Blood Cortisol Levels On Cardiopulmonary Bypass After Methylene Blue Administration

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Citation

Abstract
Initiation of cardiopulmonary-bypass leads to a remarkable decrease in mean arterial pressure. We designed this study to investigate if methylene blue which is a cyclic-Guanosine Mono Phosphate inhibitor can substitute phenylephrine in this hypotensive situation.
Seventeen mongrel dogs were organized as three groups. Methylene blue was administered to seven subjects and phenylephrine to another seven. No manipulation was made in the control group. We took blood samples just before the drugs were administered, after the initiation of cardiopulmonary-bypass and at approximately 15th minute of cardiopulmonary-bypass. Mean arterial pressure levels of the control group after initiation of cardiopulmonary-bypass were significantly lower than methylene blue and phenyephrine groups. In the methylene blue and control groups, final cortisol levels were statistically similar but lower than the phenylephrine group.
We conclude that single dose methylene blue can be used effectively for hypotensive periods after the initiation of cardiopulmonary-bypass with less stress hormone release.

INTRODUCTION
Once cardiopulmonary-bypass (CPB) is initiated, the patient’s systemic venous blood is diverted from the patient’s right heart, maintaining the pump’s venous reservoir volume. During this transition, the ventricles receive progressively less blood during diastole, so a progressive decrease in pulsatility on the arterial and pulmonary artery pressure (PAP) monitors is seen. Once “full flow” is achieved, systemic venous blood is (ideally) draining from the patient to the pump/oxygenator. Therefore, the central venous pressure (CVP) and PAP should decrease to near zero (2 to 5 mmHg is common), whereas systemic flow, arterial pressure, and oxygenation are maintained at desired values.

Initiation of CPB is usually accompanied by a fall in mean arterial pressure (MAP) (30 to 40 mmHg), which combined with hemodilution, effect peripheral perfusion. Phenylephrine HCl, metharaminol, even norepinephrine are common medications that are used in such these situations.

Methilene Blue (MB) has been advocated especially as an adjunct to conventional vasoconstrictors in vasoplegic syndrome following cardiac surgery. Merely preoperative administration of MB stabilized systemic vascular resistance (SVR) during total CPB, and reduced the incidence of vasoplegic syndrom in high risk patients undergoing cardiac surgery. Shanmugam reviewed many studies about usage of MB in vasoplegic syndrome especially in cardiac surgery, and concluded that single dose methylene blue injection is very effective on low SVR.

Methylene blue was used by Yiu et al. to reverse refractory hypotension after CPB as an alternative to the other vasoconstrictors. They concluded that it is also possible to hypothesize that proinflammatory mediators arising from cardiopulmonary-bypass do not act by stimulating nitric oxide release but through activation of the final common pathway of nitric oxide.

Cardiopulmonary bypass often causes a stress hormonal response with subsequent changes in hemodynamics and organ perfusion. Open heart surgery is associated with acute perioperative changes in plasma levels of neurohormonal stress factors leptin and cortisol. In patients there has been
shown a significant rise in cortisol levels in the early postoperative phase, with a partial recovery toward baseline values observed at 24 hours postoperatively.

**Figure 1**

Table 1: Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviations or Acronyms</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACT</td>
<td>Activated Coagulation Time</td>
</tr>
<tr>
<td>BSA</td>
<td>Body Surface Area</td>
</tr>
<tr>
<td>BW</td>
<td>Body Weight</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic Guanosine Mono Phosphate</td>
</tr>
<tr>
<td>CO</td>
<td>Cardiac Output</td>
</tr>
<tr>
<td>CPB</td>
<td>Cardiopulmonary-Bypass</td>
</tr>
<tr>
<td>CVP</td>
<td>Central Venous Pressure</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>HR</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenously</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean Arterial Pressure</td>
</tr>
<tr>
<td>MB</td>
<td>Methylene Blue</td>
</tr>
<tr>
<td>MPAP</td>
<td>Mean Pulmonary Artery Pressure</td>
</tr>
<tr>
<td>PAP</td>
<td>Pulmonary Artery Pressure</td>
</tr>
<tr>
<td>PCWP</td>
<td>Pulmonary Capillary Pressure</td>
</tr>
<tr>
<td>SVH</td>
<td>Systemic Vascular Hindrance</td>
</tr>
<tr>
<td>SVR</td>
<td>Systemic Vascular Resistance</td>
</tr>
<tr>
<td>SVRI</td>
<td>Systemic Vascular Resistance Index</td>
</tr>
<tr>
<td>η</td>
<td>Viscosity</td>
</tr>
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</table>

Cortisol is a steroid hormone released from the adrenal cortex in response to adrenocorticotropic hormone (produced by the pituitary gland). Normal values at 8 a.m. are 6 to 23 µg/dl. Higher and more prolonged levels of cortisol in the bloodstream have been shown to have negative effects, such as: impaired cognitive performance, and suppressed thyroid function, lowered immunity and inflammatory responses in the body, as well as other health consequences.

Although stress isn’t the only reason that cortisol is secreted into the bloodstream. Cortisol gets higher due to an -adrenergic stimulation, but not after cyclic guanosine mono phosphate (cGMP) inhibitors. The aim of this experimental animal study is to investigate the effect of methylene blue on plasma cortisol levels after the initiation of cardiopulmonary-bypass.

**METHODS**

All procedures were approved by the ethical committee (January 17th, 2002), and were consisted with the 1996 ‘Guide for the Care and Use of Laboratory Animals’. All acronyms and abbreviations were shown in Table 1.

Seventeen healthy mongrel dogs of either sex (9.5 1.3 kg) were included in the study. Seven subjects were treated with 2 mg/kg methylene blue (Group M) and another seven subjects threatened with 10 µg/kg phenylephrine (Group P) and the rest three subjects in the control group (Group C) were not treated.

All subjects were preconditioned to the laboratory environment at least 2 days before the experiments. The dogs were cared for in the facility using standard procedures. Food was withheld from the dogs for 12 hours prior to experimentation.

After intramuscular premedication with midazolam (0.2 mg/kg) and butorphanol (0.1 mg/kg), catheters were inserted percutaneously into the femoral vein and neighbouring artery with over the needle polyethylene catheters (18 G branules B.Braun, Germany) for blood pressure monitoring, fluid administration and blood sampling. Anesthesia was induced with 1.5 mg/kg ketamine intravenously (IV) and xylazine (1.5 mg/kg IV). Following endotracheal intubation, anesthesia was maintained with sodium pentobarbital (30 mg/kg IV) and vecuronium bromide (5 mg IV) were used. Ringer’s lactate was administered IV at a rate of 10 mL/kg/h. Ventilation was controlled using a mechanical volume-cycled ventilator (Dräger Evita. Dräger, Lubeck Germany) to maintain normocapnia (PaCO2: 35-45 mm Hg).

The left thoracic wall and the neck of the dog were shaved and prepared aseptically. A lead II electrocardiogram (ECG) was used to monitor the occurrence of arrhythmias. Heart rate (HR) was determined from the ECG. After median sternotomy, A thermodilution cardiac output catheter (size 7F,Sorenson, Abbott, Montreal, Quebec) was inserted from main pulmonary trunk and directed into the pulmonary artery.

**CARDIOPULMONARY-BYPASS**

After the animal was supine positioned, a median sternotomy incision was made through the midline. Heparin was administered (300 units/kg IV) and the activated clotting time (ACT) was determined (Blood Coagulation Timer,
Hemocron, Technidyne Corp., Metuchen, New Jersey) after 5 minutes and every 20 minutes thereafter. The ACT was maintained above 400 seconds by readministration of heparin (150 units/kg IV) if needed. The arterial cannula (size 12-14F, Jostra AG, Hirrlingen, Germany) was inserted into the ascending aorta for systemic arterial perfusion during CPB. The bicaval venous cannula (size 18-20F, Jostra AG, Hirrlingen, Germany) was positioned in the right atrium for the return of systemic venous blood to the oxygenator. A cardiopulmonary console (Model 5000, Sarns, Ann Arbor, Michigan) with a membrane oxygenator (Medos Hilite®. 2800. Pediatric Hollow Fibre Oxygenator) was used for perfusion. Pediatric polycarbonate tubing set was used (The Jostra HLM Tubing Set, Jostra AG, Hirrlingen, Germany). The extracorporeal circuit was primed with 600mL of Ringer's lactate for all groups. The pump was started at an initial flow of 70 ml per kg in minute and adjusted to maintain a mean arterial blood pressure between 60 and 90 mm Hg.

DATA COLLECTION

The data collected throughout the procedure included mean systemic arterial, mean pulmonary arterial (MPAP), pulmonary capillary wedge (PCWP), and central venous pressures. Cardiac output (CO) was determined by thermodilution. (The injectate volume was 5 mL of 0.9% sodium chloride in water, cooled to 2-5°C.)

Each cardiac output value was an average of 3 successive measurements (Cardiac Output Computer 3300, Abbott, Montreal, Quebec) performed during expiration. Arterial and mixed venous blood samples were collected anaerobically in heparinized plastic syringes and analyzed within 2 min following blood sampling (Acid-Base Laboratory ABL3, Radiometer A/S, Copenhagen, Denmark) for blood gas PaCO2 and PaO2, and pH.

The hemoglobin concentration of arterial blood was measured by the cyanmethemoglobin technique. Hematocrit was determined by capillary tube centrifugation and by a refractometer, respectively. Body surface area (BSA) of each dog was calculated from body weight (BW) using the formula: BSA = (10.1 X BW2/3) /100. Mean arterial pressure and systemic vascular resistance index (SVRI) were calculated as followed: MAP = Diastolic arterial pressure + (Systolic Arterial Pressure – Diastolic Arterial Pressure) / 3 (mmHg), SVRI = (MAP - CVP) / BSA x 79.9 (dynes*s/cm2).

Values were corrected for body temperature. Plasma levels of cortisol was measured by enzyme-linked immunosorbent assay. Data were collected at three stages: 10 min before the onset of CPB (pre-CPB), immediately after the onset of CPB, and at 15th minute of CPB. For each collection, all the measurements were performed within 3 minutes.

STATISTICAL ANALYSIS

All statistical analyses were performed by using the program GraphPad InStat® version 3.06. All values were expressed as mean ± standard deviation (SD). Hemodynamic data and plasma cortisol levels were compared with analysis of variance. Kruskal-Wallis test for comparisons between groups, and Friedman test for comparisons within groups were performed. Dunn’s multiple comparisons test as post test was used. A p value of less than 0.05 was considered significant.

RESULTS

Detailed hemodynamic results are presented in Table 2 and Table 3.

Heart rate values remained steady in all subjects. After CPB initiated, MAP decreased in comparison to pre-CPB values in all groups; in group M from 82.6 ± 5.4 mmHg to 58.9±5.5 mmHg (p<0.01), in group P from 79±5.9 mmHg to 58.7±3.5 mmHg (p<0.01) and in the control group from 78±3.6 to 42.7±6.4 (p<0.05). The final MAP values of the control group were lower than the treated groups (p<0.05). The alterations between three CO and SVRI values within groups were also statistically not different (p>0.05) except control group. SVRI values were decreased significantly only in control group.

Cortisol level gradients are presented in Figure 1. Blood cortisol levels decreased by the initiation of CPB in all groups (In group P, decrease was not statistically significant but as rank sum difference between first and second values is +7.0, we can consider it clinically significant). In phenylephrine group, final cortisol levels were significantly higher than methylene blue group and control group.

Hemodilution occured by cardiopulmonary-bypass, but there was no statistical significance between groups (Table 4).

DISCUSSION

Methylene blue is a water-soluble thiazine dye and widely employed in clinics to treat methaemoglobinemia. It has been used in the treatment of septic shock, endotoxic shock, anaphylaxis, Systemic Inflammatory Response Syndrome. The onset of hemodynamic effects of methylene blue is relatively
Evora and colleagues reported their experiences since 1994 in methylene blue to restore the arterial pressure and SVR. Donati and colleagues confirmed that the acute vasoconstrictive and positive inotropic effects of methylene blue were not associated with changes in blood volume, myocardial diastolic function, or pulmonary vascular permeability assessed by extravascular lung water. Gordon et al. proposed that this could be explained by the acute reduction of blood viscosity that results from hemodilution with non-blood priming solutions.

In our subjects hematocrit levels decreased by cardiopulmonary-bypass (Table 3). These investigators proposed systemic vascular resistance (SVR=[MAP-CVP]/CO) to be the product of blood viscosity (?) and inherent systemic vascular hindrance (SVH): SVR = \? x SVH.

**Figure 2**

Table 2: Raw Hemodynamic Values of The Dogs

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Treatment</th>
<th>MAP1</th>
<th>MAP2</th>
<th>SVRI</th>
<th>SVR2</th>
<th>SVR3</th>
<th>CO1</th>
<th>CO2</th>
<th>CO3</th>
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<td>75</td>
<td>66</td>
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<td>5.55</td>
<td>5.30</td>
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<td>56</td>
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<td>1790</td>
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<td>5.20</td>
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<td>1580</td>
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<tr>
<td>Dog 9</td>
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<td>1578</td>
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<td>1500</td>
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<tr>
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<td>1444</td>
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<td>65</td>
<td>1588</td>
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<td>1912</td>
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<td>5.10</td>
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<td>control</td>
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<td>50</td>
<td>1468</td>
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<td>1204</td>
<td>5.35</td>
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<tr>
<td>Dog 16</td>
<td>control</td>
<td>75</td>
<td>40</td>
<td>1358</td>
<td>1224</td>
<td>1198</td>
<td>5.24</td>
<td>5.04</td>
<td>4.96</td>
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<tr>
<td>Dog 17</td>
<td>control</td>
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<td>1224</td>
<td>5.18</td>
<td>4.95</td>
<td>4.80</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. (SVRI: Systemic vascular resistance index)

a: p<0.01 versus first mean arterial pressure values in methylene blue and phenylephrine groups,
b: p<0.05 versus first mean arterial pressure value in the control group,
c: p<0.05 versus first mean arterial pressure values in methylene blue and phenylephrine groups,
d: p<0.05 versus second mean arterial pressure value of methylene blue and phenylephrine groups,
e: p<0.05 versus third mean arterial pressure value of methylene blue and phenylephrine groups,
f: p<0.05 versus first SVRI value within the control group,
and p<0.05 versus third SVRI of methylene blue and phenylephrine groups.

**Figure 4**

Table 4: Hematocrit Values

<table>
<thead>
<tr>
<th>Groups</th>
<th>Methylene Blue</th>
<th>Phenylephrine</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=7</td>
<td>n=7</td>
<td>n=3</td>
<td>p</td>
</tr>
</tbody>
</table>
| Hemoctocrit 1 | 0.34±0.01    | 0.33±0.03     | 0.34±0.01 | NS 0.83
| Hemoctocrit 2 | 0.30±0.01    | 0.20±0.02     | 0.29±0.01 | NS 0.94
| Hemoctocrit 3 | 0.30±0.01    | 0.29±0.01     | 0.29±0.02 | NS 0.89

Data are presented as mean ± standard deviation.

With initiation of hypothermia-induced vasoconstriction and/or with increasing levels of endogenous catecholamines and angiotensin, mean arterial pressure increases. Treatment with ?-agonists is usually not necessary. Of concern is the
potential for myocardial and cerebral ischemia because hypothermia has not yet been achieved.

Cerebral blood flow is lower than desirable when mean arterial blood pressure during normothermic or moderately hypothermic cardiopulmonary-bypass falls below about 40 mmHg. Reich and colleagues reported that during cardiopulmonary-bypass, hypotension was a predictor of mortality.

In our study after cardiopulmonary-bypass initiated, mean arterial pressure values decreased in all subjects (treated ones and control group respectively, p<0.01 and p<0.05). Mean arterial pressure reduction was more significant in the control group in which no vasoactive manipulation was carried out (p<0.05).

Phenylephrine has remarkably positive effect on SVR, but reduces stroke volume, renal and other organ perfusion. In this study only in control group, SVRI values decreased in comparison to basal values in treated groups. In methylene blue and phenylephrine treated groups as correlated with the literature, drugs kept SVRI stable even 15 minutes after cardiopulmonary-bypass on the contrary to the control group.

Figure 5
Figure 1: Cortisol levels before and during cardiopulmonary-bypass procedure.

Blood cortisol levels decreased as the initiation of cardiopulmonary-bypass in all groups. Accused reason for reduction can be hemodilution. Subsequently at the third phase in the metylene blue group and the control group cortisol levels were remained statistically unchanged. It means methylene blue affect cortisol levels as much as control group unlike phenylephrine. However in phenylephrine group, the levels elevated significantly (p<0.002). It was surprising to observe this rapid elevation in cortisol levels in such a short period of time.

Cardiopulmonary-bypass also induces some inflammatory processes involving alterations in vascular permeability and regional blood flow and changes of coagulation and complement systems. It has been reported that an abnormal release of vasoactive substances during cardiopulmonary-bypass, like nitric oxide, could play a role in the inflammatory process. Nitric oxide stimulates guanylate cyclase, and activates the production of cGMP, resulting in vessel relaxation. Increases in the intracellular concentration of cGMP are followed by relaxation of myocardial and vascular smooth muscle. Methylene blue inhibits guanylate cyclase enzyme, and avoids the dependent vasorelaxant effect of nitric oxide in the vessels smooth muscle.

Blood cortisol levels decreased as the initiation of cardiopulmonary-bypass in all groups. Accused reason for reduction can be hemodilution. Subsequently at the third phase in the metylene blue group and the control group cortisol levels were remained statistically unchanged. It means methylene blue affect cortisol levels as much as control group unlike phenylephrine. However in phenylephrine group, the levels elevated significantly (p<0.002). It was surprising to observe this rapid elevation in cortisol levels in such a short period of time.

Stress must not be the only reason that cortisol is secreted into the bloodstream. Cortisol may raise due to ?-adrenergic stimulation, but not after cGMP inhibitors. Methylene blue increased blood pressure but the cortisol levels remained relatively unchanged in comparison to phenylephrine.

CONCLUSION
We suppose the rapid elevation on cortisol levels in phenylephrine group is related to the significant alpha effect of the drug. We conclude that single dose methylene blue administration can be used effectively for refractory hypotension after the initiation of cardiopulmonary-bypass. The advantage of methylene blue seems to be the less remarkable cortisol hormone release.
References

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