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Introduction

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The red blood cell (RBC) is a magnificently engineered apparatus. Manifestations of a deficiency or genetic error of red cells or their principal constituent, hemoglobin (Hb), are varied and may be severe or fatal. Recognition of the need for augmentation of native red cells from an external source initiated and propelled the fields of blood banking and transfusion medicine, and contributed substantially to the development of hematology and immunology.

Initial transfusion of blood from animal and human sources was performed more than three centuries ago [1]. The subsequent path has been tortuous and at times tortured. Discovery of human blood types by Landsteiner [2, 3] and subsequent development of knowledge of immunology and the science and technology of blood and blood component storage has allowed for the current relative safety of transfusion, while at the same time permitting the advancement of several areas of clinical medicine such as surgery, anesthesiology, and hematology.

However, regulatory criteria for blood or RBC approval do not definitively address efficacy or safety. The US Food and Drug Administration (FDA) requires that blood or RBC units for transfusion have a mean unit RBC recovery of $\geq$75% at 24 hours after transfusion, with a standard deviation of $\leq$9%, and hemolysis of $<$1.0%. These types of criteria were developed in the 1970s, and although they have slightly been modified since then, they cannot be regarded classically as specifying efficacy and safety. Thus, it is not surprising that there is conflicting evidence for both of these issues.

Red-cell efficacy has never been a condition for regulatory approval. The decrease of 2,3-DPG [4, 5] and increase of Hb affinity for oxygen (decreased p50) with storage

Chemistry and Biochemistry of Oxygen Therapeutics: From Transfusion to Artificial Blood, First Edition.
Edited by Andrea Mozzarelli and Stefano Bettati.

duration (see Chapters 17 and 18, and [5]) led many to hypothesize that stored transfused red cells could not offload oxygen until 2,3-DPG was regenerated, a process that takes many hours [6, 7]. An experiment in normal healthy humans found that RBCs stored for three weeks are as efficacious as fresh RBCs (stored for $\leq$3 hours) in reversing anemia-induced cognitive deficits [8]. However, there are no large clinical prospective randomized trials examining *in vivo* efficacy of stored RBCs.

Similar to the issue of efficacy, safety has never been a condition for FDA approval for unmodified blood or red cells (aside from donor screening and testing for markers of selected infectious diseases). Red-cell transfusion is not without risks, including disease transmission (viral, bacterial, parasitic), transfusion-associated lung injury (TRALI), hemolytic transfusion reactions, graft versus host disease, immunomodulation, circulatory overload, and perhaps risks associated with the age of stored red cells (see Chapters 13 and 15). The risk of transfusion-transmitted infectious disease is at an all-time low in many countries [9, 10], but is substantially higher in many others. Furthermore, there is a continual possibility of emergence of new pathogens or mutation of existing vectors, or of introduction of an existing pathogen into a geographically naive population or blood supply owing to increased international travel. Current blood-banking procedures and processes center on donor selection, testing of donor blood, and quality control. However, these measures do not address storage lesions or potential resultant clinical outcomes.

Many RBC changes that occur with storage likely do not have important clinical safety implications. However, laboratory studies have shown that lysophosphatidyl cholines accumulate with blood storage [11], and when added to perfusates of isolated rat lungs [12] or injected in rats, produce pulmonary injury, as do plasma [12] and lipid extracts of plasma of blood stored for 42 days [12].

Numerous publications, including original reports, reviews [13], and commentaries [14], have sought to examine a potential association of storage duration of transfused RBCs with adverse outcomes, with both positive and negative findings. The original reports are largely retrospective examinations of databases of varying sizes and scopes, with disparate results. For example, Koch *et al*. noted that transfusion of blood stored for more than 14 days compared to blood stored for a lesser period was associated with a substantial increased risk of mortality, renal failure, and sepsis or septicemia in their retrospective analysis of a single-center database of 2872 patients who underwent cardiac surgery [15]. On the other hand, more recently Edgren *et al*. examined the Danish and Swedish database of nearly 400 000 transfused patients and found no association between duration of storage of red cells and mortality within seven days of transfusion, and a 5% risk for long-term follow-up, which they attributed to confounding factors [16]. Retrospective database analyses are subject to many potential confounders, including severity of illness [16] and number of units transfused [17]. Some retrospective analyses, when corrected for either or both of these, find that a potential association no longer pertains [18]. Most importantly, retrospective analyses cannot account for the clinical reason for the transfusion: frequently blood is transfused in an attempt to obviate complications in a more sick population or to treat complications that tend to occur in more sick patients. In as much as there are no prospective randomized controlled trials adequately examining such a putative association, four such trials (two in Canada and two in the USA) have been initiated.

Issues surrounding transfusion have prompted alternative approaches. The rationale and the science and clinical application(s) for synthetic or semisynthetic oxygen carriers have undergone substantial development over the many years of investigation. There is little question that the various molecules that have been developed carry and offload oxygen. A wide variety of laboratory experiments attest to that, and need not be described here. There are several potential clinical applications for the use of these biologics: (i) in preference to transfusion of RBCs to prevent or treat ischemia, while avoiding the potential adverse effects of transfusion (see Chapter 10); (ii) for delivery of oxygen to tissues that may be inaccessible to red cells (e.g. to provide oxygen to tissues beyond a tight arteriolar fixed stenosis); (iii) when compatible or any red cells are not available (e.g. pre-hospital trauma; mass disaster; recipient rare red-cell type); (iv) in cases of recipient refusal of transfusion (e.g. religious reasons) (see Chapter 14); (v) for tumor therapy; and (vi) as a "place holder" to conserve red cells in cases of substantial hemorrhage and transfusion. These potential applications are all discussed in this volume and do not require elaboration here. Other therapies that increase Hb and red-cell mass, such as erythropoietin or iron therapy (oral or intravenous), do not act sufficiently rapidly to satisfy these indications for an acute need. Hence the need for development of oxygen carriers capable of providing oxygen rapidly to tissues/organs with these acute needs.

The first infusions of free Hb in humans are attributed to Sellards and Minot, who used a preparation of unmodified hemolyzed RBCs [19]. Substantial development with partial purification of Hb by Amberson *et al*. followed in the 1940s [20]. Further experimentation was delayed owing to the observed deleterious renal effects [20]. In the 1960s and 1970s Rabiner [21, 22] and Savitsky [23] infused somewhat purified Hb solutions and although these investigators named their preparations "stromal-free", stroma removal was incomplete (more than 1% of stroma remained) [23] and the Hb molecule was unmodified, allowing for dissociation to dimers and monomers. These preparations did not eliminate renal function impairment or hemoglobinuria. Chemical crosslinking and/or polymerization of native human or bovine Hbs [24], or crosslinking of recombinant human Hb at the genetic level [25–27], and improved purification techniques resolved the renal toxicity of at least some [28], but not all [29, 30], of these preparations.

The central difficulty for the clinical development of non-red-cell oxygen carriers has been real and perceived issues of safety and adverse effects. The first phase III clinical trial conducted under the aegis of 21CFR50.24 [31], exception from informed consent, in the USA resulted in increased mortality in those patients receiving the hemoglobin-based oxygen carrier (HBOC) [32], casting a pall over further clinical development. Several HBOCs have undergone phase II and phase III clinical trials and while at times they have shown potentially promising efficacy, they simultaneously produced a variety of adverse events, including myocardial, vascular, renal, and gastrointestinal disturbances. The thought that these might be a class effect in part led to an FDA/National Institutes of Health (NIH)-sponsored conference in 2008. Publicly available information regarding the safety profile of human exposure was reviewed, largely confirming the increased incidence of these adverse events [29, 30]. More recently, another phase III clinical trial in pre-hospital trauma failed to meet its end point of mortality non-inferiority [33]. The incidence of adverse events and failed clinical trials poses substantial challenges to further development of HBOCs.

Many at the 2008 FDA/NIH conference suggested that further understanding of the mechanism of the noted adverse events should precede clinical development. Many believe that Hb scavenging of nitric oxide [34] can explain all of the noted adverse findings [29, 30]. Included in these effects could be: limitation of vascular dilation with resultant hypertension; inability to respond to tissue/organ hypoxia, perhaps causing the myocardial and renal injury; constriction of the sphincter of Oddi, resulting in symptoms in unmedicated conscious humans [28] and perhaps the elevated pancreatic and hepatic enzymes; and, as has been suggested [29, 30, 35], the interaction of NO with platelets [36, 37] and the inflammatory response (see also Chapters 4, 5 and 17).

As always, regulatory approval and clinical use of any drug, biologic, or device, requires judgment as to the balance of the risk and benefits of a proposed therapy, drug, or biologic and the alternative. Thus, adverse effects of HBOCs should not be examined in isolation, but rather in juxtaposition with the alternatives: red-cell transfusion or lack of transfusion – that is, acute anemia. Some of the risks of RBC transfusion have been highlighted above.

Not transfusing RBCs when Hb concentration requires augmentation (acute anemia) has the risk of inadequate tissue oxygenation. Normal humans demonstrate reversible (by red-cell transfusion [8, 38] or breathing oxygen [39]) inadequate cerebral oxygenation at Hb concentrations of 5 g/dL and 6 g/dL [8, 38–40]. Retrospective database analyses have found an association of mortality with anemia [41–44]. Full examination of the risks of transfusion or acute anemia (not transfusing) is beyond the scope of this introduction or this volume, but has been reviewed succinctly recently [45].

The NIH/FDA conference and the symposium that prompted this book contained forward-looking elements. The former suggested that populations be studied where there is a favorable balance of benefits and risks. However, risks for acute anemia, transfusion, and likely HBOCs are not uniform across all populations. Formal analysis of HBOC risk by patient age, disease processes, and other important risk factors might identify populations of improved benefit : risk. The symposium, as delineated in this book, focused on current research intended to improve the balance of benefits and risks. The reader will find approaches that include development of encapsulation of Hb (see Chapter 27), further molecular modifications (see Chapters 24, 25 and 26), large naturally occurring Hbs, and red-cell "pharming" (see Chapter 19). Only time will tell whether any of these will succeed. In addition to the issue of resolution of HBOC-induced adverse events, other questions remain. While the pharmacodynamics of HBOCs has received much attention, the pharmacokinetics has not. What should be the ideal pharmacokinetic profile remains an unresolved issue. Clinical development of HBOCs must also focus on indications that have valid end points for which practical and feasible clinical trials with reasonable cost can be implemented and completed. This area of endeavor has vigor and hope, but can retain that only insofar as these questions can be resolved.

One symposium or volume cannot cover this entire field of research and development. This book emanates in large measure from the XXII Symposium on Blood Substitutes held in Parma, Italy in 2009. The intention of that symposium and this book is to relate and discuss the current state of the art. A perusal of the chapter titles and their authors should quickly impress the reader that the meeting organizers and editors have gathered the experts on these subjects, who have succeeded admirably in that endeavor.

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