To Delay the Process of Blood Coagulation Using Electrolysis Technique in Sheep Blood

Alireza Jahanbani, Seyed Mohamad Sajjadi Dezfouli, Ashkan Javaherifar, Mohammad Reza Yourdkhani, Mohammad Goudarzi

Abstract— The objective of this study was to evaluate the impact of electrolysis technique on blood coagulation process (BCP), hematological and biochemical parameters involved in BCP. A healthy ram was used as an experimental model. Using the electrical power-supply and two pieces of platinum as the non-reactive electrodes, a range of 200 to 1050 mV electric Charge induced to the blood sample that had been taken from the ram's jugular vein and then by means of capillary tubes, BCP was examined every 30 seconds during Electrolysis. After 12 minutes and 40 seconds, the control blood sample clotted while the blood of electrolysis container took 30 more minutes to coagulate. Partial thromboplastin time and prothrombin time increased significantly. Prothrombin activity, calcium, fibrinogen and total protein decreased since other factors confirmed the delay in BCP. As an innovation it can be noted that the electrical current potentially can be used as an anticoagulants agents. But measuring the quality of the method and quality of the blood under the influence of the electric current, requires extensive experiments.

Keywords— Blood, Coagulation, Electrolysis, Heparin, Platinum, Prothrombin activity, Sheep.

1 Introduction

Inspite of extensive progress in related studies on the blood coagulation process and anticoagulant agents, there is still no alternative method for chemical anticoagulants which contains less adverse effects [10], [32].

In blood coagulation process (both intrinsic and extrinsic blood clotting pathways) calcium ions plays a fundamental role [10]. This role is how important which most of the anticoagulants used in the laboratories e.g. EDTA, Oxalates and Citrate containing agents, work by eliminating calcium ions in the clotting process [1], [2], [10]. Due to adverse effects that eliminating calcium ions in the form of complex from the blood may cause, application of calcium binding anticoagulants which forms complex with calcium ions in using of cardiopulmonary bypass (CPB) machines and dialysis machines is not recommended [10], [13], [15]. For this reasons Heparin is being used in mentioned machines above as anticoagulant agent [1], [10], [13] mean while Heparin has also its adverse effects.

Using high amount of Heparin during cardiac surgeries dilutes the blood [1], [4], [5], [13], [14], [15]. On the other hand, Heparin can cause; releasing of tissue factor pathway inhibitor, platelet dysfunction, increasing fibrinolysis, severe reduction of Antithrombin III, shock, thrombosis and even type III heparin induced thrombocytopenia (HIT) [1],[10], [12]-[15].

Generally after long term of taking heparin, in order to reversing heparin effects its specific antagonist protamine sulfate is being used [1], [2], [3], [10]. Protamine Sulfate can cause blood pressure reduction, decreasing heart motion and releasing notable amounts of Histamine in the body [1], [2], [3], [10], [13], [14]. Hypersensitivity reactions to animal tissue derived heparin also can lead to anaphylaxis in patient [10], [11]. At the moment the actions to reduce the intensity of these allergic reactions are being taken by using low molecular weight heparin (LMWH) [1], [2], [3], [9]-[14]. Despite of the urge of surgery, cardiovascular surgeons never recommend patients with Hemophilia, Thrombocytopenia, Hepatic diseases, gastrointestinal ulcerative lesions, intracranial bleeding susceptibility, low level of blood prothrombin, thrombocytopenic purpura, Asthma susceptibility, Hemolytic uremia syndrome (HUS) and heparin resistance to undergo a heart surgery or repeated dialysis due to the problems mentioned above [1], [2], [3], [10]-[15]. Heparin may also have interaction with medicines like Cardiac glycosides, Aspirin, some specific antibiotics and some Quinidine drugs which makes the condition more complicated [1], [2], [3], [10],[16].

Performing non-spontaneous oxidation and reduction reactions by using direct electric current is called Electrolysis [18]-[20], [32]. During electrolysis process, anions move toward the positive electrode (Anode) and cations move toward the Negative electrode (Cathode) in the electrolyte solution. Anode is where oxidation or releasing of electron in the solution occurs. Cathode is also where reduction or electron absorption takes place. In

- *First Author: DVM, Member of Young Researchers and Elite Club Islamic Azad University, PhD student of Biochemistry, University of Shiraz, Shiraz, Iran.*
  E-mail: jahanbanimahakeman@gmail.com
- *2nd Author: Veterinary Medicine Student, Member of Young Researchers and Elite Club Islamic Azad University Garmsar Branch, Garmsar, Iran. E-mail: Sajjadi.mohamad@hotmail.com*
- *3rd Veterinary Medicine Student, Islamic Azad University Garmsar Branch, Garmsar, Iran.*
- *4th Author:* Master of Analytical Chemistry, High school teacher of Garmsar. Iran
- *5th Author: Department of Electrical Engineering, Faculty of Engineering, Garmsar branch, Islamic Azad University, Garmsar, Iran.*

chemistry, the loss of electrons is called Oxidation, while electron gaining is called reduction [9], [18], [30], [31], [32].

Several experiments on blood electrolysis, different body fluids electrolysis or artificial fluids similar to the blood electrolysis have been done [4]-[8], [32]. Electrolysis technique is usually used for assessing the specific ions or substances. In such techniques ion-selective electrodes (ISEs) is used generally [16]. Electrolysis is also used for blood pH and electrolytes measurements [7], [16]-[22], [32] but none of these studies were aimed to prevent or delay the process of blood coagulation.

The hypothesis of current study is; by using the low voltage direct electric current during electrolysis technique, ionized calcium which has an essential role in blood clotting, will be absorbed by negative electrode.

Since that the majority of blood proteins including coagulation factors have negative electric charge in normal blood pH [22], [23], [24] they can be also absorbed by the positive electrode. Blood coagulation process can be delayed or even stopped without using of any specific chemical substance like heparin or any other chemical agents which forms complex with calcium.

The impact of the electrodes and the electrical current on the biochemical and hematological aspects of the blood should be mainly considered.

Eventually the reduction in calcium ions by electrolysis technique can prevent blood coagulation. how much this reduction will be and whether this reduction puts the patient's life at risk or not, and to see if stopping the electrical current will absorbed calcium by negative electrodes be released back to the blood flow again or will be remained on the electrodes.

2 Materials and Methods

A healthy adult ram has been chosen for this study. The ram was examined by a veterinarian and the initial laboratory tests confirmed the ram's health. The ram had been kept in veterinary medicine faculty barn for two weeks and after repeating the examinations a health certification was given to the ram. After first two weeks and certifying the ram's health, it was kept in the veterinary faculty to adapt itself to the new environment. In this period of time the ram was fed with a standard ration according to the season. We provided it with fresh water all days long being there. The ram experienced a one hour walk every day. In this period blood parameters were measured with an interval of two weeks (Table 1).

Two 999/5 karat platinum plates with 2*2 cm dimensions also were used as electrodes for the electrolysis technique [4], [6], [16], [30]-[33]. Protek-DF17305B5A power supply and ESCORT-3136A Multimeter was used in this study.

TABLE 1

BLOOD PARAMETERS OF THE RAM

Blood Parameters	Date 9/17/2015	10/19/2015
PTT (Sec)	48	50
PT (Sec)	12	16
INR	1.3	1.7
Ca^{++} (mg/dl)	9.1	9
Total Protein (g/dl)	6.5	6.3
Sugar (mg/dl)	67	61
Prothrombin Activity (%)	84	65

The ram was taken to the veterinary hospital of the faculty in a stress free environment to get the first phase of the study started. After a proper restraining of the ram, 60 cc of blood was taken from ram's jugular vein by respecting all the animal rights and aseptic principles [26]. 20 cc of the blood divided equally into two Polypropylene containers immediately which one of them was for the main test and the other one was for control sample [5]-[8].

To assess the hematological parameters the remaining blood transferred to three tubes; EDTA, Citrate and a tube containing no anticoagulant agent.

Two platinum plates which supplies 250-800 mV were put in the main blood container as a non-reactive electrode [4]-[8], [21], [29], [32], [33], and then the container of blood was being shaken gently to increase contact surface between the blood and the plates [8]. The main test was conducted with fixed electric current (Ampere).

At minutes 2, 4 and 6 samples were taken from the main and control blood container and transferred in the EDTA, Citrate and non- anticoagulant agent tubes .

The time was recorded from the beginning. Every 30 seconds the blood was checked for coagulation by a capillary tube [10], [12].

After 12 minutes and 45 seconds the blood in the control container totally came to clot while the blood in other container with an electrode was still normal. In minute 18 another blood samples poured into three EDTA, Citrate and non-anticoagulant tubes .

30 minutes and 12 seconds after observing coagulation in the control container, a gradual reduction in macroscopic quality of blood in the electrocell occurred until minute 42 appearance of the blood had been totally changed macroscopically and seemed much diluted but no clot was observed.

Blood samples transferred to the university hospital lab. Citrated blood samples were allowed to stand vertical for an hour [10]. After RBCs sedimentation, PT and PTT tests was done with the use of USA Fisher laboratory kits.

Non-anticoagulant containing blood samples were centrifuged 3000 RPM for 3 minutes [17]. Different biochemical parameters were examined after serum separation with the use of ES-200 Autoanalyzer. The amount of fibrinogen concentrations were measured by MR96A ELISA reader. The fibrinogen concentrations of some of the samples were also measured randomly by CLAUSE method and no difference was observed in results compared with the ELISA outcome .

All the mentioned procedures have been repeated again 2 weeks later. The ram was being kept in a steady circumstance with no change in food diet and environment. A certain recovery time was given to the ram to get back to a normal physical condition and the amount of lost blood replaced helpfully.

3 Results

According to the results showed in table 2, blood coagulation time delayed more than 10 minutes by induction 200 to 800 mV electric charge. PTT of the control blood sample increased from 47 seconds to 56 seconds in the blood influenced by electric current which this shows a delay in partial thromboplastin time.

The amount of calcium ions decreased from 9.5 mg/dl in the control sample to 8.1 mg/dl in the blood underwent electrolysis and the blood was not clotted which it can be concluded that under the influence of electric current, the blood calcium ions either absorbed to the negative electrode or were deionized and neutralized.

Prothrombin time (PT) in control blood sample was 14.8 seconds while this increased to 16.7 seconds in the sample under the experiment. The prolonged PT and PTT and lack of the blood coagulation suggest that the induction of electric charge to the blood is effective for preventing the blood from clotting. This delay may be due to elimination of calcium from both the intrinsic and the extrinsic pathway of the coagulation cascade, or the effect of electric charge on the protein involved in the coagulation process, or simultaneous effect of both actions is causing a delay in blood clotting time [4]-[7].

Since there was a reduction in total protein value from 6.7 g/dl in control sample to 6.5 g/dl in electrolysis blood (Table 2) this hypothesis that the electric current affects the blood proteins can be strengthen. Additional experiments and electron microscopic imaging of the surface of the platinum plates can contribute to confirm or deny the hypothesis [32].

TABLE 2

BLOOD PARAMETERS MEASURED IN THE FIRST EXPERIMENT

Blood Parameters	First experiment Control Blood	First Experiment Electrolysis Blood 200-800 mV Electric Charge
PTT (Sec)	47	56
PT (Sec)	14.8	16.7
INR	1.5	1.6
Ca++ (mg/dl)	9.6	8.1
Total Protein (g/dl)	6.7	6.5
Sugar (mg/dl)	61	64
TAG (mg/dl)	15	15
Prothrombin Activity (%)	80	68

Next time the experiment was performed with an interval of 14 days after the first test and the following results were obtained (Table 3). On this time with the use of a variable power supply device, the amount of electricity voltage increased from 800 mV to 1050 mV and the time of blood coagulation delayed more than 30 minutes. (The Electeromotive force of calcium is -2.866).Finding shows in the Table 3 confirms this delay. PTT increased from 48 seconds at the beginning of the sampling to >120 seconds at the end of 30 minutes and PT increased from 14 seconds at the beginning of the sampling to >60 seconds at the end of 30 minutes.

The blood calcium levels at the beginning of sampling were 9.5 mg/dl which this fall down to 6 mg/dl at the end of 30 minutes .This finding can confirm the effect of electric charge induction on the delay in blood coagulation process. Reduction of fibrinogen levels which play a fundamental role in coagulation process from 161 mg/dl at the beginning to 102 mg/dl at the end of 30 minutes is also another confirmation.

TABLE 3

BLOOD PARAMETERS MEASURED IN THE SECOND EXPERIMENT

Blood Parameters	Second Experiment Control Sample At the Beginning of The Blood Collecting	Second Experiment Control Blood At 8 Min	Second Experiment Electrolysis Blood 800-1050 mV At 8 Min	Second Experiment Electrolysis Blood 800-1050 mV At 14 Min	Second Experiment Electrolysis Blood 800-1050 mV At 30 Min	Second Experiment Electrolysis Blood 800-1050 mV After Cutting off the Electric Current
PTT (Sec)	48	43	56	61	>120	>120
PT (Sec)	14	16	17	17.8	>60	>60
INR	1.3	1.6	1.5	1.9	It was not reported	It was not reported
Ca++ (mg/dl)	9.5	9.2	8.3	8	6.8	6
Total Protein (g/dl)	6.3	6.2	6	6	5.3	4.8
Sugar (mg/dl)	72	70	59	49	41	56
Fibrinogen (mg/dl)	161	173	151	131	102	It was not reported
Prothrombin Activity (%)	84	58	61	52	It was not reported	It was not reported

Notably, while the amount of fibrinogen decreased by 60 units and is expected to be consumed in the coagulation process, but there was not seen clot and it can represent the effect of induction of electric charge on the protein. Perhaps this protein has been attached to the platinum surface and or under the influence of electricity, their efficiency has been lost and additional testing needs more studies to be proved. Moreover, the Prothrombin Activity in the blood samples that collecting at beginning sampling was 84 percent, 10 minutes after becoming apparent of the first symptoms of clot formation in control sample, the container that containing the electrodes with electric current, the Prothrombin Activity had fallen to 52 percent.

All these findings confirmed the delay in the blood coagulation process. In addition to these findings, control blood was totally clotted in 12 minutes while the blood underwent electrolysis was not clotted until 30 minutes macroscopically. Coagulation process was evaluated with the use of capillary tubes throughout this period.

While there was no clot in the electrolyte container after 30 minutes but appearance of the remaining blood was different with the fresh blood.

4 Discussion

To delay blood clotting, without using any usual anticoagulation chemical substance and only with the use of electric charge induced by low voltage and Non-reactive platinum electrode, at first glance could be new in its kind, but what is more important than this initial result, changes that induced due to the electric charge in blood compounds. Perhaps one of the most important parameters that must be taken into account, the amount of free radicals produced during this experiment. Moreover, blood parameters have changed that the results of them which are listed, are very important.

Of course, understanding the fact that in the course of electrolysis what changes and how much is done, needs too many specialized and complete studies. If we considering to our basic information about the blood (that contain at least More than 300 different proteins, variety of ions, different blood cells, blood gas and other vital substances, sometimes little change in either of these cases could threaten the health of organisms seriously and even lead to death), Then will understand the necessity of conducting numerous studies in this field. For example, if the sodium ion that is regulating the osmotic pressure of the blood, By electrolysis modified slightly or in the most optimistic circumstance not absorbed to an electrode and only deionized, can cause many harmful chain changes that will not be compensated.

The results of this experiment, including of a significant reduction of calcium concentration, increased prothrombin time and significant reduction of fibrinogen compared to the control sample, bring different assumptions to justify. This means that serum calcium has been deionized and proteins that involved in the blood coagulation process that has negatively charged at a normal blood pH, have been neutralized during the test or even have been destroyed (considering significant decreasing of fibrinogen), Which if correct any of the hypotheses that additional studies be determined them, Can be verified reducing the quality of blood and inability to restore blood into the body. Which case to eliminate any possible defects, new hypotheses can be presented. One of the downsides this study was the absence of evaluating the morphology of blood cells are sampled at different times that because of some inconsistencies samples were not reliable, that the flaw is that must be corrected in the next study.

At its best, must first, as L. Duic and et al in 1973 or D. Aurbach and et al in 1991 and M. Daraio in 1981 that have done research separately on each blood factor or on synthetic fluid like blood, the effect of electrolysis process on blood should be studied. After completing and confirming the tests, mutual influence on each of these parameters during electrolysis must be checked. Doing these researches needs to financial and hardware resources. What Benjamin R.E. Simona in his Ph.D. thesis entitled as "Interfacial electrochemistry of blood coagulation factors: Fundamentals and applications" Which has done in 2015 on this subject are also another confirmation. Our knowledge about of the effect of Electrolysis on blood is so little that still has to be worked on techniques and basic concepts [33]. While this study only indicates the answer of one question and the question is; whether induction electric charge can

inhibit or delay time blood coagulation? And the answer is YES.

Research conducted and examples of them mentioned in the above, so focused on the details of the process of electrolysis and its chemical aspects and were left unanswered this question. Also implementation and using directly from this technology in clinical aspects has been postponed while knowing the answers to these basic questions, motivate greater incentives to conduct research on this subject and its importance and make it more functional.

According to the excessive application of electrolysis and related technology in medicine, particularly in the assessment of the contents of body fluids that have made significant progress today is a common laboratory and medical equipment, Perhaps replacing this technology to using of certain drugs and chemicals substances is not so unthinkable.

Certainly for the applying this study in sophisticated devices like cardiopulmonary machine or in blood dialyzer or in tests such as complete blood cell count, which needs to whole blood samples, the most comprehensive study is needed.

The next step in clinical practice nor in the molecular and cellular field maybe is returning the uncoagulated blood based on electrolysis to the body of animal testing that It is also considering the ethical issues and the rights of animals requires permission by the Medical Ethics Committee and the religious references.

In the end, it should be noted, with all of these requirements and limitations, further research must be done quickly so maybe can introduce a healthy and safe technology to prevent blood clotting.

5 Conclusions

There is no doubt that delaying the blood coagulation process without any chemical substances and only by using electric current is a remarkable innovation. In order to making this procedure a practical method, Complementary experiments are needed to evaluate most of the blood parameters. This study only confirms this method may be possible but measuring the quality of the method and quality of the blood under the influence of the electric current, requires extensive experiments.

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