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Mitochondrial Potential and Nd1 gene Expression Analysis in Platelets for quality Assessment

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Abstract

Background: Platelets are the tiniest blood particles, and their shape shows how lively they are. They are largely concerned with hemostasis, or the process of starting blood coagulation. The diverceful activity of platelet and molecules released from platelet storage granules are both necessary for these various activities. Initiating homeostasis, an inflammatory response, and an immune response at the site of injury during the acute phase of wound healing, as well as encouraging angiogenesis and tissue regeneration during the repair phase, is examples of how these platelet activities interact synergistically. Therefore mitochondria function/quality plays various important roles in several disease processes. Several studies have demonstrated mitochondria dysfunction in various chronic degenerative diseases (ex: cardiovascular diseases; Type 2 diabetes). Hence, there is need for quality assessment platelet mitochondrial DNA for platelet functions.

Materials and Methods: Platelets were incubated with Rhodamine 123 exclusive dye to assess the membrane potential. The cytotoxicity of platelets were assed at different dilution point using MTT assay. The activity of platelet mitochondrial (mtDNA) was assessed using ND1 gene.

Results: The aggregation of platelets with Rhodamine dye indicates the active state of platelets. The decline of platelets with high concentration MTT assay was observed. The activity of mtDNA in platelets the ND1 gene and internal control 12s gene were expressed at same indicating the active presence of mtDNA in platelets.

Conclusion: The Rhodamine 123 stain with platelet indicates that the presence of mitochondrial membrane potential. The ND1 gene expression indicates the quality of mitochondrial DNA in platelets.

Keywords: Platelets, Rhodamine-123, MTT, ND1 gene

Introduction

Platelets were discovered in 1882 by Giulio Bizzozero, but the dynamic and multifunctional nature of platelets remained a focus of study mainly for biologists for many decades. Platelet mitochondria were thought of solely as ATP producers, using the OXPHOS process to meet a large portion of the energy demands of dormant cells [1]. Platelets are abundant in blood and help to maintain homeostasis. Platelets, on the other hand, are activated in a variety of inflammatory responses through the release of their diverse arsenal of mediators. Platelet cytoplasmic granules (dense, and lysosomes) fuse with the plasma membrane upon activation, allowing the contents of the granules (microparticles) to be released into the extracellular milieu [2]. Human platelets (PLTs) have a short shelf life under in vitro storage conditions. Regulations in most countries limit the maximum storage time to 5 to 7 days due to concerns about bacterial contamination. PLTs quality deteriorates with storage, as evidenced by a loss of discoid morphology, a decrease in aggregation and hypotonic shock response changes in cell surface antigens, and a reduction in the ability to respond to a Ca2+-elevating agonist like thrombin. Taken together, these changes represent the so-called PLTs storage lesion. Although PLTs lack a functional nucleus, they do contain mitochondria, which have been shown to play an important role in the generation of proapoptotic signals in a variety of other cell types. The collapse of the mitochondrial membrane potential ($\Delta\Psi$), which results in 'cytochrome c' release and caspase activation, triggers at least one apoptosis cascade [3].

Platelet Mitochondria Are Essential for Platelet Function and Survival

The presence of DNA, the presence of a double plasma membrane, and the ability to divide during the cell cycle are all characteristics shared by mitochondria and nuclei. The mitochondria, also known as the "powerhouse of the cell", play a unique role in energy production and metabolism. The mitochondria contain the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS), both of which are involved in the production of adenosine triphosphate (ATP). Other than energy production, mitochondria have been linked to a variety of processes, including the production of reactive oxygen species (ROS), Ca2+ homeostasis, apoptosis regulation, and ER-stress response mechanisms. Mitochondrial health and dysfunction appear to play a role in ageing and neurodegenerative diseases (such as Alzheimer's and Parkinson's disease) [4,5].

Mitochondria and Energy Metabolism in the Platelets

Most human nucleated cells have hundreds, if not thousands, of active mitochondria, whereas platelets have only 5–8 mitochondria per cell. Platelets have a much higher rate of ATP turnover than resting mammalian muscle cells, indicating that they are metabolically active. Because several key processes within the platelet, such as calcium homeostasis, require a constant supply of energy, ATP is required for proper platelet function. Platelets have been shown to have metabolic flexibility, allowing them to meet this energy demand; activated platelets have a glycolytic phenotype while still functioning mitochondrial. The platelet's ability to adapt to a variety of situations, including hypoxia and the presence of mitochondrial inhibitory agents, is due to its ability to use glycolysis or fatty acid catabolism instead of OXPHOS (mitochondrial ATP production) [6].

Platelet Mitochondrial DNA (mtDNA)

Mitochondrial DNA is a helpful tool for evolutionary research due to its small size, reasonably maintained gene content, and high mutation rate. Unlike nuclear genetic code, mitochondrial genetic code in vertebrates is made up of 60 sense codons that encode 20 standard amino acids, with TAA, TAG, AGA, and AGG serving as the remaining four ending codons. The genetic code is universal, with the exception of mitochondrial DNA. Only six codons in animal mitochondrial DNA that change during evolution have been identified; these codons are TGA, ATA, AAA, AGA, AGG, and TAA. The MT-ND1 gene is one of 13 mitochondrial DNA protein-coding genes involved in respiration. It encodes the NADH dehydrogenase 1 enzyme and is a component of the mitochondrial respiratory chain's complex I. The mitochondrial DNA is a covalently closed, double-stranded molecule with a size of around 16.6 kb that encodes 2 rRNA, 22 tRNAs, and 13 polypeptides. Hence in this study the expression of ND1 for platelet mitochondrial DNA (mtDNA) assesment included [7].

Fluorescent imaging of mitochondria is a crucial technique for mitochondrial function research. Under some situations, the staining of mitochondria with potential-indicating dyes, such as rhodamine 123, fades quickly when the transmembrane potential is lost. 1-(Rhodamine B)-4-(2′-chloroacetyl)-piperazine amide (RB-CAP) was found to be electrophoretically accumulated into mitochondria, where it formed covalent bioconjugates with intra-mitochondrial protein sulfhydryl's, allowing the mitochondrial staining to persist despite subsequent transmembrane potential collapse. To assess the m component of p, numerous lipophilic cationic dyes (tetramethylrhodamine methyl (TMRM) and ethyl (TMRE) ester, Rhodamine 123 (Rho123), DiOC6 (3) (3, 3'-dihexyloxacarbocyanine iodide), and JC-1 (5, 5', 6, 6'-tetrachloro-1, 1', 3, 3. According to the Nernst equation, dyes will accumulate within mitochondria in inverse proportion to 'm' as positively charged molecules, given that suitable experimental controls and assay conditions are used. More polarized mitochondria (hyperpolarized mitochondria with a more negative interior) collect less dye [8] Microscopy is used to identify fluorescent dye buildup in mitochondria. Platelet rich plasma (PRP) as emerging role of the important therapeutic in various diseases, as platelets derives its major energy of mitochondria OXPHOS, the therapeutic value also depends on the quasi of the platelets. Standard methods adopted for mitochondrial function such as MTT assay which is one of the most flexible and widely used assays. Viability test that relies on live cells converting substare to chromogenic product. The water-soluble yellow dye MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) is converted in the MTT test.

Platelets is a simple way to obtain viable mitochondria, and sampling is less invasive than muscle or skin biopsy. Platelet mitochondrial changes have been seen in a variety of diseases, mostly affecting other organ systems, as well as in the ageing process, and have thus been proposed as a possible marker of systemic mitochondrial dysfunction. Platelets are also good candidates for mitochondrial functionality assessment. In order to stain mitochondria, any probe has to entry into the cell and to reach the organelles. The membrane potential of mitochondria indicates the stability of mitochondria we tested the viability of platelets stained with rhodamine 123 (R123), and MTT assay to analyze the quality of platelets. The presence of mitochondrial DNA (mtDNA) in platelets assessed by gene expression analysis.

Materials and Methods

The study was approved by Institutional review board, Deccan college of Medical Sciences. About 5 to 10 ml of blood sample was collected in plasma tube from a healthy donor. Theplatelet rich plasma was purified by ultracentrifugation at 800g for 5 minutes. Platelet rich plasma was separated and used for further analysis.

Platelet Mitochondria membrane potential(Δψ) with Rhodamine 123 (Rho-123) stain

Platelets were taken into dilutions. The platelets were then diluted with Rhodamine-123 dye to assess the membrane potential and observed under microscope. The same concentration and dilution factor of platelets with rhodamine 123 dye were incubated at room temperature for 30 minutes then observed under fluorescent microscope.

Plateletcytotoxicity assay with MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide)

The tetrazolium salt 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT, Sigma) has been used to measure the metabolic activity of Platelets, essentially as described by Xia et al and colleagues. In short, a sample of Platelets were diluted in 1XPBS to a different concentration, in each well of 96 well microplate about 50µl (10^3) of samples added according to dilution and about 50µl of DMEM complete media was added and incubated for 10-15 minutes at 37^0C, after incubation in each with 50µl of MTT (5 mg/mL) along with blank in duplicates and incubated for 4 hours at 37^0C. The absorbance was measured in a microplate reader at a wavelength of 570 nm after adding 50µl of DMSO (Dim) and incubating for 30 minutes at 37^0C.

Gene Expression analysis using ND1 gene

The platelet mitochondrial DNA (mtDNA) was extracted by in house developed lab protocol. The concentration of mtDNA was quantified using nanodrop reader at absorbance 260/280nm ratio of approximately 1.8 and less than 2.0 was considered for pure mtDNA. The mtDNA

expression was studied using ND1 gene which is a complex I marker of mitochondria. The primers for ND1 gene were Forward primer 5'- CTACTACAACCCTTCGCTGAC -3' and reverse primer 5'-GGATTGAGTAAACGCTAGGC -3' and internal control 12s universal primers were used. The amplification was done using 10ul of SYBR green PCR master mix with 0.5µl forward and 0.5ul reverse primer with 2µl of mtDNA. The RT-qPCR conditions were 94^{0}C for 5 minutes, 94^{0}C for 30s, annealing at 50^{0}C for 30s and extension 72^{0}C for 30s followed by a melt curve.

Statistics

The platelets were counted using Fluorescent microscope on Heamatocytomter. The mean expression folds of ND1 gene and 12s gene were calculated using excel.

Results

Platelet Mitochondria membrane potential (Δψ) with Rhodamine 123 (Rho-123) stain

The platelets diluted with different concentration with Rho-123 stain were showed in Figure 1, showing that the concentration of platelets at different dilution points with PBS. The platelets were resuspened in PBS then mixed with rhodamine 123 dye at different concentration. The intensity of the dye at different concentration was high. Fluorescence intensity was directly correlated to the amount of the dye used, independently from membrane potential. At all concentration of rhodamine-123 dye had no effect on the mitochondrial loading probes (Figure 2). The MTT assay showed the toxicity at higher concentration (Figure 3).

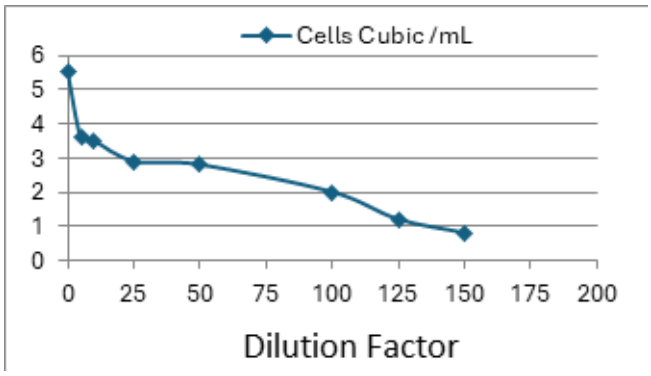

Figure1: The count of platelet viability at different dilution point.

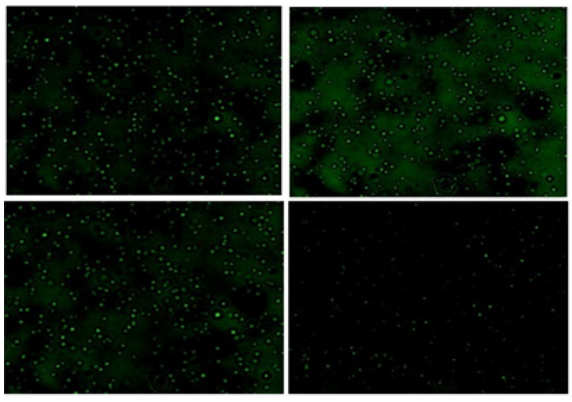

Figure2: The aggregation of platelets at different concentration of rhodamine-123 dye.

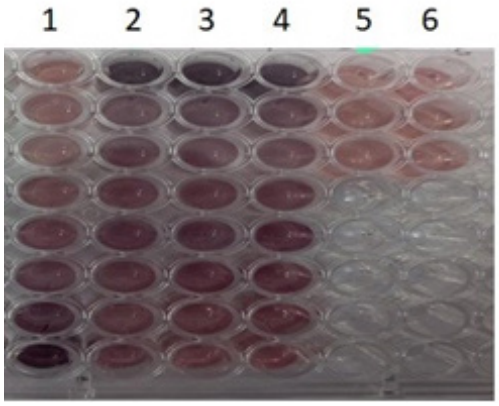

Figure 3: Platelet cytotoxicity assay with MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide). The well number 1 indicates the standard low to high concentration, well number 2, 3, and 4 were platelets with different concentration (High to low), well number 5 and 6 were negative controls.

Expression of Platelet mtDNA with ND1 gene

Further we have been assessed the presence of platelet mtDNA with ND1 gene using expression study and 12s as internal control. The mtDNA mean CT values were showed high in both with ND1 gene and 12s (Figure 4).

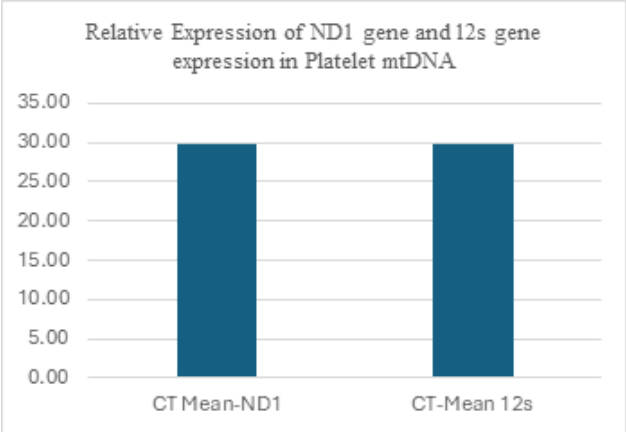

Figure 4: Relative expression of ND1 gene and 12s gene in Platelet Mitochondrial DNA.

Discussion

Mitochondrial activity is critical for platelet health and longevity because platelets require high energy levels generated by OXPHOS to respond to environmental challenges and blood vessel damage [9]. Present data demonstrate that the platelet mitochondria is strong in energy production. The mitochondrion is important in the regulation of apoptosis in nucleated cells, and permeabilization of mitochondrial membranes is required for apoptotic cell death. The permeabilization of mitochondrial membranes during nucleated cell death is caused by two distinct mechanisms [10, 11]. The mitochondrial permeability transition

pore (MPTP) opens in the inner mitochondrial membrane in the first mechanism, allowing water and molecules up to 1.5 kDa to pass through. Multiple apoptotic stimuli can cause the MPTP to open, resulting in depolarization of the Δψm and swelling of the mitochondrial matrix, followed by permeabilization of the outer mitochondrial membrane and release of proteins normally confined to the intramembranous space, such as cytochrome c and apoptosis-inducing factor. Members of the Bcl-2 family of apoptosis-regulating proteins act directly on the outer membrane in the second method [12-14]. In addition to being engaged in energy metabolism and ATP synthesis in platelets, mitochondria are also major drivers of platelet activation and apoptosis, both of which are critical for platelet function and lifetime. The pathophysiological significance of platelets and their mitochondria in many systemic disorders is still being researched. Therapies that target platelet mitochondria may eventually be effective in such disease processes.

Conclusion

As the powerhouse of the cell, mitochondria play essential roles in metabolism and energy production. Distinct from these processes, mitochondria also participate in the regulation of cell apoptosis, reactive oxygen species (ROS) generation and the ER-stress response. Similar to their roles in nucleated cells, mitochondria in platelets also regulate metabolism, ATP production, platelet activation and apoptosis, which are fundamental processes for platelet function.

Considering that platelets contain mitochondria which play crucial roles in platelet function, future studies delineate the contribution of platelet mtDNA role in various diseases and its usage for better understanding in diseases.

Conflict of Interest

No.

Acknowledgement

No.

References

1. Doery. J.C.G, HirshJ. and CooperI. (1970) "Energy Metabolism in Human Platelets: Interrelationship between Glycolysis and Oxidative Metabolism". *BLOOD*.; 36, No. 2.

2. Salvioli S, Ardizzoni A, et al; (1997) "Jc-1, but not DiOc6 (3) or rhodamine 123, is a reliable fluroscent probe to assess Δψ changes in intact cells: implications for studies on mitochondrial functionality during apoptosis". *FEBS Letters*; (411), pp77-82.

3. Verhoeven AJ, Verhaar R, et al; (2005) "The mitochondrial membrane potential in human platlets: a sensitive parameter for platelet quality". *TRANSFUSION*.; pp45.

4. Huebsch LB, Harker LA (1981) "Disorders of platelet function-Mechanisms, diagnosis and management (Medical Progress)". *West J Med*.; (134), pp109-127.

5. Lim KM, Kim HH, Bae ON, et al. (2009) "Inhibition of platelet aggregation by 1-methyl-4-phenyl pyridinium ion (MPP+) through ATP depletion: evidence for the reduced platelet activities in Parkinson's disease". *Platelets*; 20(3): pp163–70.

6. Bartolome, F, Wu, H. C, et al. (2013) "Pathogenic VCP mutations induce mitochondrial uncoupling and reduced ATP levels". *Neuron*.; 78(1), pp57–64.

7. Arif Uddin et al; (2017) "Factors influencing codon usage of mitochondrial ND1 gene in pisces, aves and mammals". *Mitochondrion*; doi: 10.1016/j.mito.2017.06.004.

8. Perry S.W, Norman J.P, et al; (2011) "Mitochondrial membrane potential probes and the proton gradient: a practical usage guide". *Biotechniques*. 50(2): pp98-115.

9. Melchinger, H., Jain, K., et al; (2019) "Role of Platelet Mitochondria: Life in a Nucleus-Free Zone. Front. Cardiovasc". *Med*.; 6, pp153.

10. Bernardi P, Scorrano L, Colonna R, et al. (1999) "Mitochondria and cell death. Mechanistic aspects and methodological issues". *Eur J Biochem*.; (264), pp687–701.

11. Kroemer G, Reed JC. (2000) "Mitochondrial control of cell death". *Nat Med*.; (6), pp513–519.

12. Danial NN, Korsmeyer SJ.(2004) "Cell death: critical control points". *Cell*. (116), pp205–219.

13. Green DR, Kroemer G. (2005) "Pharmacological manipulation of cell death: clinical applications in sight?" *J Clin Invest*.; (115), pp2610–2617.

14. Kroemer G, Galluzzi L, Brenner C. (2007) "Mitochondrial membrane permeabilization in cell death". Physiol Rev.; (87), pp99–163.