Effect of Coffee Beans Towards Conversion of Blood Group B to Blood Group O

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Abstract- ***ABO and RhD variants are the most studied blood groups . ABO incompatible blood transfusions are one of the leading causes of harmful and even fatal transfusion reactions. To prevent this, a universally transfusable blood group has to be created. Progressions are being made towards the modification of blood group B to blood group O. This can be done by removing the antigens from the RBC surface and converting it to universal blood group and this idea was pioneered by Goldstein and colleagues at the New York Blood Center in the early 1980s. The conversion of group B RBCs to O was carried out using α-galactosidase extracted from coffee beans especially Coffea arabica and Coffea canephora. However, because this enzyme ha poor kinetic properties and low pH optimum, the process was not economically viable but clinical trials showed positive results. The ECO-RBC product can be characterized by various methods like blood group serology, SDS PAGE Western blot, flow cytometry, ELISA etc. Presently, safety and efficacy of ECO RBCs are evaluated by performing more clinical trials.***

Indexed Terms- ***Transfusion reactions,α-galactosidase, Coffea arabica, Coffea canephora, ECO RBC***

I. INTRODUCTION

Coffee bean extracts has been shown to have antioxidant, anti-obesity and hepatoprotective activity. And coffee bean extracts showed stimulatory effect on the immune system. Globally, blood is an essential part of modern healthcare. Every 2 seconds someone in the US requires blood. Approximately, 29,000 units of red blood cell is required everyday in US. And in the United States alone,5 million patients receive approximately 14 million units of red blood cells donated each year according to the estimation given by American Association of Blood Banks and National Blood Collection and Utilization survey. Green coffee beans has shown to reduce blood pressure and body compostion by influencing 11-β HSD1 Enzyme in healthy individuals i.e. weight loss. Numerous patients with the need of blood are facing challenges in blood supply. Blood is being discarded because of infectious disease testing continues creating a problem. A shortage of human blood of all types persists and American Red Cross warned that without sufficient blood donations some surgeries need to be delayed. Shortages are deteriorating because of shrinkage in donor base. Even if the 60% of population is eligible for donation, but normally <5% of the population donates the blood annually to meet the needs of the whole population(American Red Cross, America's Blood Centre , Ad Council and American Association of Blood Bank).

Once the donation has been made, the blood centres and transfusion services are responsible for providing the safest possible unit for the patients requiring transfusions because ABO incompatible blood remains one of the leading causes of harmful and even fatal transfusion reactions reported.

One possible solution for this would be to create universally transfusable RBCs either by covering or eliminating the antigens present on the surface of the RBCs. For this purpose, Polyethylene Glycol(PEG) is used which hides the antigenic structures by covalent binding to the cell membrane has been done to make stealth RBCs. However, reduced RBCs survival has been reported when tested in a rabbit model and production of anti-PEG antibodies were seen (Garratty,2004). The alternative approach was to use

an enzymatic process to eliminate the antigens and convert all non-O blood to universally accepted blood O.

II. THE ABO BLOOD GROUP SYSTEM

Currently, there are 44 blood group systems present with 354 red cell antigens and clinically the most important one of which is still the ABO system discovered by the Karl Landsteiner in 1900. His extensive research on serology lead to the identification of major blood groups-A,B,O,AB, compatibility testing and later transfusion safety and its practices. Later on he was awarded with Nobel Prize in 1930. Dr. Jack Goldstein used the glycolytic enzymes to alter the RBC surface for transfusion purposes. Chains of sugars carried by the glycoproteins and glycolipids present on the cell surfaces, all have same basic sequence of terminal α-1-2 linked fucose linked to the penultimate galactose called as H-antigen. The major distinction between the human blood groups lies with the presence of additional α-1-3 monosaccharide that branches off from the penultimate galactose. In case of A cells , this sugar is α-1-3 linked N-acetylgalactosamine(GalNAc). In case of B cells, this sugar is α-1-3 linked galactose(Gal). Group O cells lack either of the monosaccharides at the terminal, while AB cells bear a mix of A and B chains.

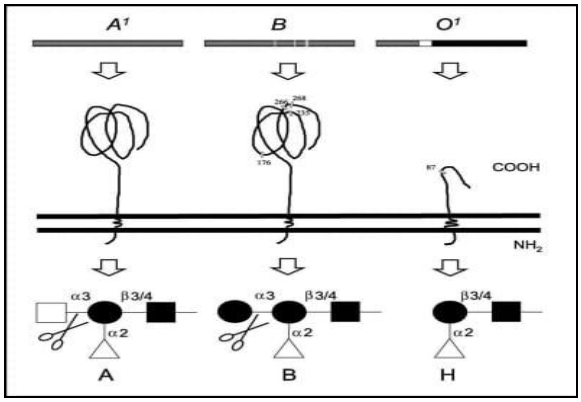

Figure: Illustrating the single carbohydrate difference between blood groups A, B and O.

III. SELECTION OF ENZYMES FOR CONVERSION

Previous methods used for selection, identification and characterization of exoglycosides are relied on the use of simple monosaccharide derivatives as substrate to identify the saccharide and potential linkage specificity. While the substrate provide easy, fast and inexpensive tools to identify glycosidase activities, but may not reflect kinetic properties. Earlier,α- galactosidases and α-N-acetylgalactosaminidases used for stripping off A and B antigens from RBC have been mainly identified and characterized by pNP monosaccharide derivatives. All α-galactosidase and α-N-acetyl galactosaminidase enzymes used in the past studies to attempt removal of A and B antigens on cells are related, as evidenced by significant DNA and amino acid sequence similarities.

IV. CONVERSION OF BLOOD GROUP B TO BLOOD GROUP O

For conversion of blood group B to O and produce "universal blood", 100ml of group B RBC's are subjected to 50ml purified and recombinant coffee bean α-galactosidase in sodium phosphate/citrate/chloride buffer at 26°C for 2 hours. The remaining α-galactosidase was removed by washing with 0.9% NaCl solution. The specific activity of coffee bean α-galactosidase was reported to be 32U/ml using para –nitrophenyl(pNP) α-D-Gal as substrate. Enzymatic conversions are done at pH 5.5-5.6 with approximately 6mg/ml at 80-90% hematocrit. The resulting converted O cells seemed to functioned normally in transfusion experiments and no significant adverse reactions were seen. The experiments were performed in gibbons because of similarity in blood group of gibbons with ABO blood system of humans. After this, studies on humans were conducted, the first one was conducted in 1982. Clinical phase 1 trials were conducted using B-ECO RBC's given to healthy group A and group O volunteers. The trials moved from small infusions to single RBC unit and later multiple and repeated full unit transfusions. The results showed that the transfused group B-ECO RBC's survived well in the circulation of the recipients irrespective of their blood groups. At the end of clinical phase 1 trials, the investigators concluded that ECO RBCs are effective. The next step Phase 2 clinical trials conducted in 2000. 21 patients underwent transfusion with full units of B-ECO RBCs. 18 of them were also given

control transfusions with blood group identical untreated RBCs. The hemoglobin increments were comparable between ECO RBCs and control transfusions. An increase in anti-B titre was noted in five of the patients. While the serum samples from random groups A, B and AB individuals were non-reactive with ECO RBCs.

V. PRESERVATION OF ENZYMATICALLY CONVERTED O RBCs

To determine the validity period of α-galactosidase converted red blood cells, the ECO RBCs were stored in monoammoniumphosphate(MAP) nutrient solution at 4°C. The ECO RBCs were analyzed every week. The ECO RBCs were analyzed for their appearance whether there were any clot, hemolysis, any bubble formation, presence of supernatant clear fluid and container integrity and for their volume, specific volume, pH , α-galactosidase residual, presence of bacteria or any endotoxins etc.

VI. CHARACTERIZATION OF ENZYMATICALLY CONVERTED BLOOD GROUP O

After the conversion of blood group B to universally accepted blood group O, the ECO was re-evaluated. More than 30 parameters are tested and buffers, additives, concentrations, incubation conditions including time and temperature are optimized and checked.

The ECO RBC product can be characterized by various independent method which includes blood group serology, functional assays, ECO RBCs identification by flow cytometry, ECO RBCs volume by density formula, hematocrit, blood group by direct agglutination and indirect antiglobin test, residual amount of α-galactosidase by ELISA , bacterial contamination by automated blood culture system, SDS PAGE western blot(for assessment of glycoproteins) and thin layer chromatography and mass spectroscopy (for assessment of glycolipids). ECO RBCs behave similarly to normal RBCs except for their blood group serological properties, which are changed. They type as blood group O in all assays.

Non-ABO blood groups tested remained the same as measured by agglutination or flow cytometry.

A large panel of monoclonal blood grouping reagents was screened to monitor and optimize the ECO process. The reagents with the highest sensitivity were selected.

The chosen murine monoclonal antibody (ES-15) can detect weak A antigens on group B RBCs and has lower A antigen site density on A-ECO RBCs compared to subgroup A x RBCs and group B RBCs. The ECO process involves converting A, B, or AB red cells to group O using enzymes in an automated device.

Gel column agglutination technology is used to perform blood grouping of ECO RBCs, and the results are comparable to native RBCs.

The gel technology is based on size exclusion, where a positive reaction leaves agglutinated RBCs on top of the gel column.

CONCLUSION

Now, it is practically possible to convert the blood group B to universal blood group O by the use of coffee beans , the proofs for which are established by successful clinical trials. However, further work has to done to make the process even more efficient because a large volume of coffee beans and high temperature are required to strip off the antigens from the red blood cells . And coffee beans are not so proficient in stripping off ‘A’ antigens because of the presence of different subtypes of A antigen.

To solve the problem , some scientists are working and they are creating an enzyme through a process known as “Directed evolution”. This is a method of protein engineering that is based on natural selection and allows to evolve a protein usually enzyme. Starting with an original enzyme, mutations are inserted into gene that codes for it. For this process efficient mutants are selected that are most suitable in removing antigens and the process is repeated again and again. The scientists believe that this process can yield an enzyme that is 170 times most effective. This would have an enormous impact on transfusion

medicine which can solve perennial blood bank problems and prevent fatal transfusion reactions.

Another problem associated with conversion process is that the enzyme is able to remove vast majority of antigens but not all the antigens. As our immune system is incredibly sensitive to blood groups, even presence of small amount of residual antigens can trigger an immune response. So utmost care has to be taken when converting the blood group and even during the transfusion. So, providing transfusion services to the blood banks while maintaining viable blood inventories and proper conditions for conversion process has to be the main goal.

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