IBBJ
Spring 2016, Vol 2, No 2

Short Communication

Potatoes: Source of Laboratory Reagent for Human Platelets Research

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Submitted 13 Sep 2016; Accepted 07 Oct 2016; Published 26 Nov 2016

Irish potatoes is rich in carbohydrate, with varied glucose levels. It is also a very good source of apyrase, an enzyme used for platelet storage. The aim of this study was to explore Irish potatoes as an easily available source for apyrase extraction and purification. Apyrase was extracted from 5 Kg of Irish potatoes. Platelets were derived from 5-10 ml of human whole blood and G6PD activity was assessed in platelets stored for 48 h either in presence or absence of apyrase (commercial or extracted from Irish potatoes) using fluorescent spot test and electrophoresis. Platelets stored in apyrase showed G6PD activity under both fluorescent spot test and electrophoresis method, whereas those stored without apyrase enzyme for such a time did not show G6PD activity. Therefore, potatoes could be used as a valuable source for preparing apyrase and allowing platelet storage for long duration for *in vitro* studies or transfusion purposes, especially in bleeding disorders which will be complicated without adequate conservative medium, due particularly to the short half life of this blood cell.

Keywords: Potatoes, platelets, apyrase, G6PD

Potatoes are both tropical and temperate food crop but highly grown in the temperate regions. In Nigeria, it is mostly available in the northern parts of the country, particularly in Jos Plateau confirming the cold climatic preference of this food item. There are at least two varieties of the potatoes species, the pinkish coated type and the light brown. The former, is sweet whereas the light brown coated has no sweetness and such biochemical characteristics lies the health relevance of potatoes (1). Perhaps this is the reason of the high rate of consumption of the popular light brown coated potatoes which is also small in size, and called the Irish potatoes. Of course not only consumed by the Irish people, but by the whole world. It is rich in carbohydrate, with varied glucose levels (2-4). Apart from the nutritional values of Irish potatoes. It is also a very good source of biochemical substance, a protein (enzyme) called apyrase used for platelet storage and as washing medium for physiologic functioning of this blood cell *in vitro* (5).

Platelet is an important blood cell to study due mainly to its small size, complicated features but very responsive in its functional state to bleeding situation though, the mechanism of platelet activation during injury is not fully understood, but

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its functions are fully understood in adhesion, and aggregation potentiated by ADP, cAMP, calcium ions and thromboxane but regulated by its receptors (6), for final arrest of bleeding (7-8). However, platelet alone cannot arrest bleeding during tissue damage without interplay of the coagulation system. But even with this system, platelet is very paramount in ensuring perfect hemostasis and in clinical management of bleeding (9). There is normally a correlation between bleeding time and platelet count. Bleeding will be less with adequate platelet count and increases with low platelet count or structural defects (10). Thus, platelet is a very relevant cell in understanding haemostatic mechanics. Such relevance led to the search for a non defective physiologic medium for the *in vitro* study of this blood cell also in a physiologic state. Platelet has a short half-life outside the body and without adequate medium to keep it in a normal state, *in vitro* platelet studies would be difficult. This necessitates sourcing for locally available raw material for the preservation of platelet in apyrase enzyme from potatoes. The study was also for the preservation of an important metabolic enzyme, Glucose 6-phosphate dehydrogenase (G6PD). G6PD is an enzyme that is present in virtually all cells. It catalyses the first and rate limiting step of the hexose monophosphate pathway which generates nicotinamide adenine dinucleotide phosphate (NADPH). Lipid production in platelets depends on NADPH though the platelets have the capacity for *de novo* synthesis of lipids as a result of their content of acetyl CoA, (11). For example, an important lipid with coagulant activity called platelet factor three (PF3a) depends on NADPH for its production showing the relevance of G6PD enzyme in the biochemical activities of platelet (12). In G6PD deficient platelets where NADPH generation is impaired, haemostatic disorder(s) from impaired PF3a may result (13). The *in vitro* study of platelet biochemical activities in the presence of apyrase from locally available potatoes will enhance the possibility of investigating this important blood cell outside the body. The present study demonstrates the research potentials in sourcing for materials in our environment such which compete qualitatively with the imported apyrase.

Materials and methods

Extraction and purification of apyrase

The method of Molnar was used for apyrase extraction (5). Briefly, 5 kg of Irish potatoes were homogenized in a blender after peeling the coats, and washed for 10 min with $^{1}/_{10}$ volume of distilled H_2O (500 ml). It was stirred for 20 min and pressed through cheese cloth. The homogenate was then centrifuged at 450 g at room temperature for 10 min in order to remove the sedimenting starch. All consecutive steps were carried out at 0-10 ^{o}C. 520 ml of the extract was brought to 0.024 M $CaCl_2$ by addition of appropriate volume of 1M $CaCl_2$ solution. The mixture was stirred for 10 min and any precipitate of calcium phosphate was allowed to settle for 1 h. A dark color formed was decanted and the sediment was centrifuged for 20 min at 1000 g. It was resuspended in 1 M $CaCl_2$ ($^{1}/_{10}$ volume) of the original extracting volume (520 ml) and was stirred for 1 h. The procedure resulted in the elution of the apyrase enzyme from the calcium chloride precipitate and was centrifuged at 1000 g for 20 min. The supernatant (40 ml) was dialyzed against 0.1 M kCl for 20 h, an aspect of purification. A small amount of precipitate which formed after dialysis was removed by further centrifugation. 30 ml of the enzyme was added to tyrode solution and used for platelet washing and suspension.

Blood collection

5 – 10 ml of venous blood was collected from human subjects aged between 15-40 years.

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Extraction of platelets from human whole blood

10 ml whole blood was centrifuged at 120 g for 10 min, to obtain platelet rich plasma (PRP). The PRP was further centrifuged at 6000 g for 30 min to obtain pure platelets.

G6PD activity assessment in platelets

Both platelets stored in apyrase and those without apyrase were lysed using triton X-100. The lysing resulted in the elution of G6PD enzyme examined in the study.

Fluorescence spot test and electrophoresis were the two tests carried out to confirm the presence of G6PD enzyme.

For electrophoresis, 250 ml of Tris buffer pH 8.6 was measured into each compartment of the tank. The shoulder piece was adjusted to obtain a gap of 5 cm. Filter paper pads were used as wicks. They were impregnated with the buffer solution and placed over the Perspex-shoulder pieces with one edge immersed in the cathodic and anodic compartments of the buffer solution. The titan III paper was soaked for 10 min in the Tris buffer and set firmly in place using tension rod for maximum contact. It was set at a voltage of 260 V and 1 mA current per cm of the strip. The lysates of platelets and red blood cells as control were applied on the cellulose acetate strip using multiple applicators that deliver appropriately 2µl aliquot of each sample and electrophoresis was carried out for 20 min. The lysed samples were applied on titan paper III and after electrophoresis, migration of the platelet G6PD was observed with red cell G6PD as control, indicating the presence of the enzyme in platelets.

For fluorescence spot test, 10 ml of blood was added to 100 ml of G6PD screening mixture in a test tube. A drop of this mixture was added on a filter paper using pasteur pipette and observed under UV light for fluorescence. For platelet G6PD assessment, a preparation similar to that of red blood cells was done.

The G6PD screening mixture was prepared by adding 2 ml of a 10 mM Glucose 6-phosphate sodium salt solution (obtained by the dissolution of 305 mg of disodium salt or an equivalent amount of the potassium salt in 100 ml water) to 1 ml NADP disodium salt (60 mg of NADP and 60 mg of dissodium salt were dissolved in 10 ml of water), 2 ml saponin 750 mM, 30 ml Tris Hcl 200 mM pH 7.8, 1 ml of 0.49% reduced glutathione (GSSG), and 2 ml H2O.

Results

Platelets stored for up to 48 h in apyrase extracted from Irish potatoes showed G6PD activity under fluorescent spot test and electrophoresis, whereas those stored without apyrase enzyme for such a time did not show G6PD activity. Also there was no difference in activity with the commercially available apyrase and the locally extracted one as tested by the activity of the G6PD enzyme in the platelets.

Discussion

In the present study, the extracted apyrase from potatoes proved its potency by maintaining the physiologic activity of the platelets *in vitro* as demonstrated by the presence of the G6PD enzyme in a functional state. However, platelets without apyrase had no G6PD activity. The report is in keeping with platelet G6PD kinetics in apyrase (14). The difference with this report is the quantitative and qualitative methods (spot test) and electrophoresis, applied in platelets G6PD activity studies and comparative potency of apyrase enzyme.

Platelet storage in long duration for transfusion especially in bleeding disorders are with complications without adequate medium particularly with the short half life of this blood cell.

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In the present study, platelets were stored for 48 h with intact physiologic activity in apyrase as compared with imported apyrase. Imported apyrase is expensive and it takes a long time to arrive when ordered from developing countries.

Irish potatoes are highly available and in large quantities in our environment and will form a good source of foreign exchange earning if exploited at the manufacturing level. Moreover, apyrase could be easily available in local health institutions particularly the tertiary ones if this chemical is produced locally. Such availability will enhance health care delivery and promote cellular researches in bleeding diathesis.

Conflict of interest

The authors declared no conflict of interest.

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