Review Article

Can an Apheresis Therapy become an Effective Method for Anti-Aging Medicine?

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Abstract

During last 30 years, apheresis technologies had helped to treat many diseases by removing pathological macromolecules or pathological cells from patient’s blood. They included the cryofiltration to remove cryogel and the thermofiltration to remove LDL cholesterol. Unfortunately during these 30 years, developments of new apheresis technologies were not actively pursued by apheresis practitioners in the world including our group. Some diseases once treated by apheresis procedures were also shrank substantially. However, we believed that apheresis therapy had many possibilities to treat the major disease categories including heart diseases, diabetes mellitus and cancer. Even aging process might be able to be prevented by apheresis therapy.

In this paper, we should go over current membrane apheresis technologies and should introduce our concept of “Juzo” as an Anti-Aging artificial organ which was presented in 1995. Then we should introduce the new types of the 2nd generation apheresis technologies by referencing recent papers which supported our study directions. The new technologies included in this paper were the cryoaggregate filtration to remove pathological globulin molecules for the treatment of cardiomyopathic diseases, cryoreactive albumin removal apheresis (CRARA) for the treatment and for the prevention of diabetic complications of diabetic patients by removing pathological albumin and bioincompatible apheresis system for cancer treatment. In conclusion, apheresis therapies could remove pathological molecules accumulated excessively in the aging living body. The return to the normal levels of these molecules might be an effective interventional method as an Anti-Aging medical therapy.

KEY WORDS: apheresis, Anti-Aging, cryogel, cyrofiltration, 2nd generation apheresis

Introduction

The paper which was entitled “Effect of double filtration plasmapheresis (DFPP) in male patients with borderline hyper-LDL-cholesterolemia: Lipid removal and inflammation suppression” was reported by Hibino et al. on this journal in 2009 1). Interestingly, it showed whether DFPP, which could remove LDL cholesterol and other pathological molecules causing atherosclerosis, was effective physically and mentally using the Anti-Aging QOL Common Questionnaire. However, DFPP was performed only two times and removal of LDL cholesterol was not fully achieved. Based upon our experiences, in order to expect any clinical effects of DFPP, it is essential to impose at least 5 sessions of treatments or more. In addition LDL cholesterol was only one of the potential molecules to produce atherosclerosis. In order to treat atherosclerosis, additional disease causing molecules including fibrinogen and autoantibodies should be removed also.

Therefore, the result showed that DFPP group was not effective compared to control group significantly. It only showed that DFPP might suppress inflammation and improve tissue environment in visceral adipose and liver tissues with fatty deposits. While we were not satisfied with its result, the way of thinking, that suppression or improvement of atherosclerosis by an apheresis therapy might have made aging processes to be suppressed, is consistent with the direction of our thinking. We also think that the possibility of apheresis therapy for aging prevention should be investigated more in detail. In this paper we would like to show that an apheresis therapy might be effective as the Anti-Aging medical therapy.
**Apheresis therapy**

**History of Apheresis**

Originally, “apheresis” means withdrawal. Now to remove plasma components or cellular components from the blood is called apheresis therapy in the clinical medicine. Separation of plasma from the cellular components of the blood was achieved by either the membrane plasma separator or the centrifugal method. However, the centrifugal method used widely in USA did not produce a clear separation of plasma from cells. Blood cell losses, particularly platelets, are unavoidable. Accordingly, plasma treatments except plasma exchange needed to wait until the development of a membrane plasma separator in 1977.

Membranous plasma separation and plasma treatments were developed by Yukihiko Nosé and Paul S. Malchesky about 30 years ago. By 1972, the development of membrane technology was such that hydrophobic membranes with a porosity of 0.1 to 0.5 microns were available and initially aimed to develop the membrane oxygenators. IO Slayer together with Y. Nosé was developing the Monsanto hollow fiber membrane oxygenator. It was discovered by Y. Nosé that the polymeric hollow fiber device showed reasonable gas exchange characteristics but also produced a large amount of plasma leakage through the membrane walls. The membrane was not hydrophobic enough to be that of oxygenators; thus, this membrane was not considered practical for oxygenators.

But, Y. Nosé thought that this type of hydrophilic membrane was effective for plasma separation in 1972. When there were slight pressure differences between the gas side and the blood side, relatively large amounts of plasma were separated. Unfortunately, the protein content of the filtrate was in the range of 50% of plasma protein with a filtrate flow of 10 ml/min and a blood flow of 100 ml/min. In order to develop a clinically effective plasma separator, it was necessary to develop a microporous hollow fiber membrane of having not only larger pore structures but also more hydrophilic membrane material. The device required further development, and Monsanto decided not to pursue the project.

Meanwhile, the Cleveland Clinic group lead by Y. Nosé and P.S. Malchesky devoted their efforts towards the development of a membrane for a plasma separator and plasma fractionators systems. Initially a plate type plasma separator was utilized at Cleveland Clinic Foundation (CCF) in 1976. In 1977, a hollow fiber plasma separator was available from Asahi Kasei Medical Co., Ltd. (Chiyoda-ku, Tokyo). N. Inoue and Z. Yamazaki in Tokyo performed the first clinical application of this filter for hepatic failure patients. CCF group initiated its clinical application by this filter in 1977 also.

The following plasma treatment methods were developed based upon these microporous membrane filters. Each procedure and related filter, cartridge, and equipment were described in more detail in following sections.

**Various types of apheresis methods**

**Plasma Exchange**

Utilizing a plasma separator (either membrane or centrifuge), the diseased patient’s plasma was separated on line and discarded. At the same time the healthy patient’s plasma or albumin solution was replaced for the lost plasma volume.

**Double Filtration Plasmapheresis**

After plasma was separated with a plasma separator, the diseased plasma was filtered through a secondary filter called a plasma filter or plasma fractionator. The unwanted sized molecules were removed by the secondary plasma fractionator module. LDL cholesterol was a very larger molecule; it was very easily removed by the proper plasma fractionators while retaining HDL cholesterol. In order to enhance efficiencies of plasma filtration, the cryofiltration and the thromofiltration were also introduced.

The plasma filter or plasma fractionators separated unwanted larger sized molecules inside the secondary filter and retained these larger sized molecules there. Filtered plasma without unwanted larger molecules was returned to the patient.

**Plasma Adsorption**

Specific adsorption columns could remove unwanted macromolecules in plasma. This procedure was called plasma adsorption. In order to enhance its efficiencies, multiple adsorption columns were very often necessary to be included.

**Cryofiltration (Figure 1)**

Selective separation of globulin fractions or larger molecules from albumin or smaller molecules was theoretically possible, but the molecular size of albumin (68,000 daltons) and globulin (150,000 daltons) were quite similar in dimension, and it was rather difficult to separate them effectively by simple membrane filtration systems. Realizing cryoproteins were very often present in the plasma of patients suffering from autoimmune diseases, an attempt was made to utilize temperature as a variable in the membrane separation of pathological macromolecules in the plasma. At a low temperature below 4°C, heparin used as an anticoagulant for the extracorporeal circulation established fibrinogen-heparin-fibronectin complexes and attracts antibodies, immunocomplexes, pathological lipids and other cryoreactive molecules inside of these complexes in the plasma. This gel-type residual inside the secondary filter was coined “cryogel” by Y. Nosé (Figure 2, 3). While normal values existed in non-diseased states of the subject, concentrations of cryogel over 10 times higher were not uncommon in the autoimmune disease patient.

Taking advantage of cryogel formation, cryofiltration was introduced by P.S. Malchesky and Y. Nosé. Cryofiltration was the technique of cooling (at 0 ~ 4°C) and filtering of plasma on line. Cryogel was formed and retained in the membrane plasma fractionation filter. The filtered plasma was reunited with the whole blood, warmed to physiological temperature, and returned to the patient. Cryogel removal was achieved with the bulk of the plasma being returned to the patient accompanied by the passage of albumin and smaller molecular weight molecules inside of the plasma. The composition of cryogel was shown in Figure 3.
Fig. 1. Double filtration plasmapheresis (cryofiltration). When the separated plasma was cooled down at 0 – 4°C, the cryogel was removed from the plasma by the plasma filter.

Fig. 2. The cryogel removed by cryofiltration.

Fig. 3. Composition of cryogel.
Therapeutic artificial organs

For the removal of only pathological lipids such as LDL cholesterol, the formation of cryogel was not desired. Thus, for the removal of LDL cholesterol, thermofiltration was developed by Y. Nosé and P.S. Malchesky 10, keeping the secondary filter’s environmental temperature at around 37°C ~ 40°C. Since cryogel formation was prevented by this method, effective removal of LDL cholesterol was established for the treatment of atherosclerosis. Once or twice/month sessions of thermofiltration should be sufficient to maintain the LDL cholesterol level in reasonable ranges. Later we learned from Dr. Michael E. DeBakey that atherosclerosis was produced not only by LDL cholesterol alone but also other pathological molecules including fibrinogen and autoantibodies 9. Dr. DeBakey performed aorta replacement surgeries on more than 60,000 patients during 60 years of his professional life. Clinical application of thermofiltration was replaced by cryofiltration.

Current Situations About Apheresis Therapy

International apheresis registry of 2007 was reported by P.S. Malchesky 10. These authors were surprised that still more than 60 % of procedures were performed by old fashioned plasma exchanges. In the USA, most apheresis procedures were conducted by centrifugal methods. Some diseases once treated by apheresis procedures were also shrank substantially. During 1980s, our group alone at the Cleveland Clinic performed approximately 1,000 session/year of membrane apheresis therapy at the Department of artificial organs for the treatment of various types of metabolic and immunological diseases.

After introduction of the apheresis technologies approximately 30 years, it should be the time to introduce the second generation apheresis technologies to expand the application of apheresis to major disease categories including heart diseases, diabetes mellitus and cancer.

Artificial organs and Anti-Aging

In 1995 Y. Nosé put forward a concept of “Juzo” as an Anti-Aging artificial organ 9). This concept was based upon “Therapeutic artificial organs” which was proposed in 1983 by Y. Nosé 9). He insisted that “Juzo” might be an artificial organ which made aging processed to be suppressed and kept physical and mental youth due to removal of cryogel from the blood using apheresis technologies. The levels of cryogel inside of the blood increased by increasing of the ages of the patients. The following were his explanations during last 30 years.

Therapeutic Artificial Organs

As it was well known, many end-stage organ failures were introduced by immunologically and metabolically mediated diseases. If we apply our artificial organ technologies properly, we might be able to reverse the disease processes, and some disease states could be cured completely, or intensities of diseases could be contained in such a way that the standard surgical and medical therapies could control the pathophysiology of these diseases. Certainly some of the disease states could not be controlled by therapeutic artificial organ technologies alone; however, the need for human spare parts would be reduced substantially.

One type of the therapeutic artificial organs was included utilization of various types of artificial organs for the bridge to functional recovery of natural tissues and organs. They included utilization of the heart lung machine for lung functional recovery, the ventricular assist device for heart functional recovery and functional augmentation with the artificial kidney for drug overdose. Certainly apheresis is the most important therapeutic artificial organs.

Cryogel and Aging Process

As we mentioned above, cryogel in plasma especially increased in the patients with autoimmune diseases (Figure 4). Cryogel mainly consisted of fibrinogen-heparin-fibronectin complexes. These cryogel complexes also contained immunoglobulin, auto antibodies, immune complexes and many other cryoreactive macro and mini molecules which were considered to be pathological when they were increased higher than physiological levels. Cryofiltration could remove these pathological molecules by removal of cryogel. And it was well known that cryofiltration was effective for the treatment of autoimmune diseases including rheumatoid arthritis. The reason of this effectiveness was due to effective removal of pathological cryoreactive globulin or autoantibodies. Increase of such pathological globulin in plasma also contributed calcification of vascular walls.

Fig. 4. Kinetics of cryogel in aged and diseased individuals (graphic display).

The cryogel also increased in our plasma with the normal aging processes. That’s why our normal level of fibrinogen which was a major component of cryogel increased with the aging process (Figure 5). Increase of fibrinogen induced increase of viscosity of the blood and deterioration of microcirculation. Also higher levels of fibrinogen in plasma introduced microemboli easily. Such microemboli on the surface of the vascular wall were considered to be atherosclerotic origins. Reduction of fibrinogen from the plasma reduced such chances of microemboli formation and atherosclerosis on the surface of vascular walls. The fibrinogen could be removed from the blood by cryofiltration.

Moreover, low density lipoprotein which was strongly related with atherosclerosis and cardiovascular diseases could be removed.
by various apheresis methods including cryofiltration. As it was described before, atherosclerosis would be induced typically by following three types of macromolecules as suggested by Dr. Michael E DeBakey. They were (1) Increased level of fibrinogen (Blood clot induced atherosclerosis), (2) Increased level of LDL cholesterol (Lipid induced atherosclerosis), and (3) Increased level of IgG (Calcium deposited atherosclerosis).

The effectiveness of these apheresis methods for the removal of these pathological macromolecules was already well established. These molecules which increased with aging processes could be removed by using apheresis technologies, although all these molecules did not affect living body adversely when concentrations inside of the blood were maintained near normal levels.

Figure 6 demonstrates that after we removed cryogel from plasma, lymphocyte function became more normal. In order to expect normal lymphocyte function, it was essential to keep the humoral macromolecules level at a normal level. In other words, if we had pathological macromolecules in our plasma, removal of pathological macromolecules by cryofiltration might be able to improve abnormality of cell function and retained the youth status of the patients.

Figure 5. Aging and increased level of fibrinogen in plasma.

**Juzo** as an Anti-Aging Artificial Organ

The aging processes produced an increase of pathological macromolecules in blood and subsequently altered cellular functions. This situation produced either immunostimulation or immunodeficiencies depending upon what kinds of cellular and humoral immune systems were activated or suppressed. If the immunostimulation status was dominant, then the patient suffers various types of autoimmune diseases. However, in general the aging process produced immunodeficiency status. In this situation, infection and malignant tumors had a tendency to occur more frequency. If the hypothesis described above was the actual aging processes taking place in our body, certainly we knew medically how to prevent it with our apheresis technologies. We would like to call such an age preventing artificial organ “Juzo”, the organ for a longer life, in Japanese. We assumed that when human being became old and not being able to produce children, our creator thought there was no reason to keep them alive longer. So our creator intentionally forgot to install the age preventing organ inside of the body. So our life was limited in less than 100 years old. In order to keep us alive longer, it should be necessary to install the so called aging preventing artificial organ or “Juzo” into the patient. So we believed we could slow down the physical aging process by apheresis technologies. This new contribution of artificial organs together with other already proven contributions of artificial organs, such as supplemental organ functional support and replacement of a failed organ, could certainly contribute to the health and well being of elderly patients.

**The second generation apheresis therapy**

We began to initiate for the development of the second generation apheresis therapy five years ago. Heart diseases, diabetes mellitus and cancer were selected as target diseases. These diseases were strongly related with aging. Therefore, to establish the second generation apheresis therapy for Anti-Aging medical therapy should be developed to prevent aging related diseases.

**Cryoaggregate Filtration**

At lower temperatures (4°C ~ 30°C), the diseased heparinized plasma developed cryoaggregates. This method is to remove cryoaggregates formed at lower temperatures from the plasma. Almost all of cryoaggregates existed between 0.1 and 0.01 µm under below 20°C. Therefore, cryoaggregates would be removable by the plasma fractionator having its pore structures between 0.01 ~ 0.1 µm (10 ~ 100 nm). We developed 2 kinds of cryoaggregate filtration systems which could be performed online as PAT CAT (Pressure and temperature controlled apheresis therapy) and offline as Off-LAPPET (Off-line automatic plasma purifier for exchange transfusion).

This procedure (Off-LAPPET) was approved by the US FDA and currently this pilot study on non-ischemic cardiomyopathy patients was under way. For this patient population effective removal of pathological globulin would be considered to be clinically beneficial. The initial preliminary results revealed improvements of cardiac functions after 4 weeks of 5 sessions Off-LAPPET procedures (6 weeks from the initiation of the
treatment). The cryoaggregate filtration should be able to remove not only pathological globulin effectively, but also low density lipoprotein and fibrinogen effectively. Therefore, we are planning to treat ischemic cardiomyopathy patients by this method in the future also.

**Cryoreactive Albumin Removal Apheresis (CRARA) Therapy**

Currently, diabetic complications (nephropathy, retinopathy and neuropathy) were considered to be generated by increased plasma levels of glycated albumin (GA) and other glycated proteins. These glycated proteins, increased in diabetic patients, caused heart and vascular diseases and complication. We investigated whether cryofiltration removed GA from cooled heparinized plasma of the hemodialysis patients due to type 2 diabetic nephropathy by using filter. The plasma was cooled down at 4 °C and filtration was made in vitro through 0.2 µm filter. The plasma samples from 5 diabetic patients with 5 non-diabetic patients were subjected for cryofiltration. The increased GA was effectively removed as the cryoreactive albumin by cryofiltration, but non-glycated albumin was not removed. Namely, it showed that cryofiltration could remove selectively only cryoreactive GA from the patient’s plasma as pathological molecules.

Cryoreactive albumin was removed by the heparinization and cooling process of the plasma at 4°C by 0.2 µm filter. However, more effective removal of GA was established by the CRARA filter of 0.05 ~ 0.2 µm pore structures. Also the CRARA therapy was supposed to be performed at 5 ± 5°C.

The size of albumin molecules (68,000 daltons) were smaller than the size of globulin molecules (150,000 daltons), so in order to remove albumin cryoaggregates effectively, lower temperatures (5 ± 5°C) than the removal of cryoaggregated globulin (15 ± 10°C) were necessary to be employed. With these filtration pore sizes and temperatures, the CRARA therapy removed not only cryoglobulin but also cryoaggregates. It demonstrated more effective than the simple cryofiltration. Effective removal of pathological albumin from diabetic patients’ plasma by CRARA therapy should be able to reduce or eliminate microvascular complications occurred for the end stage diabetic patients.

**Bioincompatible Apheresis System for Cancer Therapy**

Now we are investigating a bioincompatible apheresis system for applying treatment of cancer patients. This concept was suggested to Y. Nosé by Professor J. Mikami in 1958, Chairman of the Department of Surgery, Hokkaido University School of Medicine (Sapporo-city, Hokkaido). In 1958, Professor Mikami told Y. Nosé that he had treated 3 patients with inoperable stomach cancer who demonstrated complete remission, out of approximately 5,000 cases. It happened that all 3 of these patients received ABO incompatible transfusions and survived. Professor Mikami stated, “The immunological control shock might have had therapeutic effects on the cancer patients”. Thus, it is expected that a bioincompatible apheresis system may be effective for immunostimulation or immunoactivation. We will be able to report about this project in near future.

**Apheresis therapy, Anti-Aging**

Recently, papers which indicated that an apheresis therapy might be effective as an Anti-Aging medical therapy have been reported. We will introduce 2 papers of them.

**Heparin Cryoprecipitation, Atherosclerosis**

At first, Meilin et al. in Israel reported whether heparin cryoprecipitation which was an in vitro method of plasma purification using centrifuge removed non-traditional risk factors for atherosclerosis from cooled heparinized plasma of the patients with hemodialysis. Their method was based upon a cryofiltration method. Since cryogel was formed by heparin and fibrinogen under cooled temperature, they tried to remove the cryoprecipitation by centrifuge. Their result showed that treatment of hemodialysis plasma with heparin cryoprecipitation (freezing -20°C, thawing 4°C, centrifugation 800g, 4°C) significantly reduced fibrinogen, carbonylated fibrinogen, carbonylated albumin and TNF-α to control levels which were simultaneously found in the cryogel. They also compared differences of removal effect between albumin and carbonylated albumin or fibrinogen and carbonylated fibrinogen by their method. Interestingly, it was revealed that carbonylated albumin and carbonylated fibrinogen were selectively removed from patient’s plasma. These carbonylated proteins were produced as a result of strong influences of the oxidative stress. This oxidative stress is strongly related with not only atherosclerosis but also aging. Therefore, their removal method could remove not all plasma molecules non-selectively but undesirable plasma molecules for living body selectively.

**Heparin-Induced Extracorporeal LDL Precipitation, Diabetic Foot Syndrome**

This study reported the effect of the heparin-induced extracorporeal LDL-precipitation (H.E.L.P.) as a novel therapeutic approach in patients with severe diabetic foot syndrome. H.E.L.P. apheresis is a kind of LDL apheresis. The method of H.E.L.P. is based upon an increase of the positive charges on LDL and Lp(a) particles at acid pH, allowing them to form an insoluble network with heparin and fibrinogen. Protective lipoproteins (HDL with apo A1 and apo A2) as well as albumin or immunoglobulins were not affected.

Seventeen diabetic patients with septic foot lesions recruited from the diabetic outpatient clinic underwent H.E.L.P. apheresis regularly until fibrinogen levels were stabilized at 3g/l or infection was controllable as evidenced by alleviation of necrosis. Patients were subsequently followed up for 2 to 73 months. Fibrinogen levels were reduced by 68% after H.E.L.P. treatment. No severe complications were noted. Necrosis could be confined in sixteen patients. Minor amputations were indicated in twelve patients. Three patients underwent major amputations of the lower limb and two patients received surgical reconstruction. To reduce fibrinogen and LDL cholesterol level of plasma was very effective for diabetic microangiopathy.
Conclusion

Generally, aging process should be a physiological phenomenon. However, if its speed is too fast or too strong, it might produce a disease. If this hypothesis would be true, “apheresis therapy”, which could remove pathological molecules accumulated excessively inside of the living old body and could return them to the normal level, should be an effective interventional method as an Anti-Aging medical therapy. For such purpose, the cryofiltration or cryoaggregate filtration should be an ideal therapeutic artificial organ called “Juzo” or to prevent aging processes of patients. We should proceed to investigate and expand the possibility of “apheresis therapy” as an active Anti-Aging medical therapy in the future.

References

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