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EXCHANGE TRANSFUSION THERAPY AND ITS EFFECTS ON REAL-TIME MICROCIRCULATION IN PEDIATRIC SICKLE CELL ANEMIA PATIENTS: AN INTRAVITAL MICROSCOPY STUDY

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Abstract

Periodic blood exchange transfusion is a treatment modality commonly used to manage pediatric sickle cell anemia (SCA) at the University of California Davis Medical Center. The goal of exchange transfusion therapy is to ameliorate vasoocclusion and improve tissue perfusion by removing sickled red blood cells (RBCs) and introducing normal RBCs. Using computer-assisted intravital microscopy, pre- and post-transfusion microvascular characteristics were analyzed. In this study, the bulbar conjunctiva exhibited a “blanched” avascular appearance in all six pediatric SCA patients prior to transfusion, indicative of tissue hypoperfusion and ischemia. Immediately following transfusion, substantial improvement in vascularization and tissue perfusion resulted, reflected by the enhanced appearance of capillaries and arterioles. In addition, a decrease in red cell velocity was observed. These observations provide evidence that exchange transfusion therapy is beneficial in ameliorating vasoocclusion and improving tissue perfusion. However, with the paradoxical post-transfusion decrease in red cell velocity presumably due to induced hyperviscosity from the large transfusion volume, blood flow is still impaired. This decreased velocity may thwart efforts to improve oxygen delivery via transfusion and may, to some extent, promote vasoocclusion instead. This paradoxical result warrants further investigation on the effects of transfusion volume and viscosity in the exchange transfusion process.

Keywords

Sickle cell anemia; microcirculation; exchange transfusion; vasoocclusion; tissue perfusion; intravital microscopy

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INTRODUCTION

Sickle Cell Anemia (SCA) is an autosomal recessive disease that afflicts ~1 in 500 African American births as well as an emerging population of ~1 in 20,000 Hispanic American births each year. Moreover, SCA is the most common inherited blood disorder in the United States affecting 70,000 to 100,000 Americans [1]. SCA is the prototype of hereditary hemoglobinopathies arising from a single nucleotide mutation in the β -globin chain, which leads to a concomitant polymerization of sickle hemoglobin (HbS) when it is deoxygenated at low oxygen tension. Polymerization of hemoglobin (Hb) causes conformational changes that subsequently transforms the molecules into a network of insoluble fibrous polymers, ultimately resulting in sickling of the red blood cells (RBCs) in SCA patients [2, 3].

Sickled RBCs are characteristically rigid and are adherent to the intraluminal endothelium of blood vessels [4], causing changes in the mechanical shearing of the endothelial surface which, in turn, leads to endothelial dysfunction and related microvascular complications commonly seen in SCA patients [5, 6]. In addition, vasoocclusion, hyperviscosity and blood sludging can cause drastic changes in flow dynamics and tissue perfusion (hypoperfusion), which can result in end-organ complications, tissue ischemia and morbidity in SCA.

Periodic exchange or simple blood transfusion is an accepted treatment modality commonly used to manage SCA with the intended goal of ameliorating vasoocclusion and improving tissue perfusion and blood flow. Several investigators have presented results that confirmed efficacy of transfusion therapy in the primary and secondary prevention of stroke, acute chest syndrome and symptomatic relief of severe anemia [7–10]. Though also used for other indications (e.g., priapism, splenic sequestration), the practice is less evidence-based. Presumably, removing sickled RBCs and replacing them with normal RBCs improves oxygen carrying capacity and tissue perfusion. Complications of transfusion therapy include alloimmunization, hypersensitivity reactions, iron overload, and hyperviscosity [11–15]. Moreover, there may be changes in blood flow dynamics which have not been previously studied.

In the pediatric Sickle Cell Clinic at the University of California Davis Medical Center (UCDMC), periodic exchange transfusion (not simple transfusion) has been adopted as a management modality of choice for children with SCA. Numerous studies have been performed to demonstrate post-transfusion improvement in oxygenation, which can be routinely measured by pulse oximetry [16–18]. However, a search in the literature revealed that real-time changes in blood flow dynamics and microvascular characteristics as well as improvement in tissue perfusion have not been directly investigated [19–21].

The paucity of information related to real-time microvascular changes in SCA arises from a lack of non-invasive, *in vivo* technology which can be used to document and quantify microvascular characteristics and longitudinal changes in SCA patients. Computer-assisted Intravital Microscopy (CAIM) has been developed in our laboratory as a tool to non-invasively study and quantify real-time microvascular characteristics in vascular diseases, including SCA and diabetes [5, 6, 22–24]. A unique operational feature of this technology lies in its ability to identify individual conjunctival vessels and to relocate them in longitudinal studies, with each vessel serving as its own reference control. This makes CAIM an ideal platform to be used in studying pre-transfusion microvascular characteristics and post-transfusion changes.

MATERIALS AND METHODS

Patient Recruitment and Study Design

Six pediatric SCA patients diagnosed with SCA (HbSS) between the ages of four to fifteen years from the UCDMC Sickle Cell Clinic were recruited and enrolled to participate in this study. The subjects were randomly selected from patients who were receiving transfusion therapy for either primary or secondary prevention of disease complications.

The pre- and post-transfusion microcirculation in the bulbar conjunctiva of the eye (conjunctival microcirculation) was imaged via video documentation in the clinic. Videotape sequences on each patient were analyzed for pre-transfusion microvascular characteristics and post-transfusion changes using image analysis procedures developed in this laboratory. The experimental protocol and the study on human subjects were approved by the UCDMC Institutional Review Board. Signed informed consent was obtained from the patient(s) or parent(s)/guardian(s) whichever appropriate. This study was conducted in accordance with the Declaration of Helsinki human-use guidelines.

Exchange Transfusion

In the UCDMC clinic, SCA patients were scheduled for exchange transfusion once a month. Upon arrival at the clinic, pre-transfusion hematocrit and blood pressure measurements were made for each patient to ensure that an exchange transfusion was warranted. Post-transfusion blood pressure measurements were made for future reference. The exchange transfusion procedure was based on a 1:1.5 (v:v) ratio. A *specific* volume of blood (10ml/kg body weight) was phlebotomized from a patient and a 15ml/kg body weight of donor blood was immediately infused. Venous blood sample was obtained from each patient prior to the transfusion process for blood typing and determination of Hb level, percentage HbS, hematocrit (Hct), and other related blood chemistry parameters. Pre- and post-transfusion measurement of oxygen saturation was performed via pulse oximetry.

Computer-assisted Intravital Microscopy (CAIM)

The CAIM technology was used to non-invasively study the conjunctival microcirculation and to quantify pre-transfusion microvascular characteristics and post-transfusion changes in this study. The procedural details have been presented in previous reports and summarized briefly as follows [5, 6]. Patients were asked to relax for at least five minutes and cautioned against touching or rubbing the eye. Non-medicated sterile saline drops were administered in case of eye irritation and excessive drops were blotted. Each patient was asked to place his/her head securely against a forehead restraint and a chin rest. The lens of the CAIM system was carefully positioned to provide a clear, focused image of the perilimbal region in the bulbar conjunctiva of the left eye to give an 8.53 mm² area view of the site of interest. Various areas of the bulbar conjunctiva were videotaped in each patient using a charge-coupled device (CCD) video camera (COHU Model CCD-6415-3000) connected to a digital video recorder (Sony GV-HD700). A fiber-optics light source (Fiber-Lite Model 3100) with a Kodak #58 Wratten green (anti-red) filter was used to enhance contrast of the vessels in image display. The basic design of CAIM utilizes macro-optics as an operational platform so that focusing for image sharpness could be performed only by adjusting the physical distance between the lens and the bulbar conjunctiva. This allows video images to be obtained at the same magnification for all pre- and post-transfusion video recordings. Approximately five minutes of high-resolution video recordings of various areas in the bulbar conjunctiva were captured for subsequent viewing and computational measurements. All recordings were viewed in their entirety and sequences were selected based on image quality by investigators for subsequent analysis and measurements via image analysis. At

least three video sequences with excellent image display from each patient were analyzed and all measurements were averaged.

Data Analysis

Specific microvascular abnormalities (e.g., avascularity, tissue ischemia, abnormal vessel distribution) were identified from viewing of selected video sequences [5, 6]. The same video sequences were analyzed using imaging analysis software (AVID; co-developed at UC Davis and UC San Diego) to generate measurements of vessel diameter and red cell velocity for longitudinal comparison. Pre- and post-transfusion video sequences on the same vessel allowed for direct comparison of real-time microvascular changes to determine any increase or decrease in blood vessel diameter and red cell velocity arising as a result of the exchange transfusion process. This image analysis technique has been previously validated and described in previous studies [5, 6, 22–24]. Of specific interest to this study, emphasis was placed on post-transfusion changes in vessel diameter, red cell velocity and appearance of capillaries and arterioles in previously poorly or non-perfused areas. Other parameters of microangiopathy were also studied but are not reported in this manuscript.

Statistical Analysis

All measurements were averaged and results reported as mean $\pm$ SD. Differences between pre- and post-transfusion groups were compared using a non-parametric rank F-test. A p-value of ≤ 0.05 was statistically significant.

RESULTS

The pre-transfusion hematocrit measurements of the SCA patients were low (25.68 ± 2.48 ; $n=6$) and comparable with previous patients who needed an exchange transfusion as a disease management modality. The pre-transfusion blood pressure measurements of the patients were also low (100.40 ± 6.50 mmHg/ 62.80 ± 10.03 mmHg; $n=5$). Pre-transfusion images of all six SCA pediatric patients exhibited a “blanched” avascular appearance in the bulbar conjunctiva, indicative of diminished presence of capillaries and arterioles (Fig. 1A, 1C, 1E). This “blanched” appearance is similar to observations made in previous studies and is a unique characteristic of SCA [5, 6]. The averaged diameters of the venules and red cell velocity from the six pediatric SCA patients were 48.51 ± 7.67 μ m and 0.95 ± 0.21 mm/s respectively -- these results were comparable with historical measurements [6]. Oxygen saturation was measured via pulse oximetry in three out of six patients and showed below normal values in all three patients (95%).

The post-transfusion blood pressure measurements of the patients showed a noticeable, albeit not significant, improvement from pre-transfusion values (104.75 ± 10.31 / 61.00 ± 7.26 mmHg; $n=4$). The appearance of the post-transfusion bulbar conjunctiva and its microcirculation in the SCA patients differed from the pre-transfusion ischemic and hypoperfused state. There was substantial improvement in vascularization of the microvasculature (Fig. 1B, 1D, 1F). In areas that were hypoperfused and devoid of capillaries and arterioles before transfusion, a significant increased presence of capillaries (and to lesser extent arterioles) was observed, indicative of improved vascular perfusion in the post-transfusion tissues. A significant decrease in averaged vessel diameter occurred immediately following transfusion ($n=6$; 36.76 ± 7.23 μ m; $p=0.015$; see Figure 2). An observable and measurable, but statistically non-significant decrease in red cell velocity occurred after transfusion ($n=6$; 0.68 ± 0.18 mm/s; $p=0.059$; see Figure 3). In addition, oxygen saturation showed significant improvement in all three patients (99%) in whom this parameter was measured.

DISCUSSION

Stem cell transplantation is the only known cure for SCA [25]. However, limited availability due to donor matching and risks of severe adverse events may outweigh its benefits [26]. Consequently, palliative treatment modalities such as transfusion therapy are more commonly utilized in the management of SCA to ameliorate or prevent complications, including vasoocclusion, blood sludging, tissue hypoperfusion and ischemia, and diminished blood flow arising from SCA [2, 4]. In addition, transfusion therapy has clear benefits in the primary and secondary prevention of stroke as well as for a variety of other indications with less compelling evidence [27–29].

Vasoocclusion is commonly identified as the underlying factor causing end-organ clinical manifestations in SCA (e.g. priapism, splenic sequestration, acute chest syndrome, cerebrovascular accident, etc). Therefore, it is expected that after exchange transfusion therapy where sickled RBCs are replaced by normal RBCs in occluded and sludged vessels, there would be improvement in tissue perfusion. With the introduction of non-sickled RBCs (characteristically non-rigid, non-adherent and more deformable), vasoocclusion and vessel sludging are ameliorated as reflected in the enhanced presence of previously inconspicuous capillaries and arterioles, thus providing direct evidence of improved perfusion (Fig. 1B, 1D, 1F). Certainly, the pathophysiologic mechanism of vasoocclusion cannot be attributed solely to RBC sickling as it is known that vasoocclusion is multifactorial [30]. There are a number of possible contributing factors in sickle cell vasoocclusion (e.g. sickle cell non-deformability, sickle blood viscosity, endothelial cell activation, adhesion molecule upregulation, central nervous system responses) which transfusion therapy may not be able to completely remedy. Post-transfusion improvement in tissue perfusion may mitigate several of these complications.

Simple transfusion is not a management of choice for pediatric SCA patients because of the small blood volume in pediatric patients. Thus, the exchange transfusion protocol is normally preferred in pediatric clinics. In most sickle cell clinics, the amount of blood to be exchanged (phlebotomized and transfused) is computed and based on the following equation:

$$\text{Exchange Volume (ml)} = \frac{(\text{Hct}_d - \text{Hct}_i) \times \text{TBV}}{\text{Hct}_{rp} - (\text{Hct}_i + \text{Hct}_d)/2}$$

Hct_d, desired hematocrit; Hct_i, initial hematocrit; Hct_{rp}, hematocrit of replacement cells (usually 0.7–0.8); TBV, estimated total blood volume in ml

The calculated amount of blood to be exchanged as based on this equation is approximately 50–60 ml/kg of body weight for an average adult. This amount of blood, which can be tolerated by an adult, is an unacceptably large blood volume in the case of a pediatric patient. In the exchange transfusion protocol used for pediatric patients at UCDMC, a volume of 10ml/kg of blood is phlebotomized and 15ml/kg of blood infused immediately. This 1:1.5 (v:v) ratio was used in the present study. From the results generated in this study, we can conclude that vasoocclusion and tissue ischemia (specifically the lack of perfusing capillaries) were ameliorated with demonstrable improvements as shown in Figure 1A–F. Despite observable improvements in perfusion, no improvement in blood flow was noted, as the averaged red cell velocity actually decreased (albeit not significantly) immediately after blood infusion in the exchange transfusion process. With the removal of sickled RBCs and introduction of normal RBCs, we expected that red cell velocity would immediately increase after an exchange transfusion. The decrease in red cell velocity was not expected though it could perhaps be attributed to a hyperviscosity condition, induced by a substantially

increased presence of normal RBCs in a non-physiological infusion volume. The velocity change we observed was in agreement with the concept of induced hyperviscosity and hemotocrit balance in an optimal transfusion suggested by Vichinsky in his discussion on the balance between transfusion volume, viscosity, hemotocrit, and blood flow dynamics [15]. The *in vivo* images we generated (see Figure 1A–F) represent the first documentation of its kind on real-time microvascular changes arising from the exchange transfusion process and may shed light on future transfusion studies. The concomitant decrease in vessel diameter which we observed could be attributed to a homeostatic feedback mechanism in an attempt to alter flow dynamics so as to maintain adequate venous return, cardiac output and mean arterial pressure via the sympathetic (T1-L2) adrenergic pathway. The exchange transfusion process is designed to improve blood flow and oxygen-carrying capacity, thereby improving tissue perfusion and mitigating vasoocclusion. However, with the paradoxical post-transfusion decrease in red cell velocity, the intended benefits of exchange transfusion may be offset by the adverse effect of increased viscosity, thus thwarting improved optimal oxygen delivery and blood flow and paradoxically promoting vasoocclusion [15, 28–30]. It is apparent that the exchange volume may need adjustment for an optimal outcome to counteract the reduction in red cell velocity, which is counterproductive in the transfusion process.

Direct implications from this study suggest that future considerations for exchange transfusion guidelines should include the measurement of whole blood viscosity and optimum Hct to prevent unintended outcomes. As discussed in previous studies in the literature, there is a delicate balance between red cell transfusion and Hct target levels which merits emphasis [15, 31]. It may be useful to utilize these added parameters to develop a mathematical model to predict the redistribution of and improvement in post-transfusion blood flow dynamics. It would be valuable to conduct follow-up studies using a larger cohort of subjects, inclusion of additional time points for observations (e.g., immediately post-transfusion, 1-, 2-, 6-, and 12-hours post-transfusion), and designing the study to measure whole blood viscosity and Hct to characterize the temporal changes in blood flow dynamics. This would provide a comprehensive evaluation of the physiologic effects of exchange transfusion therapy on the microcirculation.

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Figure 1A–1F. Pre- and post-transfusion frame-captured images showing microvascular changes in the bulbar conjunctiva of pediatric SCA patients

Optical magnification, 4.5x; on-screen magnification, 125x.

1A. An image of the bulbar conjunctiva in a pediatric SCA patient (Patient #TF-2; age 8 years old). Note the characteristic “blanched” avascular appearance in the bulbar conjunctiva and a significantly diminished presence of arterioles and capillaries. The boxed area shows the presence of a typical venule with sludged blood flow in the patient. The parallel marker indicates the location where vessel diameter and red cell velocity were measured for comparison with **1B**.

1B. An image of the same location of the bulbar conjunctiva in the patient immediately following transfusion, showing a significant post-transfusion decrease in venular diameter. In addition, a substantial increase in tissue perfusion is shown as indicated by the enhanced presence of capillaries and arterioles.

1C. An image of the bulbar conjunctiva in a second SCA patient (Patient #TF-3; age 5 years old). Again, note the “blanched” avascular appearance similar to the appearance in Patient #TF-2 in **1A**. The two boxed areas and parallel markers indicate the locations where vessel diameters and red cell velocities were measured for comparison with **1D**.

1D. An image of the same location in the bulbar conjunctiva shown in **1C** immediately following transfusion. Note the significant decreases in vessel diameter in the two locations (indicated by the parallel markers) and a substantial improvement in tissue perfusion,

1E. An image of the bulbar conjunctiva in a third SCA patient (Patient #TF-5; age 15 years old). Again, note the “blanched” avascular appearance similar to the appearance in Patients #TF-2 and #TF-3. The boxed area and parallel marker indicate the location where measurements were made for comparison with **1F**.

1F. An image on the same location in the bulbar conjunctiva shown in **1E** immediately following transfusion. Again, note the significant decrease in vessel diameter and substantial improvement in tissue perfusion.

Figure 2. Pre- and post-transfusion measurements of the conjunctival vessel diameter in six pediatric SCA patients

Vessel diameter measurement for each patient and averaged (mean $\pm$ SD) vessel diameter measurement for all six patients combined. Note the significant change in post-transfusion vessel diameter measurements ($p=0.015$).

Figure 3. Pre- and post-transfusion red cell velocity measurements of the conjunctival vessels in six pediatric SCA patients

Red cell velocity measurements for each patient and averaged (mean $\pm$ SD) red cell velocity measurement for all six patients combined. Note the substantial change in post-transfusion velocity measurements, albeit not statistically significant ($p=0.059$).