Biochimica et Biophysica Acta 1784 (2008) 1421–1427

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Review

Microvascular experimental evidence on the relative significance of restoring oxygen carrying capacity vs. blood viscosity in shock resuscitation

Beatriz Y. Salazar Vázquez [a,d], Reto Wettstein [b], Pedro Cabrales [c], Amy G. Tsai [c,d], Marcos Intaglietta [d,*]

[a] Faculty of Medicine, Universidad Juárez del Estado de Durango, 34000 Victoria de Durango, Durango, Mexico
[b] Department of Plastic and Reconstructive Surgery, University Hospitals of Geneva, Switzerland
[c] La Jolla Bioengineering Institute, La Jolla, California 92037, USA
[d] Department of Bioengineering, University of California, San Diego, La Jolla, California 92093, USA

ARTICLE INFO

Article history:
Received 26 October 2007
Received in revised form 15 April 2008
Accepted 24 April 2008
Available online 4 May 2008

Keywords:
Plasma expander
Blood viscosity
Oxygen carrying capacity
Blood substitute
Hemorrhagic shock
Microcirculation

ABSTRACT

The development of volume replacement fluids for resuscitation in hemorrhagic shock comprises oxygen carrying and non carrying fluids. Non oxygen carrying fluids or plasma expanders are used up to the transfusion trigger, and upon reaching this landmark either blood, and possibly in the near future oxygen carrying blood substitutes, are used. An experimental program in hemorrhagic shock using the hamster chamber window model allowed to compare the relative performance of most fluids proposed for shock resuscitation. This model allows investigating simultaneously the microcirculation and systemic reactions, in the awake condition, in a tissue isolated from the environment. Results from this program show that in general plasma expanders such as Ringer's lactate and dextran 70 kDa do not sufficiently restore blood viscosity upon reaching the transfusion trigger, causing microvascular collapse. This is in part restored by a blood transfusion, independently of the oxygen carrying capacity of red blood cells. These results lead to the proposal that effective blood substitutes must be designed to prevent microvascular collapse, manifested in the decrease of functional capillary density. Achievement of this goal, in combination with the increase of oxygen affinity, significantly postpones the need for a blood transfusion, and lowers the total requirement of restoration of intrinsic oxygen carrying capacity.

1. Introduction

The transfusion trigger is usually defined by an array of physiological parameters which in combination with decreased intrinsic oxygen carrying capacity of blood (circulating hemoglobin concentration, or hematocrit, Hct) determines the decision to switch from volume maintenance by means of plasma expanders to the transfusion red blood cells (RBCs). It is virtually universally perceived that the requirement for blood is determined by the impending risk of anoxia, particularly in the vital organs, and it is generally accepted that the transfusion of RBCs improves patients' conditions.

Whether the beneficial effect of a blood transfusion is due to the restoration of oxygen carrying capacity is debatable, because RBCs in general release less oxygen upon transfusion, particularly if they have been stored for some time (more than a few days) [1]. The almost universal practice of transfusing the oldest RBCs in storage ensures that in most instances there is a comparatively reduced initial restoration of oxygen carrying capacity, since full oxygen delivery is re-established several hours after a blood transfusion.

Blood transfusion are usually called for when the circulating blood hemoglobin reaches the level of 7 g/dl, this value being lower in young, normal individuals, and higher for elder persons. When blood reaches this hemoglobin concentration, Hct and all blood factors have fallen to about half of the normal value. A parameter that may decrease to a greater extent is blood viscosity, due to its approximately exponential dependence on Hct [2]. Notably blood viscosity is a parameter that directly links systemic blood pressure and cardiac output. It is a well established fact that at hemodilution levels of about 50% of normal Hct there is no fall in either blood pressure or cardiac output if normovolemia is maintained [3]. An explanation for this result is that this level of hemodilution elicits an increase of cardiac output and some level vasoconstriction, probably due to the combination of baroreceptor reflexes, and the decrease of shear stress on the vascular wall, which lowers nitric oxide (NO) production promoting vasoconstriction and the increase of peripheral vascular resistance [4].

2. Microvascular function in vasoconstriction

In clinical settings related to different hipotensive shock conditions vasoconstriction is instituted to ensure the perfusion of central organs. As an example, vasopressin is prescribed for increasing blood pressure when the organism does not respond to other interventions [5]. A

* Corresponding author. Department of Bioengineering, University of California, San Diego, 9500 Gilman Dr. La Jolla, CA 92093-0412, USA. Tel.: +1 858 534 4275.
E-mail address: mintagli@ucsd.edu (M. Intaglietta).

1570-9639/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbapap.2008.04.020

hidden component of this process is that vasoconstriction mostly occurs at the level of arterioles, whereby upstream blood pressure is increased, while pressure in the capillary compartment is lowered [6]. While in principle this accomplishes the objective of elevating the pressure necessary for perfusion, there is evidence that increasing blood pressure by causing vasoconstriction has potentially negative consequences since it lowers functional capillary density (FCD).

A critical element of this process is the evolution of FCD, a parameter used in microvascular physiology to describe microscopic tissue perfusion. It quantifies capillary perfusion by measuring the total length of capillaries that have transit of RBCs in a period of 30 s per unit area of tissue observed by intravital microscopy. The significance of FCD was first proposed by Fagrell [7], who established its relevance in tissue survival. In hemorrhagic shock survival is determined by maintenance of FCD and is mostly independent of tissue oxygenation [8].

Attempting to re-establish perfusion of the vital organs by vasoconstriction to elevate blood pressure has a negative impact on capillary perfusion because FCD is a function of local capillary pressure, which lowers downstream of the constricted arterioles. Maintenance of adequate levels of FCD is usually associated with an adequate supply of oxygen to the tissue. Additionally functional capillaries are also necessary for the extraction of the products of metabolism from the tissue which otherwise accumulate. This is evidenced in extreme hemodilution, a process that affects tissue oxygenation independently of the changes of FCD, but presents a significant difference in outcome in terms of acid base balance depending on whether the plasma expander used maintains FCD above a minimum threshold [9,6].

3. What is adequate oxygen delivery?

This question does not have a simple answer because there is no oxygen related parameter that unequivocally characterizes the need to restore or maintain oxygen delivery capacity in the organism, with the exception of obviously extreme cases. In general there is the tendency of over emphasizing oxygen delivery, and upon reaching either measured or perceived borderline conditions blood transfusions are prescribed, overlooking the fact that one in 10 transfusions presents complications, and one in 100 causes severe problems. Tissue pO_2 is often taken as a marker for blood transfusion, however this is a moving target since it is only partially related to oxygen delivery and consumption, and it is not possible to assess in the vital organs.

Conventional tissue oxymetry measures average blood saturation in the tissue, combining arteriolar and venular blood. The pO_2 of mixed venous blood is distantly related to tissue pO_2, and tissue whose metabolic function is severely compromised uses little oxygen and can have a high pO_2, since moribund tissue does not consume oxygen as shown by Cabrales et al. [10] who found that inhibiting tissue metabolism increases tissue pO_2. This study showed that performing an 80% exchange transfusion of molecular hemoglobin caused the reduction of microvascular oxygen delivery and extraction in the tissue of the hamster window chamber and the elevation of interstitial pO_2 above normal levels.

From a physiological viewpoint there are two components that define adequate tissue oxygenation. One is that the tissue must receive oxygen at a rate sufficient to sustain its metabolic requirements. Secondly and directly linked with the need to sustain metabolism, oxygen delivery must also ensure that the tissue is uniformly supplied with oxygen, in such a fashion that there are no anoxic foci, a condition also termed focal ischemia. In this context average tissue pO_2 at rest is usually found to be in the range of 20 to 25 mmHg. A recent study in the hamster window chamber window model, where measurements can be made without anesthesia, and isolated from the environment showed that tissue pO_2 had a Gaussian distribution averaging 20.2±7.7 mmHg, range 4.8-35.5 mmHg. In this study pO_2 was measured in areas 20×20 μm using the phosphorescence quenching [11] technique [12] in regions of the tissue void of microvessels. The absolute value of the standard deviation is significant because it shows what percentage of the tissue may be at risk of being anoxic. In the Gaussian distribution, two standard deviations away from the mean represent 2.5% of the total tissue, or the probability of finding a microscopic region at the risk of anoxia. It should be noted that this variability comprises both the intrinsic biological variability and that arising from the methodology per se. Furthermore there is a natural temporal variability arising from the continuous local adjustment of microvascular flow due to vasomotion [13].

The NAD/NADH (NAD: Nicotinamide adenine dinucleotide) redox state is a sensitive indicator of intracellular oxygen tension [14]. NADH fluorescence is a specific marker of tissue hypoxia and can be determined precisely by optical methods [15]. The decrease of oxygen concentration decreases NAD and other components of the mitochondrial respiratory chain which become reduced [16,17]. The critical interstitial and intravascular pO_2 associated with the switch to anaerobic metabolism is reported to be below 2 and 7 mmHg respectively, indicating that oxygen delivery is regulated at levels well above the minimum required for oxidative metabolism. The oxygen tension required at the mitochondrial level to support oxidative metabolism (critical pO_2) is <1 mmHg, whereas tissue oxygen tension reported in tissues *in vivo* is in the range 15-25 mmHg [15].

These considerations suggest that adequate oxygen delivery should fulfill both a quantitative and qualitative criteria; where for the second requirement the presence of near normal FCD plays a critical role.

4. Resuscitation from hemorrhagic shock

Hemorrhagic shock, even in standardized animal experiments, is a composite pathophysiological condition, where local and central controls interact. Regardless of this inherent complexity, it provides a fairly definitive means for understanding the interplay between perfusion and intrinsic blood oxygen carrying capacity in sustaining the tissue. A conventional shock experiment consists of withdrawing 50% of an animal's blood volume, waiting 1 h, and returning the animal to normovolemia by infusing 25% of the shed volume, using either blood or a plasma expander (the remaining 25% is contributed by autotransfusion from the tissue fluid). A variety of oxygen carrying and non carrying volume restoration fluids can be used experimentally to evaluate the merit of restoring perfusion vs. intrinsic oxygen carrying capacity including conventional plasma expanders, molecular hemoglobin based solutions, vesicle encapsulated oxygen carriers, and the transfusion of blood with modified RBCs.

A meaningful comparison in terms of outcome in considering the different biophysical properties of volume/oxygen carrying fluids could be made by analyzing the outcome of the basic resuscitation fluids that have been studied in the setting of the chamber window hamster preparation. Clearly this model is far removed from the realities of shock resuscitation in humans, however it is a standard model that has allowed investigating both systemic and microvascular parameters in the awake condition, in a tissue that is isolated from the environment, and therefore not subjected to unknown reactions due to exposure. This comparison avoids the need of justifying differences between species, methodologies and laboratory settings.

There is presently a significant background of information on the responses of the hamster chamber window model to a resuscitation following hipovolemic hemorrhagic shock. In the following we will make some basic comparisons by relating the type of resuscitation fluid, its oxygen carrying capacity, and its capacity to restore blood viscosity to near normal levels. The conventional fluids that serve as baseline are Ringer's lactate, blood, 70 kDa dextran and hydroxyethyl starch (HES). These fluids will be compared with results obtained with