

Artificial blood: going with the flow

Artificial blood has been 'on the horizon' for several decades without fully materialising. However, recent progress in the field may mean that the holy grail of emergency medicine really is just around the corner, as Chris Bird explains

On 7 April, the *New Scientist* carried a news item on modified blood that can be given to patients of any blood group. Then, on 10 May, scientists at the University of Sheffield released details of 'plastic' blood that can be stored as a thick paste. Samples will be on display at a Science Museum exhibition until January 2009. Subsequently, 23 May saw Northfield Laboratories report results of a phase III trauma study that looked at the use of a haemoglobin-based red cell substitute in 720 patients. These three stories encapsulate the different approaches to the development of artificial blood that can be stored, preferably without refrigeration, and then transfused to patients, irrespective of their blood group.

Military conflict has often highlighted the need for blood transfusion in situations where the use of human blood is just not practical. The Vietnam war in the 1960s was the spur to much research in this area. Further research in the 1980s and 1990s resulted in products that caused serious side effects such as heart attack and stroke. Human studies ended and some of the companies involved went bankrupt. Now, the conflicts in Afghanistan and Iraq have provided fresh impetus in this field of research and new products are undergoing trials.

Of course, civilian injuries, particularly those involving traumatic brain damage, also can be treated beneficially by the rapid restoration of an oxygen supply to tissues that are particularly susceptible to the effects of hypoxia. Some artificial bloods have an oxygen carrying capacity 50 times that of blood, and many blood substitutes often are termed oxygen therapeutics for this reason. In addition, there is a change of emphasis away from viewing these new products as simple

replacements for blood towards products with particular value in certain clinical situations or where conventional transfusions are impractical. The three main categories of alternatives to blood are substitutes made from animal or human haemoglobin, the perfluorocarbons and red blood cells that have been treated to remove their antigens.

HAEMOGLOBIN SUBSTITUTES

Blood transfusion has a history that goes back well over a hundred years. Initially, with no understanding of blood group differences and the possible reactions between donor cells and patient antibodies, transfusion sometimes

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proved lethal. Of course, these problems were overcome by the early pioneers in transfusion science. As a means of overcoming some of the dangers associated with blood transfusion, clinicians sometimes resorted to the infusion of stroma-free haemoglobin solutions; however, acute renal failure often was the result of this approach. Now, therapeutic solutions of haemoglobin are available. PolyHeme (Northfield Laboratories) is a chemically modified human haemoglobin with a shelf-life of 12 months. It does not require crossmatching and therefore can be used outside the hospital setting.

In a phase III study, patients in



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Haemoglobin extracted from out-of-date blood donations is being tested as an alternative to conventional transfusion, and may have a role outside the hospital setting.

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haemorrhagic shock following traumatic injury were treated either with PolyHeme at the scene and for up to 12 hours thereafter or they received saline at the scene followed by blood on arrival at hospital. The results from 720 patients were published in May. Mortality at day 1 and at day 30 were the endpoints used and, according to Northfield, the data showed no statistically significant difference between patients treated with PolyHeme and those in the control group. However, adverse events were more frequent in the PolyHeme group, and these included three patients who died from adverse cardiac events compared to just one in the control group.

Despite these findings, Northfield continues to believe that there is potential benefit to the use of PolyHeme in patients with delayed access to blood, whose expected mortality without oxygen-carrying replacement would be considerably greater.

PERFLUOROCARBONS: MADE IN MANHATTAN

Perfluorocarbons, chemicals related to the non-stick Teflon used in cooking pans, were discovered in the 1940s as a by-product of the Manhattan Project, the American wartime effort that created the atomic bomb. Scientists noticed that perfluorocarbon solutions were able to dissolve large quantities of oxygen, which could be as much as 100 times the amount dissolved in plasma. These chemicals are stable but hydrophobic; therefore, an emulsifying agent is necessary to produce solutions. Early trials in Japan proved disappointing as there were significant side-effects and little clinical benefit. The US Food and Drug Administration (FDA) rescinded the limited approval it had granted in 1993 and the product, Fluosol, was withdrawn in 1994.

The new generation of 'plastic' blood includes products such as PFC Oxycyte (Synthetic Blood International). The small plastic molecules join together in a tree-like branching structure with an iron atom at their core. Perfluorocarbon emulsions (PFCEs) are composed of liquid perfluorocarbons emulsified in water and salt. Due to the PFCE's inability to remain mixed with aqueous solutions, they must be prepared as emulsions before being used in patients. The PFCE particles are spherical, averaging about 2 µm in diameter, with a perfluorocarbon core and a thin egg-yolk phospholipid surfactant as a coating.

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The size and shape of the molecule is similar to haemoglobin, but it has a far greater oxygen carrying capacity. Furthermore, molecules such as Oxycyte can carry oxygen through damaged blood vessels that are too narrow to allow red blood cells to pass. Oxycyte is used with supplemental oxygen and patients are given 50–100% oxygen before and after its infusion. Under these conditions, Oxycyte has an oxygen carrying capacity 50 times that of blood and has undergone trials in the USA in patients with traumatic brain injury and other conditions.

Another PFCE, Oxygent, is manufactured by Alliance Pharmaceutical and Baxter Healthcare, a well-known company in the transfusion field. It is intended to reduce the need for allogeneic blood transfusion during surgery and at other times of acute oxygen deficit, and has been evaluated in 20 studies involving approximately 1500 patients.

A phase III general surgery study that included 492 patients at 34 centres in eight European countries demonstrated that the product significantly reduced the need for donor blood in the total study population, and a greater percentage of Oxygent-treated patients did not need a blood transfusion compared to those in the control group. Oxygent has a shelf-life of about two years.

Of course, haemoglobin carried in red cells works so well because of its changing affinity for oxygen and therefore its capacity to bind to oxygen in the lungs and then release it in the tissues. The oxygen affinity of haemoglobin is altered by pH, by the partial pressure of carbon dioxide, by temperature and by the enzyme 2,3-diphosphoglycerate. Shifts in the oxygen dissociation curve provide a natural regulatory mechanism for oxygen delivery. Both haemoglobin- and perfluorocarbon-based artificial bloods have been chemically altered to mimic this capacity in some respects.

ANTIGEN REMOVAL

It is extremely unlikely that artificial blood will ever replace donor blood, but can the need for selection of compatible blood and crossmatching be reduced or eliminated?

Removing the A and B antigens from red cells, thereby turning all blood into group O, represents the first step towards simplifying the transfusion process. In the 1980s scientists in New York used an enzyme from green coffee

beans to remove the B antigen, but the process proved too inefficient to be of value.

Now, researchers in Copenhagen are using bacterial enzymes to remove the A and B antigens. An enzyme obtained from the intestinal microorganism *Bacteroides fragilis* removes the B antigen, while an enzyme from a bacteria that sometimes causes opportunistic infections in humans, *Elizabethkingia meningosepticum*, removes the A antigen. The purified enzymes are highly efficient, with the enzyme from *B. fragilis* persisting a thousand times longer than the enzyme from green coffee beans. The team from Copenhagen is working with US company Zymequest to develop a product for clinical trial.

HAEMOSTATIC CONSIDERATIONS

However, blood has important functions other than to carry oxygen. Platelets are vital to the haemostatic process and several approaches have been undertaken to develop alternatives to transfused human platelets. For example, autologous red blood cells can be coated with fibrinogen or with just the multiple arginine-glycine-aspartine fragments bound to the surface. These have been called thromboerythrocytes and seem to be haemostatically effective in some animal models of thrombocytopenia.

Fibrinogen has also been used to coat microspheres or microcapsules of the human protein albumin, and several products have been evaluated in preclinical trials.

In addition, two liposome-based agents, plateletsomes and factor Xa with phospholipid vesicles, have been studied. Plateletsomes are lipid vesicles with platelet glycoproteins on their surface. Both have been shown to be effective *in vitro* and in some animal models, but the latter approach is associated with high toxicity.

SUPPLEMENTARY ROLE

Are hospital transfusion scientists likely to be out of a job in the near future? Certainly not. Human blood and blood products, donated freely by volunteers, are likely to remain the most appropriate product in most situations, and will also be the most cost-effective.

Recombinant blood products are already in use and it seems likely that they will be joined by oxygen therapeutics in the near future. However, these products are likely to be used only in certain procedures, or to supplement human blood products.

A trawl through the websites of companies researching artificial blood reveals a large number that have filed for bankruptcy, ceased to develop that product, or just disappeared. Thus, the field is fraught with difficulties, and clearly blood bank staff will be around for a few years to come. ■

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