LECTURE

The unusual properties of effective blood substitutes

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Abstract. Blood substitutes or oxygen carrying plasma expanders were originally formulated to simulate the transport properties of blood, particularly oxygen carrying capacity, viscosity, p50, and colloid osmotic pressure, under the hypothesis that blood is the most desirable fluid in volume restitution. However, changes introduced into the organism during hemorrhage adversely affect microvascular function due to reflex vasoconstriction which causes the fall of functional capillary density, and lowers tissue oxygenation, conditions that are not universally reversed with retransfusion of blood. The restoration of microvascular function is seldom complete upon retransfusion of blood. New formulations of hemoglobin molecules in solutions whose oncotic pressure is in the range of 60–100 mmHg, p50 is about 5 mmHg, viscosity 3–4 cP, and oxygen carrying capacity in the range of 4–7 g/dl equivalent hemoglobin deliver better microvascular function after resuscitation when compared to whole blood and oxygen carrying plasma expanders with transport properties similar to those of blood. The improved performance is in part due to the increased plasma viscosity which increases capillary transmural pressure which reverses capillary collapse induced during low perfusion pressures. High oncotic pressure reinforces this effect, since it brings more fluid into the circulation. Microvascular transport studies of the effects of resuscitation in shock show that functional capillary density is the primary determinant of survival, thus maintenance of an open and fully perfused microcirculation is more critical than insuring oxygen supply, since closed capillaries lead to the accumulation of slowly diffusing byproducts of metabolism which ultimately become toxic. The required combination of properties can be achieved by conjugating hemoglobin and polyethylene glycol. Resuscitation fluids based on hemoglobin containing vesicles may provide the next level of functional improvement in the formulation of volume restitution fluids since their biophysical properties can be specifically controlled through the inclusion of specialized compounds into the vesicles, and the formulation of the suspending medium. (Keio J Med 51 (1): 17–20, March 2002)

Key words: blood substitutes, hemoglobin, plasma viscosity, functional capillary density

The development of artificial blood, blood substitutes or oxygen carrying plasma expanders (OCPEs) from the onset had as a goal to reproduce the transport characteristic of blood, with some modifications aimed at improving its ability to deliver oxygen derived from the perception that lowered viscosity would improve blood flow and the facilitation of oxygen release would be beneficial. On this basis oxygen carrying capacity and oncotic pressure were prescribed to be within the range found in blood, while the experience with hemodilution suggested that improvements in transport would be obtained by lowering blood viscosity to values significantly below those of whole blood. An additional presumed beneficial modification was introduced when it was found that some of the oxygen carriers based on modified hemoglobins exhibited high p50's, a characteristic that was rationalized to facilitate oxygen unloading and tissue oxygenation. In the course of the rather extended period of development nitric oxide (NO) was found to be a fundamental element in cardiovascular regulation. As acellular modified molecular hemoglobin became the oxygen carrier of choice, it became increasingly apparent that this material is vasoactive, causing hypertension, which was determined to be deleterious in resuscitation. Since hemoglobin scavenges NO leading to vasoconstriction there is to the

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present a significant effort aimed at modifying the hemoglobin molecule so that its affinity for NO is reduced. Thus at present an optimal OCPE would have the following properties: Oxygen carrying capacity equivalent to 10–14 g/dl hemoglobin, p50 > 30 mmHg, viscosity 1 cP, oncotic pressure ~ 25 mmHg, and low NO binding.

Developments in the understanding of microvascular and systemic physiology show that all of these parameter specification are inappropriate for the formulation of an OCPE. Furthermore products that include these specifications are potentially dangerous, which has been shown in practice, and/or impractical in terms of economic considerations. The following discussion analyzes each of the assumptions presently incorporated in OCPEs being developed or in clinical trials.

Oxygen Carrying Capacity

Measurements in the microcirculation utilizing the technique of phosphorescence quenching (Torres & Intaglietta, 1993)[1] show that when hemodilution is carried out to a total remaining hemoglobin content in red blood cells of 5.6 g/dl, tissue oxygen is higher but not statistically significantly so than normal (Tsai, 2001),[2] thus conservatively one may take a total oxygen carrying capacity of 5 g/dl as an adequate blood oxygen carrying capacity, a number that is well established in the hemodilution literature (Messmer, *et al.*, 1972).[3] It should be noted that even though experiments were carried out in the hamster skin fold, acid base balance was normal in these experiments indicating that the observations reflected conditions in all the tissues. This numerical value for the needed oxygen carrying capacity is reflected also by a simple calculation that relates the whole body oxygen consumption and cardiac output, which yields a nearly identical number for the organism at rest. Therefore in principle the oxygen carrying capacity of an OCPE does not need to reproduce the value for normal blood and can be significantly lower.

The Viscosity of the Circulating Blood

Since oxygen carrying capacity is not the limiting factor at a blood hemoglobin content that is much lower than the blood transfusion trigger, the question arises why significantly higher minimum hemoglobin conditions are taken as the indication for restitution blood oxygen carrying capacity with red blood cells. Analyzing the mechanical and transport conditions of the circulation at the transfusion trigger it becomes apparent that at a hematocrit that is half of normal or lower, blood viscosity is less than half of that in the circulation. Given a notably reduced peripheral resistance the organism attempts to compensate by reflex vasoconstriction, a response that becomes increasingly prominent with further drops of hematocrit (and therefore viscosity). Vasoconstriction accompanied by low blood viscosity depressurizes the capillaries causing them to collapse, thus lowering functional capillary density (Lindbom and Arfors, 1985).[4]

Functional capillary density is a critical parameter in tissue survival, as shown by the study of Kerger, *et al.*, 1996,[5] who found that this is the only functional anatomical event that separates surviving from non surviving animals subjected to hemorrhagic shock. Conversely there was no difference in tissue oxygenation between these groups. Consequently the clinical evidence calling for the transfusion of blood is determined in no small part by capillary collapse, which causes the accumulation of products of metabolism in the tissue. It should be evident that further diluting blood beyond the transfusion trigger with any low viscosity plasma expander including oxygen carriers, further aggravates this process. When the plasma expander is also a vasoconstrictor such as in the case of αα cross linked hemoglobin the organism is placed in a precarious condition.

Reversal capillary collapse in extreme hemodilution cab be obtained by increasing plasma viscosity as demonstrated in the study of Tsai, *et al.*, 1998,[6] who induced hemodilution with dextran 70 kDa and continued unto extreme hemodilution with dextran 500 kDa, achieving a circulating plasma viscosity of 2.8 cp and a near normal functional capillary density. This condition was not attained if extreme hemodilution was performed with either dextran 70 kDa or the Biopure product Oxyglobin (Biopure Inc., Boston, MA), even though in the later case total hemoglobin content was 6.7 g Hb/dl (Tsai, 2001).[2] Figure 1 summarizes these findings. A remarkable side effect of extreme hemodilution with high viscosity plasma is that blood flow is significantly increased above control non-hemodiluted values,[6] a phenomenon attributable to the release of shear dependent generated endothelial relaxing factors (Frangos, *et al.*, 1985; de Wit, *et al.*, 1997).[7,8]

This analysis shows that a viscosity formulation leading to high plasma values is a critical factor in resuscitation, since the administration of an OCPE is made in conditions of extreme hemodilution, and given that there is no need for using these products prior to reaching the transfusion trigger.

The Appropriate Value for p50

Various cross linked or polymerized hemoglobins that have been developed as the oxygen carrier for OCPEs have resulted in a high value of p50, which has been incorporated unto the product under the assumption that it is beneficial because it facilitates oxygen

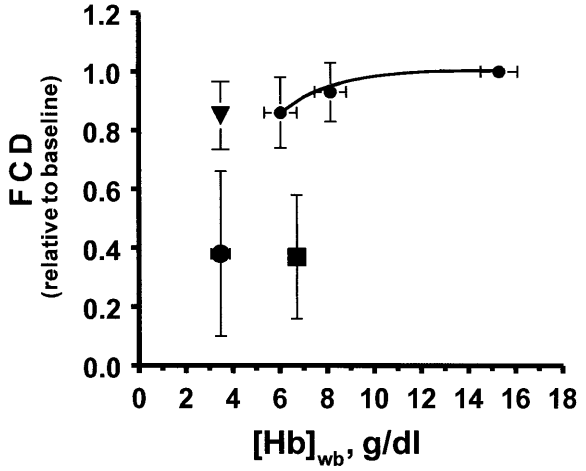

Fig. 1 Experimental findings in the microcirculation of the awake hamster model during extreme hemodilution with low and high viscosity plasma expanders. Acute moderate hemodilution is induced with dextran 70 kDa (●) until 60% of the red cell concentration has been replaced. Continuing exchange transfusions were made with different viscosity solutions and are presented as a function of hemoglobin. The solutions used were dextran 70 kDa (●), dextran 500 kDa (▼), and Oxyglobin™ (■) which have viscosities of 1.8, 6.4 and 2.8 cp. Both low visocisity solutions caused vasoconstriction, decreased functional capillary density, and low perfusion. Conversely, if plasma viscosity is restored by using 500 kDa dextran, hemodilution can be continued to extreme values. In the situation microvascular function is maintained, functional capillary density returns to normal, and tissue perfusion increase above baseline.[9]

unloading and tissue oxygenation. The problem is that the partial pressure of oxygen in the microcirculation is regulated so that there is a significant decrease in oxygen tension from the systemic circulation to the capillaries which typically have a pO_2 of about 30 mmHg as they branch from the arterioles. Since blood p50 is 28 mmHg (human, hamsters) half of the blood oxygen is delivered by arterioles in normal conditions, however if p50 of the OCPEs is above this value as in the case of Oxyglobin (p50 = 40 mmHg) most of the oxygen in blood is delivered by the arterioles.

Current results on the partition of oxygen supply between arterioles and capillaries shows that these vessels extract a significant amount of oxygen from the circulation while consuming a major portion of this oxygen flux (Tsai, *et al.*, 1998)[9] and this consumption is directly dependant on the oxygen availability in blood. The net effect of this process is that if too much oxygen is unloaded prior to arrival to the capillaries and this oxygen is consumed by the arterioles, it does not reach the tissue.

McCarthy, *et al.*, 2001,[10] identified the mechanism of facilitated diffusion as an additional process that has the potential of over oxygenating the arteriolar wall. The mechanism takes place at the blood tissue interface where oxygen traverses the red cell poor or free plasma layer between the blood column and tissue. In this space oxygen diffusion takes place by the gradient of pO_2 which also determined the gradient of oxy-hemoglobin (Nishide, *et al.*, 1997).[11] As a consequence of the large amount of oxygen bound by hemoglobin, the flux of oxygen determined by the flux oxy-hemoglobin is similar for concentrations of hemoglobin that are relevant for their use as an oxygen carrying plasma expander, therefore this flux is also determined by the p50 of the hemoglobin in solution and is increased if this has a high value. Taking these factors into consideration the appropriate p50 for a noncellular, molecular hemoglobin based OCPE should be low in such a fashion that oxygen is delivered only where it is need, *i.e.*, the anoxic portions of the tissue. This approach renders these formulations similar to therapeutic agents with targeted drug delivery, where the agent in this case is oxygen, delivered only where it is required.

Colloid Osmotic Pressure

In the design of a solution for plasma expansion it has usually been assumed that the colloid osmotic pressure should be similar to that of blood and in the range of 20–25 mmHg. However a variety of plasma expanders have zero colloid osmotic pressure (saline, Ringer's lactate) and the process of small volume resuscitation utilizes fluids with very high osmotic properties. Resuscitation with non colloidal fluids leads to tissue edema and is not universally indicated. Conversely there are some advantages in using fluids with high colloidal and osmotic pressures, since they cause tissue fluid to come in the vascular compartment. Of particular interest is that most conditions of hemorrhage are associated with endothelial edema, which has been demonstrated to be rapidly reversed upon the introduction of hyperosmotic and hyperoncotic fluids (Mazzoni, *et al.*, 1990).[12] This effect is specially relevant in view of the importance of maintaining functional capillary density as a part of the process of recovery of the injured organism.

Synthesis of an Effective Oxygen Carrying Plasma Expander

Taking this collection of factors into consideration for the design of an OCPE the fluid that emerges has fundamentally different properties than blood, since its oxygen carrying capacity is low, about one third of normal, p50 is equally low and in the neighborhood of 5 mmHg, while viscosity, so that when introduced into the circulation plasma, viscosity should be of the order of 3 cP, and colloidal osmotic pressure is also high with values greater than 50 mmHg. A fluid with these prop-

erties (Hemospan, Sangart Inc., San Diego) is achieved by conjugating hemoglobin with polyethylene glycol, and various formulations have been tested in both animal experiments and human trials with excellent results (Winslow, *et al.*, 1998).[13] Furthermore the NO scavenging characteristics do not appear to be relevant or significant since these fluids have the same NO binding constant as other formulations that are vasoactive (Rolhfs, *et al.*, 1998).[14]

These fluids may in some cases be more effective than blood because they are specifically designed to maintain functional capillary density, which is as necessary as restoring tissue oxygenation for the recovery from blood losses. This process is not intrinsic to blood transfusions, because blood does not increase plasma viscosity, which is necessary for re-pressurizing the capillaries.

In the foreseeable future OCPEs will be based on human hemoglobin therefore fluids designed according to the new paradigm are practical since their hemoglobin content is low and more than two units of blood equivalent unit of resuscitation fluid can be obtained from one blood. Finally this low oxygen carrying capacity is practical and safe because it is combined with a significant improvement of microvascular function, therefore these fluids have also therapeutic properties aimed at improving the microcirculation.

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