The Effect of Isovolemic Hemodilution on the Autoregulation of Cerebral Blood Flow

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Background: Hemodilution may increase cerebral blood flow (CBF) but the mechanism(s) remain controversial. Autoregulation is easily modified or disturbed by several conditions. The aim of this study was to evaluate the effects of isovolemic hemodilution on the autoregulation of cerebral blood flow in a rabbit model.

Methods: Stepwise hemodilution was accomplished by incrementally removing whole blood from the animals in amounts of 8 - 12 ml and replacing this with an equal volume of 6% hetastarch in saline. This procedure was continued until the target content values of approximately Hct = 18% were achieved. To evaluate the influence of pressure changes on CBF, mean arterial pressure (MAP) was increased from a baseline pressure (approximately /8 mmHg) to 145 mmHg by intusing methoxamine, and cerebral blood flow was measured at each MAP level using the hydrogen clearance method after MAP had been stabilized for 15 min.

Results: Stepwise blood replacement caused a sudden drop of Hct from 37.4% to 18.5% and a simultaneously a significant increase in local CBF of 161% in the hemodilution group. Hemodilution significantly reduced CaO2 in the hemodilution group (9.45 ± 1.7 ml O2/dl) versus the control group (18.34 ± 1.3 ml O2/dl). However, despite these decrease in CaO2, calculated cerebral oxygen delivery (DQO2) was as well maintained in the hemodilution group (24.47 ± 7.28 ml O2/100 gm/min) as in the control group (24.14 ± 8.67 ml O2/100 gm/min). MAP increases from 78 mmHg to 143 mmHg produced a significant increase in CBF from 122.4 ± 32.8 ml/100 gm/min to 170.9 ± 23.7 ml/100 gm/min in control group (39.6%) and from 218.4 ± 75.6 ml/100 gm/min to 268.4 ± 108.5 ml/100 gm/min in the hemodilution group (44.6%) (p < 0.001). These CBF increases were not significantly different in the two groups.

Conclusions: The present study demonstrates that in the normal brain the decrease in CaO2 caused by hemodilution is well compensated for by an increase CBF, and that oxygen transport to the brain is also well maintained during a Hct value of 20%. Although the present study did not show the tight CBF control within the MAP range from /8 mmHg to 145 mmHg, hemodilution did not alter the response of the cerebral circulation to increased MAP. (Korean J Anesthesiol 2005; 49: S 35-40)

Key Words: autoregulation, cerebral blood flow, isovolemic hemodilution.

INTRODUCTION

It has been supposed to increase cerebral blood flow (CBF) after hemodilution by altering hematocrit (Hct). However, there are still controversy on the mechanism whereby hemodilution increase CBF. Some authors suggested that increase in CBF is a direct rheologic effect of a decrease in blood viscosity, as opposed to being a compensatory mechanisms to decrease in O2 carrying capacity that accompanied hemodilution. Distinguishing between these two alternatives has proven to be difficult, most previous studies support the idea that reduction in arterial O2 content (CaO2) produces active cerebral vasodilation to increase CBF as the result of an autoregulatory mechanism designed to maintain constant O2 delivery to the tissue-although viscosity must be playing some role.

Hemodilution is frequently used to treat neurological conditions such as cerebral ischemia and cerebral vasospasm after subarachnoid hemorrhage in order to increase CBF, while the therapeutic effect is still controversy. In the extreme hemodilution, the cerebrovascular beds must be submaximally dilated and less response to change in blood gases and showed the reduced vasodilation reserve. It is questionable whether autoregulation is
preserved or not during hemodilution. However, few informations are available on the effect of hemodilution on cerebral autoregulation, especially on the upper limits of autoregulation. The aim of this study was to determine the effect of isovolemic hemodilution on CBF autoregulation.

**MATERIALS AND METHODS**

**Animal preparation**

Male Sprague-Dawley rats (332 ± 36 g; n = 32 Harlan Sprague-Dawley, Indianapolis, IN) were anesthetized with 4–5% halothane in 100% O2 in a plastic box. When unresponsive, animals were removed from the box, 1% lidocaine was infiltrated subcutaneously, and a tracheostomy was performed. Animals were ventilated with a small animal ventilator with an inspired gas mixture of 1.0% halothane in 40% O2-balance N2O. Ventilator settings were adjusted to achieve normocarbia. After infiltration with 1% lidocaine, bilateral femoral arterial catheters and one venous catheter (PE-50) were inserted.

Arterial pressure was continuously measured from the left femoral artery. Arterial blood was intermittently sampled for determination of pH and arterial blood gas tensions. Hct was determined by microcapillary tube centrifugation. Rectal temperature was maintained at 37–38°C with a heating pad. Animals were then turned prone and the head fixed in a stereotaxic frame. The scalp, muscle, and periosteum overlying the parietal area of the skull were resected, and bleeding was controlled with ferric chloride solution. A small craniotomy (approximately 5 mm in diameter) was made in the left parietal bone using an air-cooled drill and bone bleeding was controlled with bone wax. The dura was incised with a 27 gauge needle with aid of microscope, avoiding dural and pial vessels. A 25 μm diameter wire electrode was inserted 1.5 mm into the cortex using a micromanipulator. The electrode constructed from 90% platinum/10% iridium wire (Medwine, Mt. Vernon, NY) insulated with glass with an exposed 1.5 mm tip. An Ag/AgCl reference electrode was placed subcutaneously in the tissue of the neck. The platinum electrode was polarized to +250 mV relative to the Ag/AgCl reference. To measure CBF, H2 was added to the inhaled gas mixture and the amperage output of the polarization amplifier was recorded. When the electrode output reached a plateau (after 5–10 min), H2 administration was stopped and the washout curve recorded. CBF was calculated from the H2 clearance curve using the T1/2 method

\[
\text{CBF (ml/100 gm/min)} = 0.093 \times 60 \text{ sec} \times 100 \text{ gm} / \lambda \times T_{1/2}
\]

where \( \lambda \), the partition coefficient for H2, is taken to be 1.

**Experimental protocols**

Approximately 1 h after completion of the cranial window, baseline measurements of systemic blood pressure, arterial blood gases, oxygen content, and Hct were obtained.

**Hemodilution**

After 15 minutes, stepwise isovolemic hemodilution was performed. The first step of isovolemic hemodilution began after baseline measurements were completed. Hemodilution was accomplished by incremental removal of whole blood from the animal in 8–12 ml and replacement with an equal volume of 6% hetastarch in saline (Hespan, DuPoni Pharmaceuticals, Wilmington, DE, USA). The amount of hetastarch given was adjusted to ensure a stable MAP. This procedure was continued until the target content values of approximately Hct − 18% was achieved and Hb-bound CaO2 was checked repeatedly. Ventilation was adjusted to keep PaCO2 between 35 to 45 mmHg. The data were collected −10 min after the desired endpoints were achieved. Animals were randomly assigned to either the control group (n = 16) or hemodilution group (n = 16). To study the effect of hemodilution on pressure autoregulation, methoxamine was infused intravenously to adjust MAP to 145 mmHg in both groups. After an equilibrium period of 5 min at each pressure step, CBF was measured by hydrogen clearance in control and hemodilution animals at baseline pressure (approximately 78 mmHg) after hemodilution preparation.

**Calculations**

Total CaO2 was calculated as the sum of Hb-bounded C2 (1.39 × Hb × SaO2) and dissolved O2 (PaO2 × 0.003) and cerebral tissue O2 delivery (cerebral DO2, in ml O2/100 gm/min) equaled (CaO2 × CBF)/100.

**Statistics**

All values were expressed as mean ± SD. Statistical verification was done with Wilcoxon Rank sum test, unpaired t-test, and regression between two groups and paired t-test within groups using SAS V8.1 statistical software. Significant difference was determined to be present with P values less than 0.05.

**RESULTS**

Hemodilution did not alter arterial pH, PaCO2, temperature and MAP did not vary between groups (Table 1; P > 0.05). While PaO2 value was slightly higher in the hemodilution group than in
Table 1. Physiologic Variables

<table>
<thead>
<tr>
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<th>MAP 80</th>
<th>MAP 145</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Hemodilution</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>79.7 ± 2.0</td>
<td>76.7 ± 8.4</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.02</td>
<td>7.37 ± 0.02</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>38.9 ± 2.6</td>
<td>41.5 ± 3.1</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>204.7 ± 47.4</td>
<td>216.1 ± 46.5</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>37.4 ± 2.3</td>
<td>18.6 ± 3.6*</td>
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<tr>
<td>Hemoglobin (gm/dl)</td>
<td>12.1 ± 1.3</td>
<td>6.7 ± 0.7*</td>
</tr>
<tr>
<td>CaO₂ (ml O₂/ml)</td>
<td>18.34 ± 1.3</td>
<td>9.45 ± 1.7*</td>
</tr>
<tr>
<td>CBF (ml/100 gm/min)</td>
<td>122.4 ± 32.8</td>
<td>218.4 ± 75.6*</td>
</tr>
<tr>
<td>DO₂ (ml O₂/100 gm/min)</td>
<td>24.14 ± 8.67</td>
<td>22.47 ± 7.28</td>
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All values are mean ± SD. The obvious differences in hematocrit (Hct), hemoglobin (Hb), cranial O₂ content (CaO₂), cerebral blood flow (CBF) and methoxamine doses. There were no statistically significant differences among other variables including DO₂ (cerebral oxygen O₂ delivery) between control and hemodilution group. MAP 80: mean arterial pressure at 76 mmHg. MAP 145 mmHg: mean arterial pressure at 145 mmHg. *P < 0.01 compared with control group at each mean arterial pressure (MAP).

Fig. 1. The relationship between hematocrit (Hct) and cerebral blood flow (CBF) at baseline in the halothane-anesthetized rats. The CBF was inversely correlated to the exponent of Hct value. Regression equation is shown in graph.

The control group but there was no significance. Methoxamine dose used to increase MAP from baseline pressure of approximately 78 mmHg to 145 mmHg was more needed in the hemodilution group (1.87 ± 1.1 mI/hr) comparing with the control group (1.44 ± 0.94 mI/hr) (P < 0.05; Table 1).

As shown in the Fig. 1, there was an inverse relationship between Hct and CBF values. As above noted, stepwise hepsen replacement causes a sudden drop of Hct from 37.4% to 18.5% and simultaneously a significant increase of local CBF from 83.5 ml/100 gm/min to 218.4 ml/100 gm/min (161%) (P < 0.05; Fig. 1). A regression line was demonstrated between Hct and CBF values as follows.

\[
\text{CBF (ml/100 gm/min) = -164.82 log Hct (%) + 727.84}
\]

\[ t = -2.415, r = -0.764, P < 0.05 \]

Fig. 2. Delivery of oxygen to brain (DO₂) in control and hemodilution group. DO₂ was well maintained in hemodilution group. Values are mean ± SD.
mmHg to 145 mmHg. As MAP increased from baseline level of 78 mmHg to 145 mmHg produced a significant increase in CBF from 122.4 ± 32.8 ml/100 gm/min to 170.9 ± 23.7 ml/100 gm/min in control group and from 218.4 ± 75.6 ml/100 gm/min to 268.4 ± 106.5 ml/100 gm/min in the hemodilution group (P < 0.001, Fig. 3). The increase in percentage each are 44.6% in hemodilution group and 39.6% in control group, respectively. This magnitude of CBF increase appear to be no statistical significance between groups. In other words, the identical response of cerebral circulation to increased MAP were seen in both group. This imply CBF response to perfusion pressure change (pressure autoregulation) did not alter with hemodilution.

DISCUSSION

In normal brain, isovolemic hemodilution is accompanied by an increase in CBF. Two major mechanisms argue that either 1) CBF increases as a compensatory response to a reduction in arterial O2 content (CaO2) so as to maintain cerebral O2 delivery (DO2) or 2) CBF increases due to a reduction in whole blood viscosity, which permits flow to increase without any active vasodilation. Because the major determinant of both CaO2 and viscosity is Hct, it has been different to identify an independent effect of blood viscosity on CBF in controlling CBF after hemodilution. Some studies have reported that the primary determinant of increased CBF after hemodilution is blood viscosity. However, Paulson et al3 reported that inhalation of carbon monoxide, which caused a reduction in CaO2 without changes in arterial PO2 or blood viscosity, resulted in a reciprocal increase in CBF in normal subjects and were the first to suggest that CaO2 was an important determinant of CBF. Johns et al10 observed a similar CBF response in newborn lambs in which CaO2 was varied by changing the inspired O2 concentration at a high or low Hct in the same lamb. The authors concluded that a tight coupling between CBF and CaO2 maintained cerebral O2 transport relatively constant. Several previous studies have tried to separate these two factors by means of plasma exchange using low-viscosity plasma substrates, carbon monoxide hypoxia11 or blood exchange with methemoglobin-containing erythrocytes15 and using hyperbaric oxygenation.16 These studies all draw the similiar conclusions that the fall of CaO2, not blood viscosity, was the primary mediator of the increase in CBF with hemodilution.

CaO2 depend mainly on the volume of oxygen bound to hemoglobin (Hb), together with a relatively small amount of oxygen dissolved in the plasma. Cerebral oxygen transport, which a product of CBF and CaO2, was calculated from the product of CBF and arterial oxygen content and it reflects the oxygen availability. Hemodilution decrease CaO2 by decreasing Hb concentration. As noted, there was an inverse relation between CBF and Hct. We observed a substantial increase in CBF (161%) as Hct fell. A change in Hct from 37.4% to 18.6% cause a significant decrease in CaO2 from 18.34 ± 1.3 ml O2/dl to 9.45 ± 1.7 ml O2/dl. The calculated cerebral DO2 was 24.14 ± 8.67 ml O2/100 gm/min in control group as much as 22.47 ± 7.28 DO2 ml O2 · 100 gm/min in the hemodilution group. The study results showed that changes in CaO2 are accompanied by reciprocal change in CBF to maintain constant cerebral O2 delivery and suggested that CaO2 is a variable of fundamental importance to the regulation of CBF. Therefore the improvement in blood flow after hemodilution may be a physiologic, compensatory response to the reduced oxygen content. i.e., cerebral DO2 is well maintained until near maximal flow value are achived. This finding is consistent with several previous studies on the effect of oxygen on the cerebral circulation.3,8,10,17

However, despite the increase in CBF, data presented in some papers suggests that calculated cerebral DO2 during progressive hemodilution may not well maintained.1,17 That findings are contrast with our present results and it provide us a conflicting data on the DO2. The discrepancy come from the calculation of cerebral O2 delivery during Hemodilution is usually based on systemic Hct. Although there are still debating the exact value of Hct between tissue and systemic Hct, under physiologic condition, Hct in cerebral microvessels is known to be less that the systemic
Therefore these observation raises the possibility that tissue O₂ availability/delivery may not be adequately predicted by cerebral O₂ content calculation based on large vessel Hct and it suggest that it might be unreasonable to conclude that the CBF changes seen during hemodilution occurs in direct response to the fall in CaO₂ per se. Instead CaO₂, one conclude that tissue oxygen or tissue oxygen availability within the cerebral vascular bed is more important determinant to control the CBF response to reduction in Hct.11)

There is substantial evidence that the dependence of tissue oxygen on CBF is responsible for autoregulation in organ with high oxygen consumption. Both myocardium and brain exhibit a high degree of autoregulation and in both organ blood flow is highly dependant on tissue oxygen consumption. Several studies have shown that for any given value of CaO₂, tissue oxygenation may be well maintained during hemodilution. There are several possibility to explain for the improved tissue oxygenation and improved O₂ availability during hemodilution. Both hypoxemia and hemodilution are accompanied by increase in CBF, but tissue oxygen availability is greater in hemodilution than hypoxemia at similar CBF and CaO₂ value.11) It is attributed by the increase in oxygen extraction ratio during hemodilution which reflects a better matching of the limited oxygen supply to tissue oxygen demands.12,21,23) Another possible explanation is redistribution of red blood cell in microcirculatory network.23) With hemodilution, there are disproportionate reductions in tissue Hct compared to systemic Hct and followed by a redistribution of red blood cell flow within the network after the changes in Hct distribution. The reduction in RBC mass and the consequent decrease in viscosity enhance flow through microcirculatory beds and there are optimization of microvascular vasomotor response that affect RBC distribution through microvascular networks. Pries et al.26) noted that the changes in Hct after hemodilution are accompanied by a redistribution of red blood cell flow within the network and observed the distribution of red cells was more homogeneously to the individual microvessels. This might serve to increase tissue oxygenation in regions endangered by underperfusion. In addition to this, according to Lin et al.24) study that the mean transit time for red blood cells in microcirculation did not altered by hemodilution. This seems to be in agreement the findings of Todd et al.18) according to the increase of total cerebral volume paralles in CBF is mainly due to increase plasma volume. As a results, the low velocity of red blood cells and tissue Hct in cerebral circulation and the amount of oxygen delivered to the brain are altered very little hemodilution. These effect are supposed to improved tissue oxygenation. Therefore at any given value of CaO₂, tissue oxygenation must be well maintained during hemodilution. In the present study the identical CBF response to increased MAP were seen in both group as MAP from 78 mmHg to 115 mmHg increase. This imply that hemodilution did not alter the CBF response to perfusion pressure change, although it did not show the tight CBF control in the range of MAP from 78 Hg to 115 mmHg, which usually considered to the autoregulatory range. It speculate as to the cause of improved tissue oxygenation and oxygen availability in the condition of hemodilution as much as normal.

In summary, the present study have confirm the important of cerebral oxygen content is a major determinant of CBF and the improvement in blood flow after hemodilution may be a compensatory and physiologic responses to the reduced O₂ content and also demonstrate normal cerebral oxygen transport during hemodilution. At given value of CaO₂ (9.54 ± 1.7 ml CaO₂/l), despite of decreased value of CaO₂, cerebral tissue oxygenation must be well maintained and CBF response to perfusion pressure change (pressure autoregulation) did not alter with hemodilution.

REFERENCES


