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Blood Substitutes as Pharmacotherapies in Clinical Practice

Essay Submitted for Partial Fulfillment of Master Degree in Anesthesiology

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Contents

List of tables

List of Figures

List of Abbreviations

2,3 DPG	2,3 Diphosphoglycerate
ADP	Adenosine diphosphate
AFGPS	Antifreeze glycoproteins
AIDS	Acquired immune deficiency syndrome
ALT	Alanine aminotransferase
AML	Acute myeloid leukemia
ANH	Acute normovolemic hemodilution
ATP	Adenosine triphosphate
CAT	Catalase
CDC	Centers For Disease Control
CFU	Colony-forming unit
CSF	Colony stimulating factor
DCLHb	Diaspirin cross-linked haemoglobin
DDAVP	1-deamino-8-D-arginine vasopressin
DIC	Disseminated intravascular coagulation
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
ED	Emergency Department
ELISA	Enzyme-linked immunosorbent assay
EPO	Erythropiotin
FAMS	Fibrinogen-coated albumin microcapsules
FDA	Food and drug association
FNHTR	Febrile nonhemolytic transfusion reactions

G-CSF	Granulocyte colony stimulating factor
GM-	Granulocyte–macrophage
GP	Glycoprotein
GVHD	Graft-vs-host disease
Hb	Hemoglobin
HBcAg	Hepatitis B core antigen
HBOC	Hemoglobin based oxygen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HLA	Human leucocytic antigen
HNA	Human neutrophil antigen
Ig	Immunoglobulin
IL	Interlukin
INF	Interferon
IPMS	Infusible platelet membranes
LEH	Liposome encapsulated hemoglobin
LPI	Labile plasma iron
LPS	Lipopolysaccaride
NADPH	Nicotinamide adenine dinucleotide phosphate oxidase
NO	Nitric oxide
NTBI	Non-transfusion-bound iron

P_{50}	PO2 at which 50% of hemoglobin is saturated
PCR	Polymerase chain reaction
PFC	Perflourocarbon
PFCE	Perfluorocarbon emulsion
PMN	Polymorphonuclear leukocytes
Po_2	Partial pressure of oxygen
RBC	Red blood cell
RCT	Ranomized clinical trials
RES	Reticuloendothelial system
RGD	Arginine-Glycine-Aspartic acid
SO2	Oxygen saturation
SOD	Superoxide dismutase
TNF	Tissue necrosis factor
TRALI	Transfusion-related acute lung injury
TRIM	Transfusion-associated immunosuppression
TTBIs	Transfusion transmitted bacterial infections
UVA	Utraviolet A
WBC	White blood cell
WHO	The world Health Organization

Introduction

Developing a safe blood substitute has been a goal of medical researchers for decades, promoted by the traumas and recognition of blood-borne infections, especially hepatitis B and C, and Acquired Immunodeficiency Syndrome (AIDS). '*H*emoglobin-*b*ased *o*xygen *c*arriers' (HBOCs) prepared from various sources (human, bovine, and recombinant) have been investigated. While no HBOC is anticipated to replace allogeneic blood, a safe HBOC would facilitate hemodynamic stabilization until blood is available, and do so without concern for infectious agent transmission or transfusion reaction. HBOCs also have long shelf lives, a benefit when blood is in short supply or unavailable.

Human blood is a heterogeneous mixture of cells suspended in liquid plasma, which is itself a solution of functionally interacting proteins in an electrolyte buffer. The risks of allogeneic transfusion extend beyond microbial transmission to include allergy, allo-immunization, bacterial sepsis, graft versus host disease, transfusion-related acute lung injury (TRALI), renal failure,

volume overload, and immunosuppression ***(Despotis et al., 2007).***

Improper collection and/or storage conditions can lead to bacterial contamination that may be associated with life-threatening infections. Transfused blood is also an important cause of perioperative anaphylaxis and life-threatening hypersensitivity reactions ***(Levy and Adkinson, 2008).***

Thus, transfusions pose important risks beyond concerns about availability. Perhaps of equal importance is the development of an oxygen-carrying blood substitute for battlefield use. In shock and trauma, an oxygen-carrying agent or HBOCs could provide a "bridge to transfusion" by temporarily increasing oxygen-carrying capacity and expanding intravascular volume until hemostasis can be established ***(Greenburg and Kim, 2004).***

Blood substitutes differ from conventional crystalloid or colloid solutions in that they must transport oxygen in excess of what can be dissolved into balanced salt buffer. Many blood substitutes utilize natural hemoglobin chemistry to achieve oxygen transport ***(Stowel, 2008).*** An ideal blood substitute should also have the oxygen-carrying capacity of hemoglobin, be less antigenic,

require no compatibility testing, have a long shelf life (preferably at room temperature), have a long intravascular half life, and be free of toxicity, side effects, and pathogens ***(Greenburg and Kim, 2004).***

The two major classes of oxygen-carrying blood substitutes studied are the HBOCs and perfluorocarbon (PFC) emulsions.

Other blood substitutes include: platelets substitutes and white blood cell substitutes. The available white blood cell substitute is the granulocyte-colony stimulating factor (G-CSF), which is a proliferation, differentiation, survival, and activation factor for hematopoietic cells of the restricted neutrophilic granulocyte lineage ***(Skoda, 1999).***

Platelets must be stored in plastic bags that are permeable to oxygen and carbon dioxide, at 22° C, and have a shelf life of 5 days. This rapid outdate adds additional constraints to an already limited supply ***(Kresie, 2001).*** Platelet substitutes include: fibrinogen-coated albumin microcapsules (FAMs), infusible platelet membranes, red cells with surface-bound fibrinogen, lyophilized human platelets, freeze dried platelets and liposome-based agents ***(Wheeler, 2007).***

Aim of the work

The aim of the work is to discuss the development and current status of blood substitutes, including hemoglobin-based oxygen carriers (HBOCs) and perfluorocarbons, highlight the research in this field and its impact on the future of transfusion medicine in the operating room, as well as in trauma situations.

PHYSIOLOGY OF BLOOD CELLS

Blood consists of cellular part and non cellular part or plasma. Cellular part includes red blood cells **(RBCs)**, white blood cells and platelets **(figure 1).**

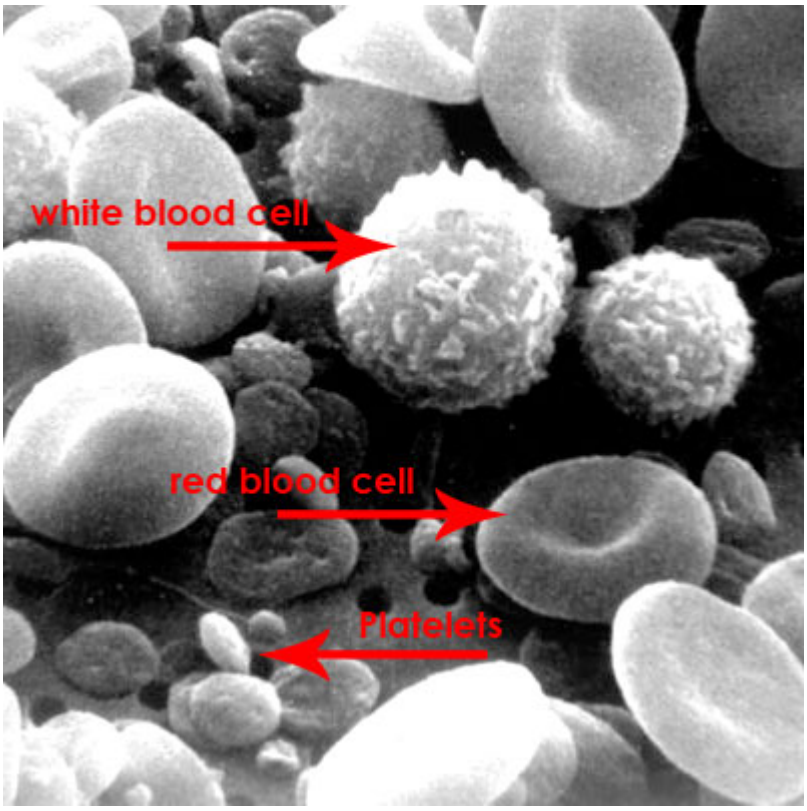

Figure (1): A scanning electron microscope image from normal circulating human blood. Photographers Bruce Wetzel/Harry Schaefer, courtesy National Cancer Institute

I- RED BLOOD CELLS

Red blood cells (Erythrocytes) are biconcave discs that are manufactured in the bone marrow. In mammals, they lose their nuclei before entering the circulation. In humans, they survive in the circulation for an average of 120 days. The average normal red blood cell count is 5.4 million/uL in men and 4.8 million/uL in women. Each human RBC is about 7.5 μm in diameter and 2 μm thick, and each contains approximately 29 pg of hemoglobin (Hb). There are thus about 3×10^{13} RBCs and about 900 g of hemoglobin in the circulating blood of an adult man ***(Ganong, 2003-a).***

The major functions of erythrocytes are to carry oxygen taken in by the lungs and carbon dioxide produced by cells. Erythrocytes contain large amounts of the protein hemoglobin with which oxygen and, to a lesser extent, carbon dioxide reversibly combine. Oxygen binds to iron atoms (Fe) in the hemoglobin molecules ***(Vander et al., 2001-a).***

Erythrocytes are completely dedicated to their job of respiratory gas (oxygen and carbon dioxide) transport. Hemoglobin, the protein that makes red RBCs red, binds easily and reversibly with oxygen, and most oxygen carried

in blood is bound to Hb. Normal values for Hb are 14–20 grams per 100 milliliters of blood (g/100 ml) in infants, 13–18 g/100 ml in adult males, and 12–16 g/100 ml in adult females ***(Marieb and Hoehn, 2007).***

Hemoglobin is made up of the protein globin bound to the red heme pigment. Globin consists of four polypeptide chains—two alpha (α) and two beta (β)—each bound to a ringlike heme group. Each heme group bears an atom of iron set like a jewel in its center **(figure 2)**. Since each iron atom can combine reversibly with one molecule of oxygen, a hemoglobin molecule can transport four molecules of oxygen. A single RBC contains about 250 million Hb molecules, so each of these tiny cells can scoop up about 1 billion molecules of oxygen ***(Marieb and Hoehn, 2007).***

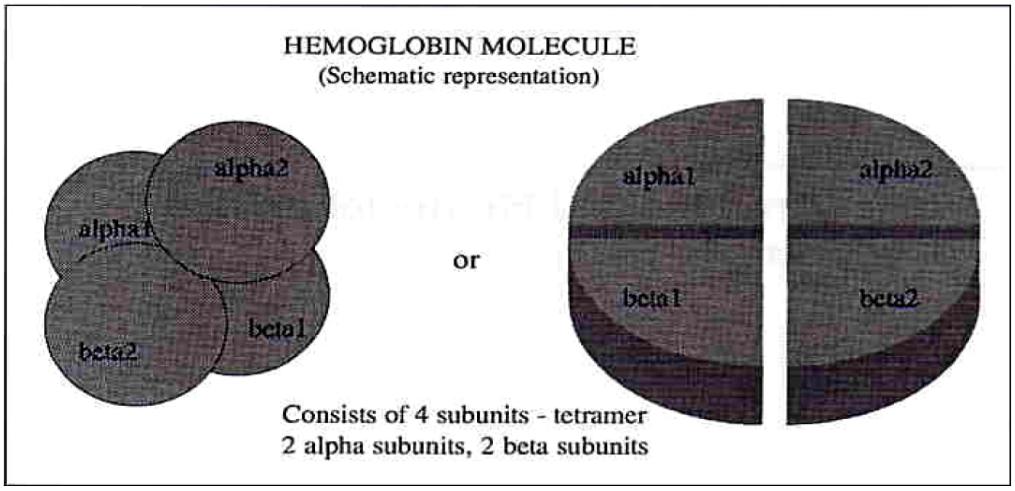

Figure (2): schematic representation of the structure of hemoglobin. Reprinted with permission from Chang TMS.Biomaterials, Artificial Cells and Immobilization Biotechnology 1992; 20:154-174.

The fact that Hb is contained in erythrocytes, rather than existing free in plasma, prevents it from breaking into fragments that would leak out of the bloodstream (through the rather porous capillary membranes) and from contributing to blood viscosity and osmotic pressure ***(Marieb and Hoehn, 2007).***

Oxygen loading occurs in the lungs, and the direction of transport is from lungs to tissue cells. As oxygen-deficient blood moves through the lungs, oxygen diffuses from the air sacs of the lungs into the blood and then into the erythrocytes, where it binds to Hb. When oxygen binds to iron, the Hb, now called oxyhemoglobin, assumes a new three-dimensional shape and becomes ruby red. In the