2,3-Butanediione Monoxime Cardioplegia: Advantages Over Hyperkalemia in Blood-Perfused Isolated Hearts

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Background. 2,3-Butanediione monoxime (BDM) has been shown to possess cardioprotective properties related to the inhibition of cross-bridge force development, the reduction of myofilament Ca²⁺ sensitivity, and the attenuation of intracellular Ca²⁺ transients. This study tested the hypothesis that cardiac arrest achieved with BDM would be as effective as that achieved with St. Thomas’ solution (StT).

Methods. Isolated rabbit hearts, studied on a blood-perfused Langendorff column, underwent 1 hour of ischemia (37°C) and 30 minutes of reperfusion. Cardioplegia was administered every 20 minutes in the form of (1) Krebs-Henseleit solution only (control), (2) 20 mmol/L of BDM, or (3) StT. Recovery of developed pressure, atrioventricular activation times, and tissue water content were measured.

Results. Recovery of developed pressure for the control, BDM, and StT groups was 44% ± 3% (p < 0.05 versus BDM and StT), 57% ± 5%, and 62% ± 4%, respectively. Atrioventricular activation times were significantly prolonged in the control group (42 ± 15 ms, p = 0.042) and the StT group (26 ± 9 ms, p = 0.034), but not in the BDM group (14 ± 8 ms). Tissue water content after reperfusion was 80% ± 0.4%, 80% ± 0.2%, and 76% ± 1.0% (p < 0.05 versus control) in the control, StT, and BDM groups, respectively.

Conclusions. 2,3-Butanediione monoxime was as effective as StT in protecting the myocardium. Unlike StT, BDM ameliorated myocardial edema and atrioventricular conduction delay after reperfusion.

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Preparation of the support animal. The support animal was anesthetized intramuscularly with acepromazine (1 mg/kg) and xylazine (17.5 mg/kg), followed by ketamine (62.5 mg/kg). Anesthesia was monitored throughout the experiment and supplemented as needed. A tracheostomy was performed, an endotracheal tube inserted, and mechanical ventilation begun with 100% oxygen (ventilator Model 683; Harvard Apparatus, Dover, MA). Ventilator settings were adjusted to maintain arterial pH between 7.35 and 7.5, PCO2 between 35 and 45 mm Hg, and PO2 greater than 200 mm Hg.

Heparin (2,500 U) was given through an ear vein. The right femoral artery was cannulated and the cannula was connected to a pressure transducer (Model P231D; Gould Inc, Cleveland, OH) and monitored continuously on our recording system (Gould ES 1000; Gould Inc). A systolic blood pressure of greater than 80 mm Hg was maintained by the transfusion of either blood collected from the donor animal or Plasmalyte (Baxter Healthcare Corp, Deerfield, IL). Serial hemocrits were measured.

The left internal jugular vein and the left carotid artery were cannulated. The cannulas were attached to silicone elastomer tubing with an internal diameter of 0.125 inch (Baxter Scientific Products, McGaw Park, IL), which was positioned in roller pumps. Arterial blood was pumped (Masterflex Model 7013; Cole Parmer Instrument Co, Chicago, IL) to perfuse a modified Langendorff apparatus described previously [16]. Indomethacin (1 mg/kg) was administered to the support animal to augment blood pressure stability [16].

Preparation of the donor animal and isolated heart. The donor animal was anesthetized, intubated, ventilated, and heparinized as described earlier. A cardiotomy was accomplished rapidly through a median sternotomy. Blood for transfusion was collected from the thoracic cavity. The aorta was cannulated with the heart suspended from a modified Langendorff apparatus and blood perfusion was begun.

A fluid-filled latex balloon was placed into the left ventricle and secured with a pursestring suture in the mitral valve annulus. The balloon was connected by polyethylene tubing to a pressure transducer (Model P231D; Gould, Inc) and amplifier (Model 13-4615-50; Gould Inc). The zero-pressure reference was set at the level of the aortic valve. Two needle electrodes were secured in the right atrial appendage and connected to a pacemaker (Model 5320; Medtronic, Inc, Minneapolis, MN).

For the duration of the study, the heart rate was maintained at a constant rate of 180 to 240 beats/min. Two additional electrodes were placed on the left ventricular epicardium to monitor the bipolar ventricular electrogram. The electrodes were connected to an isolated preamplifier (Model 11-G3407-58; Gould Inc) and a universal amplifier (Model 13-4615-58; Gould Inc) and filtered between 0.05 and 1,000 Hz. The pressure and electrogram waveforms were displayed continuously and digitized in real time using an AT-CODAS system (DATAQ Instruments, Akron, OH) at a sampling rate of 1,000 Hz.

The heart was enclosed in a water-jacketed beaker, and myocardial temperature was monitored with a probe placed in the right ventricle (temperature probe Model 0112; Shiley Inc, Irvine, CA). Myocardial temperature was maintained at 37°C by adjusting the temperature of the water bath (Model 71; Polyscience, Niles, IL). At hourly intervals, heparin (500 U) was administered to the support animal.

Experimental Protocol

Hearts that did not generate a systolic pressure of greater than 80 mm Hg at an end-diastolic pressure (EDP) of 10 mm Hg were excluded from the study. After instrumentation, the hearts were given 30 minutes to equilibrate and preischemic data were acquired. Intracavitary left ventricular pressure waveforms and left ventricular bipolar electrograms were recorded over seven balloon volumes, each corresponding with a fixed intracavitary EDP (0, 2.5, 5, 10, 15, 20, and 25 mm Hg).

The hearts were randomized to receive 50 mL of one of three cardioplegic solutions, every 20 minutes, beginning with the onset of 1 hour of normothermic, global ischemia: (1) no cardioplegia (Krebs-Henseleit solution only, control, n = 6); (2) StT (n = 6); or (3) BDM in Krebs-Henseleit solution (5 mmol/L, n = 6). Krebs-Henseleit solution consisted of 118.5 mmol/L of NaCl, 25 mmol/L of NaHCO3, 3.2 mmol/L of KCl, 1.2 mmol/L of MgSO4, 1.2 mmol/L of KH2PO4, 2.5 mmol/L of CaCl2, and 5.5 mmol/L of glucose. The dose of BDM used in this study was chosen on the basis of previous studies performed in our laboratory that documented reversible arrest in isolated myocyte preparations with the use of 5 mmol/L of BDM, as well as work by others that demonstrated myocardial protection in crystalloid-perfused hearts with the use of BDM in this dose range [12].

After 1 hour of normothermic, global ischemia, the hearts were reperfused for 30 minutes. Intracavitary left ventricular pressure waveforms and electrograms were recorded over the identical range of balloon volumes recorded during preischemic data acquisition. At the conclusion of the study, a sample of the left ventricle was excised, blotted, weighed, and dried until a constant dry weight was reached. Myocardial edema was expressed as the percentage of tissue water in the following equation: %H2O = (wet weight − dry weight)/wet weight.

Data Analysis

Digitized pressure waveforms were collected on data files analyzed using software developed in our laboratory.

End-Systolic Pressure. The end-systolic pressure (ESP) of a beat was defined as the maximum point of the digitized pressure waveform as described previously [16]. The ESP
versus balloon volume data were fitted to a linear ESP-V relation (ESPVR) with a least-squares linear regression:

\[ \text{ESP} = E_{\text{max}} \times V + k \]

where \( E_{\text{max}} \) is the slope of the ESPVR and \( k \) is the y-axis intercept of the ESPVR.

**End-Diastolic Pressure.** The EDP of a beat was defined as the point at which the slope of the pressure waveform exceeded 0.5 mm Hg/ms. The EDP versus balloon volume data were fitted to a linear EDP-V relation (EDPVR) with a least-squares linear regression:

\[ \text{EDP} = m(V - V_0) \]

where \( m \) is the slope of the EDPVR and \( V_0 \) is the balloon volume at which EDP is zero, or the x-axis intercept. A linear representation of the EDPVR has been shown to be appropriate over the range of volumes examined in this model [16].

**Developed Pressure.** Developed pressure in the left ventricle (DP) was defined as the difference between ESP and EDP for a given beat. The DP of 10 beats was averaged for each balloon volume. The DP versus balloon volume data were fitted to a linear pressure-volume relation using the following regression:

\[ \text{DP} = \text{ESP} - \text{EDP} = (E_{\text{max}} \times V + k) - m(V - V_0) \]

**Recovery of Developed Pressure.** The recovery of DP, expressed as a percentage, was calculated as the ratio of the postreperfusion developed pressure to the preischemia developed pressure at the same balloon volume. The average percent recovery of developed pressure (%DP) was determined from the following definite integral, approximated using the trapezoidal rule:

\[ \% \text{DP} = \left( \frac{100}{V_b} \right) \int_{V_a}^{V_b} \frac{\text{DP after reperfusion}}{\text{DP before ischemia}} \, dV = (V_b - V_a) \]

where \( V_b \) is the largest matching postreperfusion balloon volume and \( V_a \) is the smallest matching postreperfusion balloon volume.

**Statistical Analysis**

The results are expressed as the mean plus or minus the standard error of the mean. Analysis of variance was used for multiple comparisons. When appropriate, the Kruskal-Wallis analysis of variance on ranks was used as a nonparametric alternative. Individual comparisons between groups were made with a Student-Newman-Keuls posttest. A one-way repeated-measures analysis of variance was used for comparisons that involved sequential, time-based measurements. When appropriate, the Kruskal-Wallis analysis of variance on ranks was used as a nonparametric alternative. A t-test, or paired t-test when appropriate, was used for comparisons between two sets. Differences were considered statistically significant when \( p < 0.05 \).

**Results**

There were no significant differences in \( \text{PO}_2; \text{PCO}_2; \text{serum sodium, potassium, or calcium}; \text{or hematocrit in the support animal throughout the study. The pH of the support animal was maintained within the normal range (7.40 to 7.60) throughout the experiment.**

**Temporal Aspects of the Development of Electromechanical Arrest**

The time required to achieve electrical arrest was significantly shorter in the StT group compared with both the control group and the BDM group (Table 1). However, there were no significant differences between StT and BDM in the time required to achieve mechanical arrest, which occurred more rapidly in both treatment groups compared with the control group.

**Atrioventricular Conduction Times**

The PR interval was significantly prolonged throughout the reperfusion period compared with preischemic values in the control and StT groups (Table 1). In contrast, no conduction delay was observed in the BDM group.

**Postischemic Systolic Function**

The infusion of both BDM and StT resulted in improved recovery compared with control solution (Fig 1). Recovery of developed pressure after reperfusion in the BDM group was statistically equivalent to that in the StT group.

**Postischemic Diastolic Compliance**

Changes in diastolic compliance were measured using a fixed volume analysis. Before ischemia and after reper-

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**Table 1. Electrical and Mechanical Activity**

<table>
<thead>
<tr>
<th>Cardioplegia</th>
<th>EA (s)</th>
<th>MA (s)</th>
<th>PR Interval (ms) Before Ischemia</th>
<th>After Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>1,788 ± 435</td>
<td>580 ± 89</td>
<td>69 ± 10</td>
<td>111 ± 7d</td>
</tr>
<tr>
<td>StT (n = 6)</td>
<td>48 ± 8e</td>
<td>42 ± 7d</td>
<td>65 ± 14</td>
<td>90 ± 10b</td>
</tr>
<tr>
<td>BDM (n = 6)</td>
<td>2,020 ± 395</td>
<td>36 ± 7d</td>
<td>77 ± 7</td>
<td>90 ± 10</td>
</tr>
</tbody>
</table>

* Values are means plus or minus standard error of the mean; \( b \) \( p < 0.05 \) versus respective preischemic value (paired t-test); \( c \) \( p < 0.05 \) versus control and BDM (analysis of variance); \( d \) \( p < 0.05 \) versus control (analysis of variance).

BDM = 5 mmol/L of 2,3-butanedione monoxime in Krebs-Henseleit solution; Control = Krebs-Henseleit solution only; EA = electrical arrest; MA = mechanical arrest; StT = St. Thomas’ solution.
fusion, left ventricular EDP (LVEDP) was recorded at an identical balloon volume corresponding with a preischemic LVEDP of 5 mm Hg. The postreperfusion LVEDP in the control, BDM, and StT groups was 30.8 ± 6.2 mm Hg (p = 0.05 versus before ischemia), 4.0 ± 0.2 mm Hg (p = 0.01), and 4.3 ± 0.5 mm Hg (p = 0.23), respectively. No statistically significant loss of compliance was produced in any group, although the control group showed a trend toward decreased compliance. Compliance increased slightly in the BDM group.

**Myocardial Tissue Water**

Compared with the control group, the mean percentage of tissue water was significantly reduced only in the BDM group (Fig 2). There were no significant differences in tissue water content between control hearts and those that received StT.

**Coronary Flow**

Compared with control and StT hearts, BDM-protected hearts showed a prolonged hyperemic state on reperfusion (Fig 3). All three groups demonstrated a return to preischemic coronary flows by the end of the reperfusion period.

**Comment**

**Cardioprotective Effects of 2,3-Butanedione Monoxide**

The ideal cardioplegic agent should be able to produce rapid mechanical arrest while maintaining the myocyte in a state of minimal metabolic demand. Previous observations have suggested that BDM may be an attractive alternative to traditional cardioplegia [9–15]. 2,3-Butanedione monoxide attenuates plasmalemmal and sarcolemmal calcium flux, possibly through dephosphorylation of calcium channels [10]. Further, BDM also directly interferes with actin-myosin cross-bridge force production [10, 11], which appears to be its dominant mechanism of negative inotropy.

Although BDM has been shown to be cardioprotective during ischemia, it has not been tested as a cardioplegic agent in a clinically relevant, intact, blood-perfused heart model. This study demonstrated that BDM is as effective a cardioplegic agent as traditional StT in a more clinically relevant, blood-perfused model. The infusion of either BDM or StT solution resulted in improved posts ischemic recovery of systolic function compared with the infusion of control solution (Fig 1), and both solutions preserved the preischemic diastolic properties.

**Induction of Electromechanical Arrest With 2,3-Butanedione Monoxide**

Traditional cardioplegic solutions have been designed to induce rapid electromechanical arrest to minimize en-
ergy consumption during the period of global ischemia. In this study, BDM demonstrated an ability to mechanically arrest the heart as quickly as potassium cardioplegia (Table 1). The time required to achieve mechanical arrest in the BDM and STT groups was significantly lower than that in the unprotected, control group.

Electrical arrest times were significantly prolonged with BDM compared with STT cardioplegia, but this did not influence the agents’ cardioprotective capabilities. Previous work in our laboratory has shown that as long as there is rapid mechanical arrest, persistent electrical activity does not adversely affect functional recovery and does not result in measurable high-energy nucleotide depletion after ischemia [17]. This is due to the fact that the amount of oxygen needed to maintain electrical activity comprises less than 1% of the total basal oxygen consumption of the heart [17].

Myocardial Edema
Unlike STT, BDM cardioplegia ameliorated postreperfusion myocardial edema (Fig 2). Myocardial edema has been implicated in many of the contractile, metabolic, and electrophysiologic disturbances that occur after surgical ischemia. Cell swelling depresses left ventricular performance, results in delayed and abnormal ventricular conduction, and decreases coronary perfusion [2, 7, 18]. Moreover, intracellular volume appears to be a crucial second messenger in the regulation of cellular metabolism, hormone and transmitter release, ion transport, and even such complex functions as cell proliferation and apoptosis [19]. The ability of BDM to reduce myocardial edema may be related to its ability to attenuate transmembrane calcium flux and, subsequently, intracellular ion accumulation [10].

Coronary Blood Flow
In the STT group, the initial reperfusion hyperemia dropped to preischemic values almost immediately. In the BDM cardioplegia group, reperfusion flow remained elevated from preischemic values for 5 minutes (Fig 3). By the end of the reperfusion period, flows in both groups were similar to preischemic levels. One possible explanation for this finding is that BDM is a known coronary vasodilatory agent [12]. In the immediate reperfusion period, this effect may allow for better washout of toxic metabolites and better delivery of oxygen and substrates. An additional mechanism to explain BDM’s prolonged hyperemia may be related to its amelioration of postischemic myocardial edema, which has been associated with decreased coronary perfusion [20].

Atrioventricular Conduction
Our laboratory and others have shown that cell swelling and edema is associated with slowed ventricular conduction [2, 7, 21]. In this study, delays in atrioventricular conduction, as evidenced by significantly prolonged postischemic PR intervals, were observed in the control and STT groups (Fig 3). In contrast, no significant conduction delays were observed in the BDM group.

Previous work in our laboratory demonstrated significant delays in ventricular activation with the use of standard hyperkalemic solutions [21]. These studies suggested that the injury produced by crystalloid cardioplegia did not involve active membrane properties, but involved intracellular edema, which decreased the extra-cellular space, resulting in a higher resistance to current flow [21]. The findings in this study are consistent with this hypothesis. Postischemic edema was significantly less severe in the BDM group than in either the control group or the STT group. This implicates edema as playing an important role in postischemic conduction delays.

Advantages and Disadvantages of the Blood-perfused Isolated Heart Langendorff Model
The advantages and drawbacks of the blood-perfused isolated heart Langendorff model have been described previously [16]. The use of a support animal can introduce variability related to ionic and hormonal fluctuations. However, continuous, careful monitoring yields a stable and reproducible preparation. The more physiologic nature of this model is a distinct advantage over nonparabiotic and crystalloid-perfused models.

Summary
This study demonstrated that BDM cardioplegia is as effective as conventional STT in protecting the myocardium from global ischemic injury in the blood-perfused isolated heart model. Unlike STT, BDM cardioplegia ameliorated postreperfusion myocardial edema and prevented delays in atrioventricular conduction, which have been implicated in posts ischemic arrhythmogenesis and ventricular dysfunction. 2,3-Butanedione monoxide may be an attractive alternative to traditional cardioplegia. In vivo studies are needed for further characterization of the clinical efficacy of this cardioplegic agent.

References
8. Hebisch S, Bischoff E, Soboll S. Influence of 2,3-butanedione
monoxime on heart energy metabolism. Basic Res Cardiol 1993;88:566–75.

Discussion Groups on the Internet

The following three articles will be published in the April 1999 issue of The Annals of Thoracic Surgery, and have been selected for discussion topics on the Internet. These discussion groups can be accessed by going to The Society of Thoracic Surgeons Home Page (http://www.sts.org) and clicking on “Discussion Forums.” We encourage everyone to participate, as our goal is to make the discussion as interactive as possible.

Compression of the Central Airways by a Dilated Aorta in Infants and Children With Congenital Heart Disease

Doff B. McElhinney, MD, V. Mohan Reddy, MD, Mark S. Pian, MD, Phillip Moore, MD, and Frank L. Hanley, MD

Minimally Invasive Versus Conventional Aortic Valve Operations: A Prospective Study in 120 Patients

Heinrich E. Mächler, MD, Peter Bergmann, MD, Michael Anelli-Monti, MD, Drago Dacar, MD, Peter Rehak, PhD, Igor Knez, MD, Luay Salaymeh, MD, Elisabeth Mahla, MD, and Bruno Rigler, MD

Traumatic Aortic Rupture: Recent Outcome With Regard to Neurologic Deficit

Safuh Attar, MD, Marcelo G. Cardarelli, MD, Stephen W. Downing, MD, Aurelio Rodriguez, MD, Douglas C. Wallace, MD, Robert S. West, MS, and Joseph S. McLaughlin, MD

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