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### Oxygen carriers – where do we stand?

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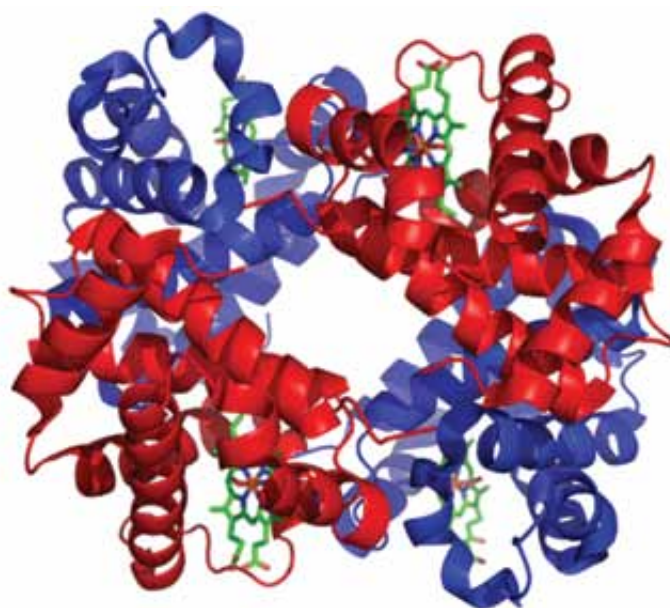
This refresher lecture will cover most of the background physiology on oxygen transportation as well as current opinions on all the available oxygen carriers.

#### Background

When blood is lost during trauma or surgery it can be replaced to a degree by crystalloids and colloids. At some point, however, it is necessary to start replacing lost blood by allogenic blood and plasma, otherwise organs cannot preserve their normal function. Blood transfusion is effective but not without significant risks. It can cause fatal haemolytic reactions, transmit blood borne infectious agents and may compromise overall immune function. It is also a scarce, short-lived and expensive commodity. Due to the risk of incompatibility problems there is a need for typing and crossmatching.

Human haemoglobin is a 64 kDa tetrameric protein with two  $\alpha$  and two  $\beta$ -globin polypeptide chains folded in a quaternary structure ( $\alpha_2 \beta_2$ ) [1] (Figure 1). Each  $\alpha$  or  $\beta$  subunit contains one iron-haem group that reversibly binds one oxygen molecule. When fully saturated with oxygen one haemoglobin molecule carries four oxygen molecules. When it reaches the tissues oxygen is offloaded, the process being facilitated by 2,3 disphosphoglycerate (DPG), an allosteric effector, situated between the two  $\beta$  subunits. As oxygen is offloaded at the tissues carbon dioxide binds to the primary amino groups of the globin chains. The resulting carbamino-haemoglobin is transported to the lungs. This transport, together with the large quantity of carbon dioxide transported by bicarbonate facilitates the offloading of the carbon dioxide in areas with a higher  $pO_2$ , higher pH and lower temperature compared with the peripheral tissues. Haemoglobin is then altered back to a state where the oxygen affinity is increased and the loop is closed [2].

Figure 1



A haemoglobin molecule

The search for a red cell substitute has been ongoing for a long time but only recently have a few candidates reached clinical testing. Artificial oxygen carriers are pharmacological substances that aim to improve oxygen delivery independently of red blood cells. They only transport oxygen and do not have any other properties of blood such as coagulation and immunological functions. Ideally, artificial oxygen carriers would have the following advantages: no need for blood group testing, immediate availability, no immunological activity, no risk of infectious transmission, long intravascular half-life and long shelf life. Currently, two groups of agents are under investigation: modified extracellular haemoglobin based solutions and perfluorocarbon emulsions. There are other carriers such as particulate haemoglobin preparations, but their development is at a very early stage and they will not be discussed in detail here.

### **Manipulating the haemoglobin molecule**

There are several ways in which the haemoglobin molecule can be altered to facilitate oxygen delivery and avoid side-effects from renal filtration [3].

#### Intra-molecular cross-linking

Preventing the dissociation of the haemoglobin tetramer is important in order to prevent renal filtration. Because the  $\alpha/\beta$  dimers are relatively stable, the goal of intra-molecular modification is to cross-link the two  $\alpha$  or  $\beta$  subunits. The cross-linking not only prevents tetramer dissociation but also reduces the affinity of the haemoglobin for  $O_2$ .

#### Polymerization

In this process, multiple haemoglobin proteins are linked together through the use of dialdehydes, such as glutaraldehyde. The increase in size of the oligomers is significant because the molecular weight of the molecule exceeds 500 kDa, compared with 64.5 kDa for unpolymerized haemoglobin tetramers. This increase in weight prevents rapid excretion of the molecule.

#### Conjugation

Haemoglobin can be conjugated by the covalent binding of the haemoglobin molecule to a biocompatible polymer such as polysaccharide in order to increase its overall size. For example, multiple polyethylene glycol (PEG) chains can be added to the haemoglobin. Haemoglobin conjugation appears to protect the molecule from renal excretion.

#### Encapsulation

This is based on recreating the natural properties of red blood cells without the presence of blood group antigens. The process involves the encapsulation of haemoglobin within lipid vesicles using a solution of phospholipide.

### **Modified extracellular haemoglobin solutions**

In general, extracellular haemoglobin dissociates into  $\alpha/\beta$  dimers which are nephrotoxic. This necessitates the molecule to be chemically or genetically modified. Modified haemoglobin has a similar  $O_2$  loading and transport capacity as blood with a sigmoid dissociation curve. However, side-effects have been observed. In particular, vasoconstriction in combination with increased systemic and pulmonary pressures due to the scavenging of nitric oxide, release of endothelin and sensitization of  $\alpha$ -receptors. Furthermore, an increase in pancreatic enzymes and bilirubin has also been observed.

#### Stroma-free hemoglobin

Stroma-free haemoglobin (SFH) is one of the original products synthesised to make an oxygen carrier. This type of carrier is now abandoned due to side-effects - mainly vasoconstriction. Furthermore, SFH has a higher oxygen affinity than native intra-erythrocytic haemoglobin because the 2,3 diphosphoglycerate (DPG) normally present in red cells is lost during purification [4].

### Human polymerised haemoglobin

PolyHeme® pyridoxal haemoglobin (Northfield Laboratories, Evanston, IL, USA) consists of outdated human red cells containing haemoglobin polymerized with glutaraldehyde. There are no apparent side-effects such as vasoconstriction or renal toxicity. Furthermore, it does not need cross-matching since it does not contain intact red blood cells which would otherwise have expressed ABO antigens. It has a shelf-life of over 12 months, can be stored at room temperature and the manufacturing process reduces the risk of viral infection. Unfortunately there is appears to be an increased risk of myocardial infarction. The manufacturers have concluded a phase III trial in 2006. The testing was done in 720 trauma patients. The study was controversial as the participants were incapable of giving consent due to their injuries. The study was approved in an FDA special category that allows use without patient consent in special circumstances. The study was designed as an 'active control, dual superiority-non inferiority trial comparing the survival of PolyHeme patients to those receiving standard treatment (salt water plus blood).' In December 2006 Northfield Laboratories released preliminary results showing increased mortality in the intervention group compared with controls. However, no peer-reviewed papers presenting the data from this study have been published so far [5].

### Polymerised bovine haemoglobin-based O<sub>2</sub> carrier

HBOC-201, Hemopure®, (Biopure, Cambridge, MA, USA) is a bovine haemoglobin polymerised by glutaraldehyde-lysine binding with the haemoglobin molecule. Side-effects from use include vasoconstriction and raised amylase and lipase implicating some effect on the pancreas. This product is commercially available in South Africa, although the clinical use has been very limited. There are suggestions that it has been used as a performance enhancing drug in sport. As a result of the side-effects it is currently banned from further human studies in the US, but some limited animal studies are under way [6].

### Hemoglobin raffimer

Hemolink®, (Hemosol Research Corporation, Mississauga, Ontario, Canada) is a haemoglobin product in which O-*raffinose* cross-links between the  $\beta$ -chains is used to form stable tetramers and bind to surface acids resulting in haemoglobin polymerisation. Its use has been associated with cardiac toxicity and its future is unclear [7].

### Diaspirin cross-linked haemoglobin

HemAssist®, (Baxter, Deerfield, IL, USA) is produced by cross-linking the haemoglobin  $\alpha$ -subunits with diaspirin, preventing their breakdown into  $\alpha$ - $\beta$ -dimers and lowering their O<sub>2</sub> affinity. Initial animals studies were promising and some clinical studies have shown a significant reduction in the use of blood products in patients undergoing cardiac surgery. When the manufacturer tried to perform studies in the prehospital setting increased mortality was observed and the phase III trial was abandoned in the US and Europe. The development of diaspirin cross-linked haemoglobin has now been abandoned [8, 9].

### Maleimide-activated polyethylene glycol modified haemoglobin

One of the major disadvantages of artificial oxygen carrying products is vasoconstriction. This is mainly due to the scavenging of nitric oxide. Excessive unloading of oxygen also contributes to vasoconstriction. Therefore, a larger and thus less diffusible haemoglobin molecule would be attractive. Robert Winslow and his group at Sangart Inc have developed a product using outdated haemoglobin molecules surface conjugated with maleimide-activated polyethylene glycol (MP4, Hemospan® (Sangart Inc, San Diego, CA, USA)) [10]. This makes the molecule larger and less diffusible. In animal studies it has been showed that these solutions cause minimal vasoconstriction but some hypertensive effects. This, however, was mainly attributed to an increased cardiac output because of the colloidal properties. Furthermore, MP4 is effective in conserving oxygen in capillaries, inhibiting vasoconstriction and is more selective in delivering oxygen to hypoxic tissues. Several phase II trials have been performed, in particular a randomised prospective safety study in 90 elderly patients undergoing hip surgery. There were no serious adverse effects and only some minor elevations of amylase, lipase and liver enzymes. There was a trend for more bradycardic events in the MP4 group. Recently, a prospective, multi-center, blinded and randomized phase III study was performed in Europe. The results from this study are expected in the spring of 2009.

### Other modified extracellular haemoglobin solutions

There are other products still in the preclinical or experimental phases of development. Enzyme cross-linked polyhemoglobin is an example of cross-linking with catalase and superoxide to prevent formation of superoxide from accumulating hypoxanthine and newly formed O<sub>2</sub> with the resulting free oxygen radicals. Other experimental products include human recombinant haemoglobin expressed in E. coli, zero-linked haemoglobin and recombinant hybrids of human- $\alpha$ -chains and bovine- $\beta$ -chains haemoglobin.

### Perfluorocarbon emulsions

This is a completely different approach relying on the fact that certain inert organic chemicals can dissolve large amounts of gases. Perfluorocarbons are removed by the reticulo-endothelial system [1]. The most widely tested solution is perflubron. Side-effects are usually mild with flu-like symptoms and myalgia; a decrease in platelet count can also occur. In 2001 a phase III study was started but then abandoned due to a reported adverse neurological outcome. This was probably not due to the perfluorocarbon itself. Nevertheless, current development has been postponed. Perfortan (perfluoromethyl-cyclohexylpiperidin, Perfortan, Moscow, Russia) has been approved by the Russian Ministry of Health [2].

### Summary

Blood transfusions are associated with serious and potentially fatal adverse effects. Artificial oxygen carriers are among the alternatives to blood transfusion. They are either modified haemoglobin solutions or perfluorocarbon emulsions. Currently, there is no market approval for Europe or North America. There are, however, some products that have passed phase III trials with promising results.

### Key Learning Points

- Blood is a scarce, short-lived and expensive commodity and administration is not without significant risks
- The search for a red cell substitute has been ongoing for a long time
- Two groups of agents are under investigations: modified extracellular haemoglobins and perfluorocarbons
- Currently, there is no market approval for Europe or North America of any of these agents

### References

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