respiratory system. The dehydrogenase enzyme mentioned here is one of many groups of enzymes involved in biological redox reactions (electron and proton transport) in living organisms, such as cell respiration and glycolysis. This group of enzymes is the most common in all species of animals, plants, and microorganisms, and belongs to the group of oxidoreductases, which are responsible for transporting hydrogen (protons) from an oxidized substrate to another type of compound (acceptor) [15]. Experimental studies in the canine model of MI demonstrate a transmural heterogeneity in the myocardial response to ischemia, suggesting that the subendocardium, where myocardial oxygen demand is greatest, is more susceptible to ischemic injury than the mid myocardium and subepicardial [18]. Strukov et al. wrote that at the site of injury of the heart muscle formed three zones with different activity of oxidative enzymes [20]. However, Furuhura et al. described the characteristics of this interstitial zone as mitochondrial damage, a slight decrease in ATP and glycogen levels, and a significant foaming of the cell cytoplasm [21]. During histochemical analysis, Fishbien et al. found that dehydrogenase enzyme activity was inhibited, glycogen was lost, and fat droplets accumulated in the interstitial zone [22]. In their study of blood circulation, Koyanagi et al. suggested that the interstitial zone is not only transmural, but also lateral [23].

In the study of Uryash et al. transmurality and fibrosis validation of coronary occlusion was performed [24]. At the end of the study (four weeks after ligation) and after all hemodynamic measurements, hearts were cut out and cut in 3, 3mm segments from apex to base parallel to the atrioventricular groove. The basal side of the segments was measured to better distinguish between myocardium stained by EB and TTC. Area at risk was measured by the Evans Blue (EB) perfusion-staining and expressed as percent of whole heart. Necrosis was measured by TTC staining and expressed as percent of each myocardial segment. To determine transmurality of the infarct

scanned images of the segments were geometrically divided into a 6-sector model using the anterior and inferior insertion of the right ventricle to the left ventricle as markers. Apical, middle and basal necrosis was defined. Fibrosis was measured by Masson's Trichrome (MT) staining and expressed as percent of the left ventricle in a segment. The middle transverse segment was sectioned and stained with MT for both wall thickness (epi-to-endocardial distance) and fibrosis (blue-stained collagen fibers) measurements. The mean left ventricle (LV) wall thickness, fibrosis and total LV area were measured from three equidistant points. Blue staining denotes (indicates) fibrosis MI-CONT vs. MI-pGz [28] were similar to the results of our study.

By simultaneously identifying the zone of inactivation of the respiratory enzyme dehydrogenase and the area of loss of blood supply at the site of ischemic heart disease, an "intermediate zone" is formed between the site of ischemic or myocardial necrosis and healthy tissue to maintain a certain blood supply. This zone is called the "prooxidant zone" as a strong correlation between the increase in area and the process of induction of fat oxidation [11, 30, 31]. Therefore, we compute the area of the ischemic site in which dehydrogenase is inactive by comparing the weight of nitro blue tetrazole stained tissue in two groups of animals and summarize the experimental results. The amount of completely dead areas not stained with nitro blue tetrazole was greater in the experimental untreated group.

We have limitation in this study. That is, the lack of systematic studies on the membrane-redox potentials three-state line system dependent-full 9 stepped cycle of proton conductance has been given a limitation. It is very important for designing the future clinical studies related to the full 9 stepped cycle of proton flow, even though we could not find data regarding to the comparison or usage of this theory.

Further, additional studies are needed to investigate the membrane-redox potentials three-state line system. In detail, we have to determine the cellular redox states and cellular metabolism under normal and pathological conditions to better understand this theory. In order to examine these conditions, in vitro experiments using cardiac cell lines for the utilizing the usage of therapeutic interventions should be carried out.

Conclusions

In coronary heart disease deficiency in experimental animals, in the 8th stage of the "The membrane-redox potentials three-state line system dependent-full 9 stepped cycle of proton conductance", bicarbonate proton input decreases within the red blood cell membrane environment due to a decrease in donor substrates at the beginning of the three-state membrane-redox potential. Ischemic standing nodule electron-proton leak and lost dehydrogenase enzyme activity occurs.

Conflict of interest

The authors state no conflict of interest.

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References

1. World Health Organization. The regional strategy for traditional medicine in the Western Pacific (2011-2020) [assessed on 16 May 2020]. Available at: https://iris.wpro.who.int/handle/10665.1/5538.
2. World Health Organization. Traditional medicine strategy 2014-2023 [assessed on 9 June]. Available at: https://apps.who.int/iris/bitstream/handle/10665/92455/9786167697581-tha pdf.
3. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. J Am Coll Cardiol 2017; 70: 1-25.
4. World Health Organization. Cardiovascular diseases. [assessed on 17 May 2017]. Available at: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
5. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. 2019 acc/aha guideline on the primary prevention of cardiovascular disease, a report of the american college of cardiology/american heart association task force on clinical practice guidelines. Circulation 2019;

140: 596-646.

6. Purev T. Mongolian traditional medicine. Ulaanbaatar, Mongolia: Munkhiin Useg Publishing LLC; 2014. p 175.
7. Surenjav T. Traditional internal pathology. Ulaanbaatar, Mongolia: Munkhiin Useg Publishing LLC; 2002. p 158-61.
8. Ambaga T. The membrane - redox potentials three - state line system dependent - full 9 stepped cycle of proton conductance the importance of new theoretical science and cognition. Ulaanbaator, Mongolia: Soyombo Publishing LLC; 2020. p 72-106.
9. Ambaga M, Kogan AH, Kudrin AN. Pharmacotherapeutic action of cardioselective blockers of H1-adrenoreceptors (OF4647 and atenolol), motley astragalus, their combinations and antioxidant-dibunol in myocardial infarction caused by persistent and transient coronary occlusive ischemia (experimental study). Moscow, Russia: Moscow First Medical Institue; 1984.
10. Michael LH, Entman ML, Hartley CJ, Youker KA, Zhu J, Hall SR, et al. Myocardial ischemia and reperfusion: a murine model. Am J Physiol 1995; 269: 2147-54.
11. Kolk MVV, Meyberg D, Deuse T, Tang-Quan KR, Robbins RC, Reichenspurner H, et al. LAD-ligation: a murine model of myocardial infarction. J Vis Exp 2009; 32: 1438.
12. Liao J , Huang W, Liu G. Animal models of coronary heart disease. J Biomed Res 2017; 31: 3-10.
13. Verma DD, Hartner WC, Levchenko TS, Bernstein EA, Torchilin VP. ATP-loaded liposomes effectively protect the myocardium in rabbits with an acute experimental myocardial infarction. Pharm Res 2005; 22: 2115-20.
14. Lindsey ML, Bolli R, Canty JM, Du XJ, Frangogiannis NG, Frantz S, et al. Guidelines for experimental models of myocardial ischemia and infarction. Am J Physiol Heart Circ Physiol 2018; 314: 43-4.
15. Heusch G. Myocardial ischemia: lack of coronary blood flow or myocardial oxygen supply/demand imbalance? Circ Res 2016; 119: 194–6.
16. Tanai E, Frantz S. Pathophysiology of heart failure. Compr Physiol 2015; 6: 187–214.
17. Hashmi S, Al-Salam S. Acute myocardial infarction and myocardial ischemia-reperfusion injury: a comparison. Int J Clin Exp Pathol 2015; 8: 8786–96.
18. Derias NW, Adamis CW. Nitroblue tetrazolium test: early gross detection of human myocardial infarcts. Br J Exp Path 1978; 59: 254-8.
19. Nachlas MM, Shnitka TK. Macroscopic identification of early myocardial infarcts by alterations in dehydrogenase activity. Am J Pathol 1963; 42: 379-406.
20. Bouchardy B, Joliat G, Majno G, Lehmann L, Mainquet J, Rissanen V, et al. The pathological diagnosis of acute myocardial infarction. J Bull Wld Hlth Org 1973; 48: 23–5.
21. Fukuhura T, Mitsunami K, Bito K. The "Border Zone" in acute myocardial ischemia. A histochemical, biochemical and ultrastructural study in the canine heart. J of Mol Cell Cardiol 1981; 13: 45.
22. Kumar S, Kasseckert S, Kostin S, Abdallah Y, Piper HM, Steinhoff G, et al. Importance of bicarbonate transport for ischaemia-induced apoptosis of coronary endothelial cells. J Cell Mol Med 2007; 11: 798-809.
23. Fishbien MC, Hare CA, Gissen SA. Identification and quantification of histochemical border zones during the evolution of myocardial infarction in the rat. Cardiovasc res 1980; 14: 41-49.
24. Koyanagi S, Eastham CL, Harrison DG. Transmural variation in the relationship between myocardial infarct size and risk area. Am J physiol 1982; 242: 867-74.
25. Uryash A, Bassuk J, Kurlansky P, Altamirano F, Lopez JR, Adams JA. Non-invasive technology that improves cardiac function after experimental myocardial infarction: whole body periodic acceleration (pGz). Plos One 2015; 10: 6-7.
26. Chen C, Chen W, Nong Z, Ma Y, Qiu S, Wn G. Cardioprotective effects of combined therapy with hyperbaric oxygen and diltiazem pretreatment on myocardial ischemia-reperfusion injury in rats. Cell Physiol Biochem 2016; 38: 2015-29.
27. Yamaki T, Iwai-Takano M, Yaoita H, Ogawa K, Tajima H, Takeishi Y, et al. Participation of mast cells in angiogenesis in the border zone of myocardial infarction in rats. J Med Ultrason 2009; 36: 119-27.
28. Chen RH, Li YG, Jiao KL, Zhang PP, Sun Y, Zhang LP, et al. Overexpression of sema3a in myocardial infarction border zone decreases vulnerability of ventricular tachycardia post- myocardial infarction in rats. J Cell Mol Med 2013; 17: 608-16.
29. Yamanishi A, Kusachi S, Nakahama M, Ninomiya Y, Watanabe T, Kumashiro H, et al. Sequential changes in the localization of the type IV collagen a chain in the infarct zone: immunohistochemical study of experimental myocardial infarction in the rat. Am J Pathol 1978; 90: 57-70.