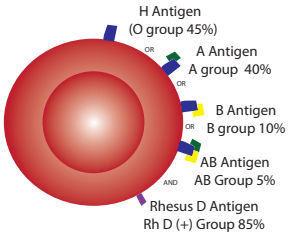
HAEMATOLOGY PHARMACOLOGY 1

Major blood groups and cross-matching

Over 400 red cell antigens have been described, the majority of which have minimal clinical significance because the antigens are weak and only develop after multiple exposures. The most important red cell antigens are ABO and Rh. ABO blood groups are determined by an three allelic genes, A, B and O genes. The A and B genes control the addition of specific carbohydrates to the terminal end of a common H antigen (A antigen corresponds to N-acetylgalactosamine and B antigen corresponds to D-galactose, O antigen is just the common H without addition). Blood is thus classified as O (45%), A (40%), B (10%) or AB (5%) according to whether none, one or both antigens are expressed. The A antigen can be further subtyped as A2 (plain A) or A1 (A and A1) and other rare groups. People normally express IgG and IgM against the AB antigens not expressed on their own red cells. Thus if blood expressing an antigen against which a high IgM titre is present is transfused, rapid agglutination of the infused red cells occurs with activation of complement and rapid haemolysis: a major transfusion reaction. This leads to circulatory collapse and renal failure. In the presence of a lower titre of IgM or IgG, agglutination and haemolysis occurs more slowly. Prior to any matched transfusion, donor and recipient blood are mixed in vitro and checked for agglutination. Rhesus antigens are expressed on all red cells. They are of three classes: C, D, and E and each antigen is expressed as one of two types (C or c, D or d etc.) The C and E antigens are not strongly antigenic. The D antigen is most antigenic so RhD is described as "Rhesus positive" and Rhd as "Rhesus negative". RhD has an 85% prevalence in Caucasians and higher in african descent. Anti-D IgG is usually only formed in Rh negative people in response to exposure to Rh positive blood. This can occur through unmatched transfusion or more commonly through carriage of an Rh positive foetus with foetal-maternal haemorrhage at delivery or earlier. Sensitization to D antigen results in expression of anti-D IgG in the mother. As IgG is transferred across the placenta, this results in haemolysis in any subsequent Rh positive foetus, called Erythroblastosis foetalis. This can be prevented by the administration of anti- D antibody at the time of likely foetal-maternal haemorrhage to remove any Rh positive blood from the mother's circulation before antibodies are expressed.

Testing Process

- ABO and Rh Recipient
- ABO and Rh Donor
- Antibody screen Recipient
- Crossmatch Recipient and Donor to detect incompatability and significant Ab



Transfusion types

Whole Blood is prepared from a single donation and consists of 450ml of blood and 63ml of anticoagulant (usually citrate). The hematocrit varies between 0.35 and 0.45. It contains no functional platelets, and levels of clotting factors V and VIII are 20% of normal. Albumin and other clotting factors are normal. Whole blood transfusion is indicated for replacement of blood loss where clotting factors are required. In general, this means haemorrhage exceeding 50% of the patients blood volume.

Packed Red Blood Cells are prepared from a single donor and consist of 220ml of packed cells and 80ml of plasma. The haematocrit is between 0.65 and 0.75. This product is used for red cell replacement in anaemia. In Australia most PRBC are in the leucocyte depleted form with most of the platelets and leucocytes removed.

Platelets are produced either as single or pooled donor (4-6) units. Each donation consists of 40-70ml of plasma with platelet equivalent to 5.5×10^{10} per L platelets. The ideal platelet transfusion should be ABO and Rhesus compatable, especially in young women. A transfusion of 5-6 units of platelets leads to an increment of between 20-40 in the platelet count.

Fresh frozen plasma is produced as a single donor product. Units of FFP consist of 250-500mL of separated plasma frozen at -20 degrees C. It includes both labile and stable factors, albumin, gamma globulin, fibrongen, and factor VIII. FFP can transmit hepatitis viruses and HIV, virological safety depends on screening of donors. Transfused units of FFP should be ABO compatible. The dose is usually 2 to 5 units depending on the INR.

Cryoprecipitate is collected in packs containing between one and six single donor units. It is collected by harvesting the precipitate that forms during slow thawing of frozen plasma. The resulting precipitate is suspended in 10-20ml of plasma. Each unit contains 150mg of fibrinogen, 150 international units of Factor VIII, and fibronectin. Between 6 and 18 units are required from haemophilia, DIC and fibrinolytic states to raise the plasma fibrinogen level by 1g/L.

Albumin is produced by fractionation of a large plasma pool followed by pasteurisation at 60 degrees for over ten hours, which is though to complete viral inactivation, although it may not inactivate prion protiens. Albumin is prepared in 5 and 20% solutions, and is inidicated in shocked states, hypoproteinaemia and liver disease.

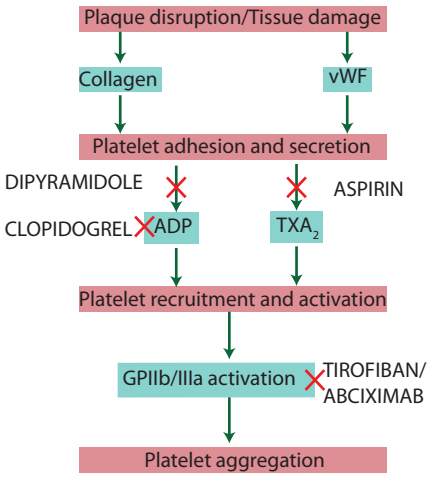
Factor concentrates are available in various forms including freeze dried separated factors VIII and IX (250 IU/vial) and both pure and impure forms of factors VII, IX and XI. There has been a shift to creating human recombinant factor VII following issues with viral transmission associated with the HIV epidemic.

Blood transfusion

At the time of collection, a blood donation is a normal venous sample. In the production of packed cells, most of the plasma and plasma proteins are removed and the remaining cells and fluid are sealed in a sterile plastic bag in an isotonic solution and stored at 1-6°C. This is an anaerobic environment in which red cell metabolism continues, though at a greatly reduced rate due to the low temperature. Hypoxia results in progressive depletion of ATP from red cells and consequent failure of the Na^+/K^+-ATPase pump, resulting in unopposed leakage of K+ from the cells. Red cells have a mean lifetime of 120 days, so a small proportion of the cells will lyse within the storage period. Almost all the white cells and platelets remaining within the unit will lyse within a few days. The gas and electrolyte changes in packed cells are usually rapidly compensated for on transfusion. Respiratory compensation occurs for the high PCO2 and low PO2. Citrate and lactate enter the TCAC, releasing Ca2+. The red cell Na^+/K^+-ATPase pump restarts and sequests much of the K+ load. Erythrocytes can also be frozen. This requires preparation in glycerol and storage at –65°C for up to 3 years. There is little change in frozen preparations from the time of freezing and near-normal function on thawing and deglycerolization. The cost of this sort of storage restricts its use to rare blood types. Problems associated with massive blood transfusion defined as replacement of blood volume in under 24 hours problems with any blood transfusion: acute and delayed reactions volume difficulty maintaining normal volume status (monitor CVP, PCWP, UO) hypothermia from infusion below body temperature (blood warmer, monitor T) dilution of any drugs in the circulating compartment electrolytes CO2 and acid load causes transient acidosis K^+ load may cause hyperkalaemia, arrhythmia (monitor ECG, gases) citrate load requires hepatic metabolism to avoid hypocalcaemia citrate is metabolized to pyruvate and HCO3 -, raising pH a little red. cells poor oxygen carrying capacity on infusion due to 2,3,DPG depletion (high FiO2, monitor SaO2) free haemoglobin from lysed cells may cause renal failure iron load clotting dilution and consumption of platelets and clotting factors results in coagulopathy treat with FFP for clotting factors and platelets other increased risk of blood-borne virus infection alloimmunization (minor RBC antigen or HLA) haemolytic or febrile reactions adenine metabolites may cause renal impairment (>60 units required)

Antiplatelet drugs

Aspirin is a salicylic acid derivative which causes irreversible non selective cycloxygenase inhibition. It is used to reduce the risk of unstable angina progressing to acute MI, and the risk of stoke. Aspirin acts by acetylating cycloxygenase and as a result platelets produce less thromboxane. The effect occurs at relatively low doses. It is rapidly absorbed with a bioavailability of 50-75%, Normal antiplatelet dose is 100mg per day. It is up to 85% protien bound and is a weak acid, therefore exists mostly in non ionised form, (which explains the rapid absorption). It is metabolised quickly by non hepatic esterases (<20mins) to salicylic acid which is active, salicylic acid is metabolised in the liver in saturable processes, leading to variable half life depending on dose. The side effect profile is due to the non selective cox inhibition. Dypyramidole acts as a phosphodiesterase inhibitor which increases platelet cAMP and inhibits platelet aggregation. It is used for the prevention of thromboembolism in patients with artificial heart valves and in the prevention of stroke (combined with aspirin). Other uses include cardiac stress testing (coronary vasodilation). The inhibition of phosphodiesterase also leads to vasodilation of arteries via actions on smooth muscle and for this reason it is used with caution in patients with aortic stenosis and recent MI. It does not affect the COX pathways. It has variable absorption, is highly protien bound, the volume of distribution is 2-3 L/kg, metabolised hepatically and has a half life 10-12 hours. It is excreted in the faeces as gluconoride conjugates. The main issues in terms of side effects are related to its vasodilatory effects and the risk of excessive bleeding from decreased platelet function. Clopidogrel a thienopyridine derivative which acts as a non competitive antagonist of platelet surface ADP, which is responsible for platelet aggregation. It is presented in oral form with tablets 75mg and its trade name is Plavix. Clopidogrel irreversibly prevent ADP from binding to its receptor on the platelet surface, thereby preventing the glycoprotien IIb/IIIa receptor transforming into its active form. It is well absorbed orally and has an onset following a loading dose of 300mg within 2 hours. Peak effect often occurs after five days administration. It extensively protien bound, and is metabolised hepatically via hydrolysis to active and inactive metabolites with excretion equally in the urine and faeces. Commonly causes GI irritation. Issues with haemorrhage. Idiosyncratically causes neutropaenia. Tirofiban binds to the glycoprotien IIb/IIIa receptor on the surface of platelets. It has a similar action to the monoclonal antibody abciximab. It is used in patients with non stable angina and NSTEMI in high risk patients. Its trade name is aggrastat. Tirofiban and abciximab act by inhibiting the platelet glycoprotien IIb/IIIa receptor and as such they block the final common pathway of platelet aggregation. They do not block platelet adhesion, secretion of platelet products, inflammatory effects or thrombin activation. Usually delivered as a constant infusion following a loading dose. Requires dose adjustment in renal failure with creatinine clearance <30ml/min, in contrast abciximab in not renally cleared. Side effects relate to haemorrhage, although spontaneous haemorrhage is uncommon. Reversal is problematic and platelet infusion is recommended if there is uncontrolled bleeding.



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