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Haemoglobin, oxygen carriers and perioperative organ perfusion

Kocian, R ; Spahn, D R

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Haemoglobin, oxygen carriers and perioperative organ perfusion

Roman Kocian, Donat R. Spahn

Roman Kocian, M.D.

Senior staff member

Institute of Anaesthesiology

University Hospital

Raemistrasse 100

8091 Zürich

Switzerland

Telephone Office : +41 44 255 26 96

Telefax : +41 44 255 44 09

e-mail : roman.kocian@usz.ch

Corresponding author:

Donat R. Spahn, M.D., F.R.C.A.

Professor and Chairman

Institute of Anaesthesiology

University Hospital

Raemistrasse 100

8091 Zürich

Switzerland

Telephone Office : +41 44 255 26 95

Telefax : +41 44 255 44 09

e-mail : donat.spahn@usz.ch

Abstract

Under normal conditions, only 20-30% of the delivered oxygen is metabolized. In normovolaemic anaemia the organism reacts with increases in cardiac output and oxygen extraction. Once these mechanisms are exceeded, allogeneic blood transfusions may be administered. However, such transfusions are associated with serious adverse effects and alternatives such as artificial oxygen carriers are being sought.

Main groups of artificial oxygen carriers are extracellular haemoglobin solution and perfluorocarbons. Preparations undergoing experimental and clinical assessment include Human Polymerized Haemoglobin (Polyheme[®]), Polymerized Bovine Haemoglobin-based Oxygen Carrier (HBOC-201, Hemopure[®]), Haemoglobin Raffimer (HemoLink[®]), Diaspirin Cross-linked Haemoglobin (HemAssist[®]), Human Recombinant Haemoglobin (rHb), Enzyme Cross-linked Poly-haemoglobin, Maleimide-activated Polyethylene-glycol Modified Haemoglobin (MP4, Hemospan[®]), Zero-linked Haemoglobin (ZL-HbBv), Recombinant Hybrid of Human- α -chains and Bovine- β -chains and Perflubron (Oxygent[™]). Research into some of these compounds has been discontinued others have advanced into clinical phase III trials, but none has achieved market approval for Europe, US or Canada so far.

Key words

oxygen transport, oxygen consumption, oxygen delivery

anaemia, anaemia tolerance, transfusion, transfusion adverse effects

artificial oxygen carriers, haemoglobin solutions, perfluorocarbon

Physiology of the oxygen delivery

The main function of erythrocytes is the transport of oxygen (O₂) from the lungs to the different organs and the peripheral tissues. Normally, about 98 % of O₂ is bound to haemoglobin (Hb), the rest is physically dissolved in the plasma. A greater proportion of dissolved O₂ can be reached with a higher inspiratory fraction of O₂ (FiO₂), which may be useful in acute anaemia.

O₂ delivery (DO₂) is the product of cardiac output (CO) and arterial O₂ content (CaO₂).

$$DO_2 = CO \bullet CaO_2$$

CaO₂ is the sum of O₂ bound to Hb and O₂ dissolved in the plasma. Normal Hb binds 1.34 ml O₂ per g of Hb at 100% of Hb saturation (SaO₂). O₂ solubility in relation to its arterial partial pressure (PaO₂) is 0.0031 ml/mmHg•dl at 37°C.

Thus, CaO₂ is:

$$CaO_2 = (SaO_2 \bullet 1.34 \bullet Hb) + (0.0031 \bullet PaO_2)$$

In analogy, mixed-venous (pulmonary artery) O₂ content (CvO₂) is:

$$CvO_2 = (SvO_2 \bullet 1.34 \bullet Hb) + (0.0031 \bullet PvO_2)$$

Total O₂ consumption (VO₂) is the product of CO and the arterio-venous difference in O₂ content (AVDO₂).

$$VO_2 = CO \bullet AVDO_2$$

If DO_2 and VO_2 are known, O_2 extraction (EO_2) can be calculated as:

$$EO_2 = VO_2/DO_2$$

Under normal circumstances, EO_2 is approximately 20 to 30%. This means that there is a physiologic reserve. Should DO_2 decrease can VO_2 be maintained by increasing EO_2 . However, if DO_2 continues to decrease, EO_2 continues to rise until reaching a point where the needed VO_2 cannot be maintained anymore – VO_2 turns dependent of DO_2 and starts to decrease with any further reduction in DO_2 . The DO_2 , at which VO_2 starts to decrease, is called critical DO_2 (DO_{2crit}). At DO_{2crit} the metabolism starts turning anaerobic and as a consequence, the serum lactate starts to increase resulting in metabolic acidosis.

Reaction to acute anaemia

During acute blood loss replaced by crystalloids and colloids, Hb falls and the physiological reaction is an increase in CO, an increase in EO_2 and right shift of the Hb dissociation curve [1]. Decrease in blood viscosity and sympathetic stimulation result in an increase in stroke volume [2-4]. Interestingly, a stroke volume increase due to anaemia has been observed even under treatment with beta-blockers [5].

In addition, blood flow is redirected to favour the vital organs (brain, heart). This autoregulation, varying regionally according to local functional and metabolic activity, is thought to be due to several factors including myogenic vascular reaction to variations in transmural pressure, endothelial vasoconstrictory or vasodilatory

alterations, vasoregulatory release of neurotransmitters and adjustments of DO_2 to O_2 demand under influence of metabolic mediators [6]. Recruitment of capillaries allows for better oxygenation of peripheral tissues. Finally, anaemia stimulated increase in erythrocyte 2,3-diphosphoglycerat shifts the Hb dissociation curve to the right, allowing for easier off-loading of Hb-bound O_2 [7]. Increase of PaO_2 has been observed under acute normovolemic anaemia (ANH). This could be due to increased CO with better pulmonary flow, decreased blood viscosity, better ventilation-perfusion relation and possibly pulmonary vasodilatation due to nitric oxide [8-11].

Anaemia tolerance

There is increasing evidence that much lower Hb concentrations can be tolerated than previously admitted. In Jehovah Witnesses, where any red blood cells transfusion is usually refused for religious reasons, the most deaths exclusively due to anaemia have occurred with Hb significantly below 5 g/dl [12]. Also healthy volunteers tolerate a Hb concentrations below 5 g/dl [13]. Case reports mention survivals with Hb concentration of only 1.1 g/dl [14].

Cardiac disease

In patients with cardiac disease, the situation is different. EO_2 in the heart is high under normal conditions already, with relatively little reserve in case of increased metabolic demand. Anaemia thus is mainly compensated by increased coronary flow. Therefore, the heart is particularly at risk of inadequate DO_2 . However, even patients with coronary heart disease tolerate moderate normovolaemic anaemia well. [15, 16].

ANH to a Hb of 9.3 ± 1.0 g/dl has been tolerated without cardiac ischemia signs such as ECG alterations, haemodynamic instability or wall motion abnormality [9, 10]. ANH to a haematocrit (Hk) of 28 % had a cardio-protective effect in patients undergoing coronary artery bypass graft (CABG) surgery, resulting in less myocardial lesions, less arrhythmias and lower need in inotropic support [17, 18]. A large analysis of over 24'000 patients with acute coronary syndrome has shown a worse outcome in those who were transfused with Hk over 25 %. However, Hk was not an endpoint of this study and several issues such as transfusion decisions have not been specifically addressed [19]. An evaluation of Hk and its influence on the outcome in patients undergoing CABG surgery has shown that anaemic patients often had other risk factors such as diabetes or congestive heart failure. The only independent factor leading to higher mortality found, was a Hk lower than 14 % during the extracorporeal circulation[16]. A comparison of patients with cardiac disease including coronary artery disease in intensive care units showed no difference in outcome between groups transfused at a Hb <7 g/dl vs. a Hb < 10 g/dl [20, 21].

It appears that it is currently difficult to determine one specific Hb value as a universal transfusion trigger in patients with coronary artery disease. The decision should incorporate the degree of the coronary disease, age and associated pathologies. Nevertheless a Hb of 8 g/dl is frequently cited as a Hb based transfusion trigger in patients with stable coronary artery disease [22].

ANH in valvular heart disease has been less frequently investigated. ANH to a Hb of 10 g/dl has been well tolerated in mitral insufficiency [23]. Also in severe aortic stenosis, haemodilution to a Hk of 28% is well tolerated and accompanied by a CO

increase; moreover, it enabled a supplemental cardiac protection with reduced myocardial injuries as heralded by an improved haemodynamical stability, lower need for inotropic support and reduced release of myocardial enzymes [24, 25].

Intuitively, an increase of CO due to haemodilution would be expected being limited by pre-existing cardiac contractile dysfunction. Nevertheless, the compensatory mechanisms were independent of left ventricular ejection fraction (LVEF) in the range of 26 % to 83 % [10]. However, there was only a limited number of patients with a LVEF less than 40 % included [10].

Elderly patients

Also elderly patients with and without coronary heart disease aged up to 88 years, tolerated ANH well and the compensation mechanisms to ANH were not modified by age itself [7, 10]. However, only a limited number of patients were older than 80 years. Therefore, any extrapolation to patients older than 80 years is fraught with difficulties.

Central nervous system

Experimental ANH from Hb 14.0 ± 1.3 g/dl to Hb 6.0 ± 0.2 g/dl in healthy volunteers resulted in impairment of cognitive functions and further dilution to Hb 5.1 ± 0.2 g/dl compromised memory whereas such alterations have not been observed at a Hb of 7.2 ± 0.2 g/dl and were reversed after transfusion to this Hb. Similarly, alterations of neurocognitive functions due to ANH from Hb 12.7 ± 1.0 g/dl to Hb 5.7 ± 0.3 g/dl have been reversed by the administration of 100 % O₂ increasing PaO₂ from 100 to 400

mmHg, which corresponds in terms of CaO_2 to Hb rise of approximately 3 g/dl [26, 27].

Transfusion of red blood cells

Red blood cells transfusion (RBCT) is associated with risk and side effects. The most frequent untoward effects are acute and delayed haemolytic reactions and alloimmunisation. Infectious risks include transmission of viral diseases (Human Immunodeficiency Virus, Hepatitis B Virus, Hepatitis C Virus, possibly other types of hepatitis), bacterial diseases (contamination of the red blood cell conserve during handling), parasites (malaria) and recently prions (new variant Creutzfeld-Jakob-Disease) [28-31].

An additional immunological complication is transfusion related acute lung injury (TRALI). TRALI is an acute respiratory distress due to bilateral non cardiac pulmonary oedema appearing within 6 hours after transfusion often necessitating mechanical ventilation [32-34]. Mortality is estimated between 3% and 67% [34-36]. Since its diagnostic is made by exclusion of other causes due to absence of any specific marker, its frequency may be underestimated.

Still incompletely understood immunological side effect of RBCT is induction of immunosuppression, with risk of increased incidence of infections and neoplastic diseases [37-41].

For these reasons, RBCT should be avoided as far as possible and alternatives to RBCT should be considered in case of blood loss.

The aim of RBCT is to increase the tissue oxygenation, not to correct a Hb value or even to treat hypovolaemia. RBCT which results in increased DO_2 but does not increase VO_2 is indeed a sheer treatment of vascular filling, without other benefits but with all transfusion associated risks. The decision of RBCT should be based on individual consideration of the patient's risk to develop any complication due to inadequate tissue oxygenation. Signs of inadequate tissue oxygenation or "physiologic transfusion triggers" [42], actually considered as transfusion trigger, are haemodynamical instability, EO_2 greater than 50 %, mixed-venous O_2 partial pressure (PvO_2) under 32 mmHg, VO_2 decreased by more than 10 %, and finally myocardial ischemia characterized by new ST-segment elevation or depression and new wall motion abnormalities in echocardiography [43, 44].

Postoperative Hb below 6,0 g/dl was associated with increased morbidity and mortality [45] and it appears that this Hb value *per se* can justify a RBCT even without physiologic signs of inadequate tissue oxygenation in most patients, particularly those with coexisting cardiac disease [45-47]. No RBCT is generally needed in patients with Hb over 10 g/dl [46]. Between these limits, RBCT should only be given to prevent or treat inadequate tissue oxygenation, heralded by apparition of above mentioned transfusion triggers.

In order to avoid unnecessary allogenic RBCT, several alternatives should be considered: autologous blood donation, acute normovolaemic haemodilution, perioperative blood recuperation, pharmacological treatment (erythropoietin, antifibrinolytics), careful anaesthesia and surgery techniques, tolerance of low Hb

levels, normothermia to enhance coagulation, ventilation with high FiO_2 , controlled hypotension in particular settings, and in future, artificial oxygen carriers. Particular attention should be given to maintaining the blood coagulation in order to avoid unnecessary blood loss.

Artificial oxygen carriers

Artificial oxygen carriers are pharmacological substances aiming to improve DO_2 independently of red blood cells. They could become a valuable alternative to RBCT in the future, avoiding the actual transfusion risks. However, they only transport O_2 and do not have any other blood capacity, such as coagulation or immunological functions.

Ideal artificial O_2 -carrier would have the following advantages: no need for blood group testing, immediate availability in sufficient quantities, no immunological activity, no risk of infectious disease transmission, long intravascular half-time and long shelf life. They should be available for blood loss substitution, but they could be used also for reperfusion of ischemic organs due to vascular strokes, for cardioplegia priming and as preservative for transplanted organs.

Two groups are currently under investigation: modified extracellular haemoglobin based solutions and perfluorocarbon emulsions. Both are capable of binding O_2 but their functional characteristics are totally different. Although experimental and clinical investigation is far advanced, none of these products has been market approved until now.

A third group, particulate haemoglobin preparations able to carry other useful substances such as enzymes are also studied, but their development is still far from any clinical application [48-51].

1. Modified haemoglobin solutions

Haemoglobin is a tetramer of two α and two β polypeptide chains, bound to an iron-containing heme group. Extracellular human haemoglobin ($\alpha_2\beta_2$ -tetramers) dissociates into $\alpha\beta$ -dimers which are nephrotoxic. In order to prevent this, the haemoglobin has to be chemically or genetically modified [50].

Modified haemoglobin has similar O_2 loading and transport capacity as the native blood, with sigmoid dissociation curve [50]. Thus even with relatively low PaO_2 a sufficient amount of O_2 can be transported. Since the modifications of the haemoglobin further decrease its affinity for O_2 , the peripheral O_2 unloading is facilitated.

Several animal studies have shown the efficacy of modified haemoglobin to transport and unload O_2 ; however, important side effects have been observed [3, 52-54]. Vasoconstriction with increase of systemic and pulmonary pressures, appears to be due to nitric oxide (NO) scavenging, probably by extravasated haemoglobin, endothelin release and sensitization of peripheral α -receptors [3, 55-59]. The most affected segment appears to be the microcirculation with a reduction of the functional capillary density [60]. It has been observed that haemoglobin solutions with different O_2 affinity caused different degrees of vasoconstrictions [61, 62]. However, the hypothesis of autoregulatory vasoconstriction due to relative hyperoxic state caused

by better O₂ unloading is in contradiction with multiples observation of better tissue PO₂ during hyperoxic ventilation [63-68].

Iron overload with administration of extracellular haemoglobin could be a concern, particularly since this might compromises the immune function – in experimental animal models, the mortality has been increased after iron supplementation [69-71]. Further frequently observed side effects of haemoglobin based solutions is elevation of pancreatic enzymes and bilirubin. Since haemoglobin based solutions are coloured substances, they may affect some colorimetric laboratory measurements [72]. They do not affect the blood group and rhesus determination [73].

Different haemoglobin based solutions have been tested: stoma free Hb (“first generation”), polymerized Hb (“second generation”), intramolecularly cross-linked Hb (“third generation”) and more recently, Hb solutions aiming to reduce vasoconstriction (“fourth generation”) [74].

Stroma-Free Haemoglobin (SFH)

The idea of substituting haemoglobin as extracellular substance is over 100 years old. The early studies showed considerable side effects, most notably renal impairment due to vasoconstriction [75-77]. As a consequence, SHF has been abandoned as blood substitute.

Human Polymerized Haemoglobin (PolyHeme[®])

Outdated human blood is haemolysed yielding haemoglobin then purified, pyridoxylated to decrease the O₂ affinity and polymerized with glutaraldehyde [54, 78,

79]. Animal experiences and clinical investigation in trauma and emergency surgery showed a good tolerance of very low haematocrit if supplemented with Polyheme, decreasing the need for RBCT [78, 80, 81]. Interestingly, no serious side effects have been observed except an increase in bilirubine and amylase, and particularly no hypertension. Another large study on 171 patients with emergency surgery showed good survival of those with low haematocrit treated with Polyheme, however the comparison with a historic group seemed not to be quite adequate [82] .

No Biological License Application has been granted by the US Food and Drug Administration (FDA) yet. Currently, a randomized phase III trial with PolyHeme[®] in 720 patients in the pre-hospital setting is being completed, and results are expected later in 2007 [74].

Polymerized Bovine Haemoglobin-Based O₂ Carrier (HBOC-201, Hemopure)

HBOC-201 is bovine haemoglobin, polymerized by glutaraldehyd-lysin binding within the haemoglobin molecule; additional filtration eliminates the non-polymerized haemoglobin tetramers [54]. Studies in vascular, orthopaedic and cardiac surgery showed substantial reduction of need for allogenic blood with up to 59% of patients avoiding RBCT; however, side effects observed with HBOC-201 included vasoconstriction, raised amylase and lipase suggesting pancreatic disturbances, abdominal pain, dysphagia, nausea, oliguria, late methaemoglobinemia and apparition of IgG antiHBOC-201 [83-86].

No Biological License Application has been granted by the FDA yet, but HBOC-201 is approved for clinical use in South Africa.

Haemoglobin Raffimer (HemoLink[™])

O-raffinose cross-links the β -chains forming stable tetramers, and it binds surface amino acids resulting in haemoglobin polymerization. In animal studies, similar tissue pO_2 could be achieved as with RBCT [87]. In patients undergoing cardiac surgery, treatment with haemoglobin raffimer allowed a reduction of RBCT as compared pentastarch [88-90]. However, common side effects of haemoglobin based solutions have been observed, including hypertension, increase in pancreatic enzymes and raised bilirubin. HemoLink™ has actually been abandoned due to cardiac toxicity observed during the clinical trials [74].

Diaspirin cross-linked haemoglobin (HemAssist)

Cross-linking of the haemoglobin α -subunits with diaspirin prevents the breakdown into α - β -dimers and lowers the O_2 affinity [91, 92]. In animal models, diaspirin cross-linked haemoglobin (DCLH) resulted in better wound healing, enhanced hepatic cell proliferation and decreased splanchnic bacterial translocation as compared with transfusion of autologous blood [93]. DCLH furthermore allowed an extreme haemodilution without signs of myocardial ischemia [94]. During clinical trials, use of DCLH allowed a significant reduction of RBCT in patients undergoing cardiac and non cardiac surgery [95, 96]. However, the administration of DCLH also resulted in higher incidence of icterus, pancreatitis and urinary complications. In trauma patients, DCLH even caused an increased mortality, possibly by aggravated blood loss due to vasoconstriction conditioned hypertension – the studies were terminated prematurely [97, 98]. Finally, the development of DCLH has been discontinued.

Human Recombinant Haemoglobin (rHb)

Human haemoglobin expressed in *Escherichia Coli* is stabilized by alteration of several of its amino acid sequences [99, 100]. This mutation decreases the O₂ affinity and virtually eliminates renal toxicity. The vasoconstriction due to NO scavenging with systemic and pulmonary hypertension remains a notable side effect of rHb. Moreover, an increase in amylase and lipase has been observed, suggesting alteration in pancreatic microcirculation [101]. However, further modification of rHb (rHb 2.0) allowed to mitigate the vascular response [102, 103].

Initial animal studies showed promising results: rHb 2.0 proved valuable in resuscitation of dogs and rats [101, 104], and allowed a nearly complete blood exchange [105]. However, despite these encouraging results, the further development of rHb 2.0 has been recently stopped.

Enzyme Cross-Linked Poly-Haemoglobin

Cross-linking of polymerized haemoglobin with catalase and superoxid-dismutase can be useful in case of organ ischemia, to prevent the formation of superoxide from accumulated hypoxanthine and newly delivered O₂ with resulting free oxygen radicals. Protection from reperfusion injury and free radicals scavenging could be demonstrated in animal model [106, 107]. Larger animal and clinical investigation have to be done before introducing enzyme cross-linked poly-haemoglobin into clinical practice.

Maleimide-Activated Polyethylene Glycol Modified Haemoglobin (MP4, Hemospan)

NO-scavenging seems not to be the sole mechanism leading to vasoconstriction with extracellular haemoglobin solutions. Different degrees of vasoconstriction could be achieved with differently modified haemoglobins having nevertheless similar NO binding [103]. Excessive oxygen unloading may have vasoconstrictive effects, particularly on arterioles; consequently, with larger thus less diffusible and less extravasating haemoglobin molecules having greater O₂ affinity vasoconstriction should be reduced [108-110].

Based on this hypothesis of autoregulatory vasoconstriction in hyperoxic conditions [111, 112], a haemoglobin solution with larger molecular size and higher O₂ affinity has been created by surface conjugation of outdated human blood haemoglobin with maleimide-activated polyethylene-glycol in order to decrease hypertensive side effect [109]. It has been shown in hamster model that although all cell-free haemoglobin solutions scavenge NO, at least a part of the vasoconstrictive effect is due to other mechanisms, particularly the molecular size of Hb. The larger molecule of MP4 resulted in less vasoconstriction than other Hb solutions. Nevertheless, all of them resulted in elevation of blood pressure, but while with other Hb solutions this was due essentially to arteriolar vasoconstriction, with MP4 the increased blood pressure was a consequence of increased cardiac output due to colloidal properties of MP4 [113].

In animal anaemia models, the functional capillary density as an expression of vasoconstriction in the microcirculation segment has been preserved by MP4. However, RBCT restored tissue oxygenation better than MP4 and the haemodynamic response and lactate levels were comparable to treatment with hydroxyethyl starch [114-116]. However, the haematocrite achieved during these

experiments was still relatively high, 15% and 21%, levels which are usually fairly well tolerated even without additional O₂ treatment. However, MP4 showed in animal model their capacity to sustain oxygenation in extreme haemodilution [114, 117].

A phase I study on healthy volunteers has shown that MP4 has no clinically significant hypertensive and gastrointestinal side effects as have had previous Hb solutions [118]. A recent phase II multicenter randomized prospective safety study on 90 elderly patients undergoing elective hip arthroplasty has confirmed these observations. MP4 had no serious adverse effects; the mild elevation of amylase, lipase and liver enzyme was not statistically significant but there were a trend to more bradycardia events in the MP4 group [119]. However, there is no information about preoperative medical treatment, particularly β -blockers.

A phase III study is currently being conducted but the results are not expected before 2008.

Zero-linked Haemoglobin (ZL-HbBv)

This recent polymerized bovine-haemoglobin based solution has no intramolecular coupling, big molecular size and high O₂ affinity [74]. It tends to extravasate less [120]. Few data are actually available about ZL-HbBv.

Recombinant Hybrid of Human- α -chains and bovine- β -chains Haemoglobin

A new haemoglobin molecule, subsequently polymerized through its surface sulfhydryl groups, with high O₂ affinity, which improved oxygenation in animal brain

ischemia model [121]. Once again, little experimental information is available concerning this substance so far.

2. Perfluorocarbon emulsions (PFC)

PFC are carbon-fluorine compounds which are chemically and biologically inert, have low viscosity and high gas dissolving capacity (O₂, CO₂, other gases) [3, 122, 123]. They are not miscible with water and have to be conditioned as emulsion with droplets of approximately 0,16 µm in order to be biocompatible. This very small size could make them interesting not only to substitute for lost blood, but also in situations where inadequate tissue oxygenation is due not to anaemia but to extreme vascular stenosis impeding the circulation of red blood cells, like a most severe form of coronary artery disease or limb ischemia. Unlike Hb solutions, the relation between PaO₂ and PFC-transported O₂ is linear; consequently higher FiO₂ is required for better O₂ transport [49].

Fluosol-DA

Fluosol-DA has been the first PFC shown to protect the heart during percutaneous transluminal coronary angiography (PTCA) and it has been market approved for PTCA in 1989 [124-128]. However, it showed no efficacy in the treatment of anaemia and has been withdrawn several years later when improvements in balloon angioplasty catheters limited its use [123, 129, 130].

Perflubron (Oxygent™)

After i.v. administration, perflubron (perfluoro-octyl bromide) is absorbed by the reticulo-endothelial (RES) system. This absorption determinates its intravascular half-

time which is dose-dependent, in order of several hours [3, 122, 131, 132]. In RES, perflubron droplets are broken down then excreted in the blood again and transported to the lungs where they are exhaled, without any known metabolism [3, 122].

Animal models of haemodilution have shown the capacity of perflubron to restore peripheral tissue oxygenation with amelioration of organ function [63, 133, 134]. This is due most likely to favourable off-loading of O₂ in hypoxic tissue and to the small dimension of perflubron droplet as compared to erythrocytes allowing it to pass even very narrow or near totally obstructed vessels. It has been observed that O₂ transported by perflubron is metabolized preferentially to O₂ transported by Hb [133]. In human studies, perflubron allowed to retard or reverse the trigger for RBCT, with significantly reduced need for allogeneic blood [131, 132, 135-137]. This effect was further accentuated and prolonged with higher doses [135].

Side effects of perflubron are usually mild and without serious clinical consequences. Flu-like symptoms with myalgia and light fever have been reported, and decrease of approximately 15% in platelet account from third to seventh postoperative day has been observed [49]. On the other hand, perflubron has no effect on coagulation tests, bleeding time and platelet aggregation [49].

In 2001, a phase III study in cardiac surgery has been suspended, due to reported adverse neurologic outcome; however, experts agree that these results were probably not due to perflubron itself but more likely to the fast blood harvesting early

during the cardiopulmonary bypass [54]. To our knowledge, no major clinical development is pursued currently.

3. Other artificial oxygen carriers

Haemoglobin Containing Liposomes (Neo Red Cells)

Since several decades, the development of small vesicles (0.1 – 0.3 μm) aims to supplement the red cell based O_2 transport by artificially created corpuscular carrier. Purified haemoglobin is conjugated with phospholipids, cholesterol and α -tocopherol [138].

Theoretical advantages of this product include longer intravascular half-time (in the order of days), less vasoconstriction and the possibility to include other useful substances such as 2,3-DPG or methaemoglobin-reductase to regulate O_2 affinity and decrease methaemoglobine formation [54, 138, 139], other enzymes such as catalase or superoxid-dismutase can be co-encapsulated as well which may prove beneficial during reperfusion of hypoxic areas for reducing the reperfusion injury [140]. Although the neo red cell technology may enable fascinating combinations of haemoglobins and diverse enzymes, a clinical application appears unlikely in the near future.

Polymersome Encapsulated Haemoglobin (PEH)

This recent preparation is bovine haemoglobin encapsulated in polymer vesicles (polymersomes) to form PEH. Unlike liposomes, polymersomes can be completely covered by polyethylene-glycol (PEG). PEG is a biologically inert polymer, used in drug delivery systems and known to be safe for in-vivo administration [48]. It

influences the permeability of polymersomes, giving them better mechanical resistance with favourable osmotic properties, thus reducing problems with osmotic pressure gradients. Further, it enables a better choice of vesicular size. The O₂ affinity has been found comparable to that of human erythrocytes [48]. PEH is still in experimental in-vitro investigation.

Allosteric modifier (RSR13)

RSR13 (2-[4-[2-[3,5-dimethylphenyl]-2-oxoethyl]phenosyl]-2-methyl-propanoic acid monosodium salt) has been developed for hyperoxic treatment of tumour in combination with radiotherapy. It shifts the haemoglobin dissociation curve to the right, decreasing the O₂ affinity thus enhancing the O₂ unloading [141, 142]. However, in animal haemodilution model it has not improve haemodilution tolerance or O₂ consumption [143]. It is therefore unlikely that this substance could have any positive influence on need for RBCT and, no major clinical development is pursued to our knowledge currently.

Summary

Increasing experimental and clinical evidence indicates that red blood cells transfusion indication should be much more restrictive and considered for every patient individually, the only reliable transfusion trigger being apparition of signs of inadequate oxygenation.

During last decades, much research has been done to develop pharmacological alternatives to blood transfusion, aiming to avoid its numerous adverse effects and to reduce the inherent costs. Different artificial oxygen carriers have been designed, most noticeably modified haemoglobin solutions and perfluorocarbon emulsions.

Despite major difficulties in the development of clinical artificial O₂ carriers, they continue to hold great promise in terms of enhanced tissue oxygenation and as a substitute for allogeneic blood transfusion. However, their introduction into clinical medicine, albeit intensively awaited, may still take a few years.

Practice points

- In acute anaemia, several compensatory mechanisms maintain oxygen consumption: increases of cardiac output and oxygen extraction, right shift of the oxygen dissociation curve and redistribution of blood flow to favour vital organs.
- Anaemia is well tolerated even by patients with co-existing cardiac diseases, provided normovolemia is maintained.
- Blood transfusions are associated with serious and potentially fatal adverse effects such as acute transfusion reactions, transmission of infectious diseases, infectious complications, transfusion related acute lung injury and circulatory overload.
- Decision for blood transfusion should be based on individual and physiologic transfusion triggers (signs of inadequate oxygenation), not on a general haemoglobin level.
- Alternatives for blood transfusion are restrictive transfusion indication based on anemia tolerance, optimized surgical and anaesthesia techniques, coagulation monitoring, acute normovolemic dilution, perioperative blood recuperation, autologous blood donation and in the future, artificial oxygen carriers.
- The main groups of artificial oxygen carriers are modified haemoglobin solutions and perfluorocarbon emulsions. Although under intensive investigation, none of them has currently achieved market approval in Europe, US or Canada.

Research Agenda

- Future research is warranted to find a bed-side monitor assessing tissue oxygenation.
- Artificial oxygen carriers are to be further modified in order to maximize their efficacy and to minimize their side effects.
- Efficacy and safety of artificial oxygen carriers need to be assessed in large scale human studies.

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