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Red Blood Cell Ageing and the Ability of their Microparticle to Protect Human Endothelial Cells from Oxidative Stress

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Abstract

Red blood cells (RBCs) have various roles that are important in making sure that the body functions properly. They are responsible for delivering oxygen and removing carbon dioxide and other waste gases to and from the body. Studies have shown that storing blood for extended amounts of time leads to more oxygen entering the blood, causing oxidative stress and also formation of microparticles. Small in size (<1 micron), microparticles are membrane vesicles that are released by RBCs and other types of cells such as platelets, leukocytes, and endothelial cells. It has been shown that microparticles are increased in the plasma of patients with cardiovascular disease, however conflicting evidence has led to the idea that the increased release of microparticles may be harmful to the patient. Other research findings suggest that the microparticles derived from RBCs are actually protective against pro-inflammatory signals in endothelial cells. The aim of this project was to test the hypothesis that microparticles from younger red blood cells protect against oxidative stress signals in human endothelial cells, and as they age, if they enhance the oxidative stress response.

The primary research methods used for this study were the staining of RBC membranes with antibodies and analysis with a flow cytometer, measuring reactive oxygen species production through reactive oxygen species (ROS) assays, and observing the structural changes of the red blood cells using bright field microscopy. Results suggested that there was only an observed increase in the microparticle events among the RBC samples that contained only calcium ionophore. The other data for the RBC samples do not seem to support the initial hypothesis that was made. Further research needs to be completed to prove whether microparticles contain protective properties or if they contribute to cardiovascular diseases.

Keywords: red blood cells, microparticles, oxidative stress

Introduction

Coronary Heart Disease (CHD) is the most common form of cardiovascular disease. CHD occurs when the arteries supplying blood to the heart become narrow and stiffen due to the build up of plaque [1]. The formation of plaque is also known as atherosclerosis, a chronic inflammatory condition characterized by monocyte recruitment to the wall of the large arteries, endothelial dysfunction, and oxidative stress, and secretion of pro-inflammatory mediators.

Red blood cells (RBCs) have various roles that are important in making sure that the body functions properly. They are responsible for delivering oxygen and removing carbon dioxide and other waste gases to and from the body. Red blood cells obtain their bright red color from the protein hemoglobin, which carries oxygen throughout the body. As the oxygen is delivered through the lungs, oxygen molecules attach to the hemoglobin. Then, as blood moves through the body’s tissues, the hemoglobin frees the oxygen to the cells. Carbon dioxide then attaches to the hemoglobin, which transports it away [2].

Research has shown that storing blood for extended amounts of time leads to more oxygen entering the blood, causing oxidative stress, and also formation of microparticles. Microparticles are membrane vesicles that are released by RBCs and other types of cells such as platelets, leukocytes, and endothelial cells. They are 1-1000 nanometer in diameter and possess pro-inflammatory and pro-coagulant properties [2]. Their formation is due to the calcium influx that is frequently caused by acrosin and cell activation [4]. Although this is a normal process that happens in the body, the formation of microparticles is believed to increase over time in stored blood.

While some research studies have suggested that there is an increase in microparticle formation in the plasma of patients with cardiovascular diseases, others have suggested that microparticles formed from RBCs actually protect against pro-inflammatory signals in human endothelial cells. Endothelial cells form the inner lining of a blood vessel and provide an endothelial barrier between the vessel wall and the blood. It has been shown that the production of microparticles is increased in the processes of inflammation, coagulation, and vascular function, which are all involved in the pathogenesis of cardiovascular diseases [5]. The primary aim of this project was to test the hypothesis that microparticles from younger red blood cells protect against oxidative stress signals in human endothelial cells, and as they age, if they enhance the oxidative stress response. This study consisted of using a cocktail of different inhibitors in order to observe the effect it had on the presence of microparticles over a period of time.

Materials and Methods

Materials:
Two blood packs with different blood types were collected and reracked into four 50 mL test tubes for the purposes of this study. To prepare buffer solution, sodium solution was used to dilute the blood samples and other solutions used as a solvent for other solutions.

The following inhibitors were used to test the activity of microparticles: 1µM calcium ionophore, 2.5µM calcium chloride solution, 1µM mitogen-activated protein kinase (MEK) inhibitor (U0126), and 1µM calpain inhibitor.

In Annex buffer, staining beads, enumeration beads were used to prepare the control tubes, as well as the sample. The controls and samples were placed in FACs tubes to be analyzed by the flow cytometer. The antibodies anti-CD105 (phycocyanin) A, anti-CD31 (peridinin-chlorophyll-protein) B and annexin V-Cy7 (Cy7) C were added to the RBC supernatants for preparation for microparticle analysis with the flow cytometer.

For preparation of the ROS assays, white opaque 96 well plates were plated with human umbilical vein endothelial cells (HUVECs), then later was incubated with 100 µM DHR, IMER10% FCS, and 20 µM hydrogen peroxide solution.

Methods:
1. Isolation of Red Blood Cells from Whole Blood Research Blood Pack
2. Staining of Scrambled RBC Membranes with Antibodies, Annexin V Cy7, and Miporipine (MACSIO)
3. Measurement of Reactive Oxygen Species Production Using Phenylfluorescein-1.23 (SHR)

References

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Discussion

The findings suggest that the calcium ionophore treated RBC sample showed an increase in microparticle formation and that the other samples did not reflect a significant increase in microparticle formation. The microparticles in the samples that did not include calcium ionophore showed relatively the same amount of microparticles from the first day (Day 1) to the last day (Day 27). Since it was known that there would be an increase in microparticle formation with the inhibition of Erk activity and the treatment of calcium ionophore, it was quite simple to see if the correlations did hold true over time. It was a similar case with the addition of the calpain inhibitor except it is expected that there will be a decrease in microparticle formation. From the observed data, it suggested that there was only an observed increase in the microparticle events among the RBC samples that contained only calcium ionophore.

The other data for the RBC samples do not seem to align with the initial hypothesis made by other research. There was also evidence in the cellular structure of the RBC samples that much change did not occur in regards to microparticle formation. The major difference between the samples from day 2 and 27 is that the day 27 RBC samples seemed to have lysed and there is no particular shape, whereas the day 2 RBC samples seem to have a distinctive shape.

Conclusion

In this study, multiple research methods were utilized and data was generated. However, there were multiple factors that contributed to some of the inaccuracies of the study. For example, the ROS assays did not prove to be a successful procedure because of some procedural errors that occurred. For future projects pertaining to this research study, a ROS assay needs to be completed to test the initial hypothesis regarding if microparticles exist as reactive oxygen species. This would then address the issue on if microparticles are a result of cardiovascular disease and if they do have pro-inflammatory properties that prevent oxidative stress.