HEMOGLOBIN BASED OXYGEN CARRIERS AS POTENTIAL ARTIFICIAL BLOOD SUBSTITUTES

Essay
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Abbreviations

AOCs: Artificial oxygen carriers

ARDS: Acute respiratory distress syndrome

ATP: Adenosine tripohosphate

AVM: Arteriovenous malformation

CAT: Catalase

CaO_2: Arterial oxygen content

CvO_2: Venous oxygen content

DCL Hb: Diaspirin crosslinked hemoglobin

DO_2: Oxygen delivery

DPG: Diphosphoglycerate

FDA: Food and drug administration

GFR: Glomerulofilteration rate

Hb: Hemoglobin

HBOCs: Hemoglobin-based oxygen carriers

ICH: Intracerebral hemorrhage

HIV: Human immunodeficiency virus

NO: Nitric oxide

OER: Oxygen extraction ratio

PEG: Polyethylene glycol

PFCs: Perfluorocarbons

PO_2: Partial pressure of oxygen

P50: Oxygen partial pressure at which hemoglobin is 50% saturated with oxygen.

RBCs: Red blood cells

RES: Reticuloendothelial system

SAH: Subarachnoid hemorrhage

SOD: Superoxide dismutase

Q: Cardiac output

VO_2: Oxygen uptake (consumption)

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Introduction

Any review of modern transfusion therapy would be incomplete without considerable discussion of hemoglobin-based oxygen carriers (HBOCs). This abbreviation is the preferred term for the class of transfusable fluids also known as ‘oxygen therapeutics’. The biologic goal of transfusion therapy is to preserve tissue perfusion in order to minimize the damage – both physiologic and structural – that results from prolonged or repeated episodes of ischemia. Oxygen-carrying solutions with crystalloid and colloid elements may be effective in many clinical situations. For example, in a simple analytical model designed to illustrate how HBOCs might be used in surgery, a red blood cell (RBC) substitute of the first generation, currently in clinical testing, is projected to replace up to 60% of the current use of allogeneic RBCs (Chang, 2004).

The need for human blood for blood transfusions is steadily increasing. However, donated human blood is riddled with many problems, such as, limited availability, short storage lifetime, possibility of transmission of infectious diseases by blood-borne pathogens, allergic reactions, and problems associated with cross-matching different blood types (Schumacher et al., 2004).

The ideal intravenous fluid for trauma resuscitation would have the following properties: provide volume expansion, carry oxygen, possess a long shelf life at room temperature, not affect coagulation, and be universally compatible, non-antigenic, non-infectious, and inexpensive. No fluid meets all of these requirements, however hemoglobin based oxygen carriers (HBOCs) currently in the advanced stages of development, bring us closer to the ideal. FDA approval for several of these carriers may occur within the next two to three years. Despite the theoretical promise and attraction that these compounds possess, their true utility remains largely unexplored (Strandl, 2004).

Aim of the work:

The aim of this work is to orient intensivests about hemoglobin based oxygen carriers as artificial blood transfusion substitutes regarding current status and future directions.

1. PHYSIOLOGICAL BASIS

1.1 Oxygen requirements

Oxygen is required for the efficient conversion of substrate to ATP, whose high-energy phosphate bond is subsequently converted into the power that drives all living processes **(figure 1.1)**. Without oxygen, ATP can still be produced by anaerobic metabolism, but the yield of ATP is much less and the product of such metabolism is lactic acid. When the supply of oxygen does not meet demand, the so-called 'anaerobic threshold' is crossed, after which an oxygen debt is incurred, lactic acid accumulates and, if oxygen is not resupplied, cells die. The concentration of oxygen within mitochondria that is required for aerobic metabolism is quite low, in the range of 2–3 mmHg, and under normal conditions oxygen is in substantial excess, with tissue PO_2 in the range of 20–40mmHg **(Guyton et al., 2003)**.

In single-cell animals, the transport of oxygen from air to mitochondria is a diffusive process, and no circulation is needed. As organisms increase in size and complexity, the distances for diffusion are too great, and circulatory mechanisms are needed to move oxygen from the lung to tissue sites of respiration. Oxygen delivery (DO_2) is the product of arterial oxygen content (CaO_2) and cardiac output (Q) **(equation 1.1)**:

$$\mathbf{DO_2 = CaO_2 \times Q} \qquad \mathbf{(1.1)}$$

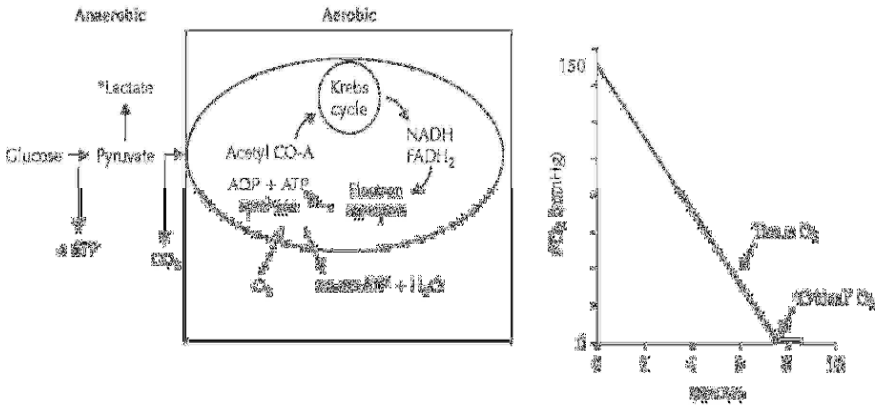

Figure 1.1 Metabolic oxygen consumption. With excess glucose and oxygen as substrates, mitochondria produce ATP for use in energy-consuming reactions (left). When oxygen supply to mitochondria is interrupted (right), *P*O2 drops to critically low levels, the much less efficient anaerobic metabolism ensues, producing less ATP and lactic acid. If the oxygen supply is not re-established, oxygen debt, acidosis and tissue death follow. Mitochondria are able to utilize oxygen efficiently to very low levels, approximately 2–3 mmHg **(Winslow, 2006)**.

Oxygen consumption (VO_2) is the product of the arterial– venous oxygen difference (C (a _ v) O_2) and cardiac output **(equation 1.2)**:

C (a-v) O_2 (1.2)× $VO_2 = Q$

However in addition to the circulatory system, containment of an oxygen carrier (hemoglobin) within the red cell is also critical, as demonstrated by a simple calculation using **equation 1.3**. If the requirement for oxygen to support aerobic metabolism in a resting human is 5 ml/min per kg, and if the heart can pump 5 l/min, and if all of the arterial oxygen is extracted, then the minimum oxygen to be carried in arterial blood is (5 ml/min per kg X 70 kg)/(5 l/min) or 7 ml/dl of blood. The : relationship between PO2 and the concentration of oxygen in plasma is

$$O_2 = \frac{\alpha \times PO_2}{760}$$

where α is the solubility coefficient in plasma (2.34 ml/dl per atmosphere). Thus, in order for plasma alone to present 7 ml/dl of blood to the capillaries, the PO2 would have to be at least (7 ml/dl X 760)/2.34, or 2274 mmHg, or about 3 atmospheres – a clear impossibility. Furthermore, mammals such as man are capable of increasing their rate of oxygen utilization many fold, reaching as high as 75–90 ml/min per kg in trained athletes. Support of oxygen transport in larger animals therefore presents two evolutionary challenges: first to transport large amounts of oxygen in blood such that it remains in the blood until it reaches respiring tissue, and second to provide mechanisms such that more blood flows to critical tissues in times of need **(Gould et al, 1983)**.

The capacity to transport large amounts of oxygen is achieved by the presence of an oxygen carrier. Invertebrates transport oxygen in a circulating hemolymph that contains either hemocyanin, a copper-containing protein, or hemerythrin, an iron-containing protein. In both of these, the metal atom is coordinated directly to the protein. However as body size increases still more, larger amounts of the oxygen carrier are required; but if the heme protein were free in the plasma it would turn over so fast that the larger organism could not possibly keep up with production. This problem is solved by the red blood cell, which packages hemoglobin in such a way that the molecules have a lifespan of approximately 100 days.

Red blood cells are uniquely suited to the task of oxygen transport for several important reasons. First, they contain a high concentration (about 35 g/dl) of hemoglobin, capable of carrying about 47 mL of O_2 per 100 mL of red cells. Hemoglobin at this concentration could not circulate, but a suspension of cells in plasma where the cells occupy about 45 per cent of the volume of blood circulates very well. Thus, the overall oxygen capacity is about 18 ml/dl. Second, red blood cells are deformable; they can squeeze into capillaries that are smaller in width than the dimension of the cells themselves. This ensures a minimal distance for diffusion of

oxygen from the alveolar space of the lung to hemoglobin, or from hemoglobin to sites of tissue respiration.

Third, hemoglobin binds oxygen cooperatively. This means that very small changes in oxygen tension result in large amounts of oxygen either taken up in the lung or released in the tissues. Finally, hemoglobin demonstrates a Bohr effect: local conditions of pH and carbon dioxide affect the oxygen-binding behavior of hemoglobin in ways that are favorable for oxygen transport. Since the supply of oxygen is critical to organ function and therefore survival, it is expected that the physiologic mechanisms that ensure this supply are complex and redundant.

1.2 Local regulation of oxygen supply

In order to ensure adequate oxygen supply to tissues and to provide an oxygen reserve for sudden increased demand, compensatory mechanisms must engage. This process, generally called 'autoregulation', is the process whereby the delivery of oxygen to tissues is matched to demand. The general mechanisms that can be altered to maintain tissue oxygenation are blood flow and oxygen extraction, and both have central nervous and peripheral components. Central mechanisms are comprised of the control of ventilation, hypoxic ventilatory responses, and carotid body reflexes and regulation of cardiac rate and stroke volume. Peripheral mechanisms are the result of local metabolic controls, and can be studied in denervated animal models. In such preparations when blood pressure is decreased, oxygen extraction increases. The oxygen extraction ratio (OER) is the fraction of oxygen that is removed from blood on a passage from the arterial to venous circulation, and is defined as **(equation 1.4)**:

(1.4)

Arterial oxygen content (CaO2), venous oxygen content (CvO2) and their difference (C(a _ v)O2) are determined predominantly by the product of the hemoglobin concentration and fractional saturation, because normally approximately

$$OER = \frac{C(a-v)O_2}{C_aO_2}$$

97 per cent of blood oxygen is carried bound to hemoglobin, the remaining oxygen being physically dissolved in plasma.

As an example of an autoregulation experiment, **Shephard et al. (1973)** studied decapitated dogs, in whom blood pressure was maintained by infusion of epinephrine. When the blood pressure was lowered by either decreasing the epinephrine infusion or reducing the level of venous blood return to the heart, cardiac output fell precipitously to about 60 per cent of its control value. At the same time, the extraction of oxygen in the circulation increased by a comparable amount, so that tissue oxygen consumption (VO_2) was preserved. Thus the end result of autoregulation was maintenance of tissue homeostasis over a range of different conditions. This is a very important concept for transfusion practice, because it suggests that need for a one-to-one replacement of lost red cells is not necessary because of the healthy body's capacity to compensate through a variety of mechanisms. The problem comes in determining when the compensatory mechanisms are stretched to their limit in a given patient.

1.3 The optimal hematocrit

What is the 'optimal' hematocrit? Obviously, there is no simple answer to this question and certainly none that would apply to all patients. Because it is easily measured, hematocrit is frequently taken as a surrogate for oxygen transport capability. The bulk viscosity of blood increases exponentially with hematocrit, and increased viscosity raises resistance to blood flow, limiting cardiac output in the absence of compensatory mechanisms. As the oxygen capacity of the blood (hemoglobin or hematocrit) increases, cardiac output decreases, and over a wide range of hematocrit there exists an optimum, defined as the point of maximal oxygen delivery. This principle have been studied theoretically, in animals **(Guyton et al., 2003)** and in humans with extensions to high altitude polycythemia **(Winslow and Monge, 1987)**, and the general conclusion is that 35 per cent hematocrit represents the best combination of cardiac output and hematocrit in healthy animals and humans. Therefore, if all patients were in perfect health a transfusion trigger could be simply defined as 35 per cent.

The problem, of course, is that patients, by definition, are not in perfect health, and the ability to compensate for loss of hemoglobin by raising cardiac output, for example, may be quite variable. In addition, it is not always simple to determine which patients can utilize compensatory mechanisms and which cannot, or which ones are in greater danger of localized tissue ischemia because of restrictions such as coronary stenosis**.**

1.4 Oxygen delivery, Oxygen uptake and the critical oxygen

The delivery of oxygen (DO2, cardiac output and arterial oxygen content) was compared to oxygen utilization (VO2) **(Cain, 1986)**. This analysis led to the demonstration that as hematocrit is decreased (decreasing DO2), there is no change in oxygen uptake until a 'critical' DO2 is reached, at which point oxygen delivery to tissue can no longer be sustained **(Figure 1.2)**. Thus, VO2 is limited by oxygen demand above the critical DO2 and limited by supply below the critical DO2. Patients are in serious danger of organ failure if DO2 is allowed to drop below the critical value, and the goal of transfusion therapy, is to maintain DO2 well above that value so that an appropriate reserve of oxygen is maintained should the patient require it because of blood loss or elevated oxygen requirement.

In summary, the capacity to regulate the supply of oxygen at a given level of arterial blood oxygen content is achieved by a combination of the ability of the heart to increase its output in response to increasing oxygen need, and the ability of the microcirculation to redirect blood flow

to capillary networks by a system of vasoconstriction and vasodilation that operates at different levels of the circulation.