

Efficacy of Contraction Uncoupling by 2,3-Butanedione Monoxime during Initial Reperfusion versus Cardioplegic Arrest for Protection of Isolated Hearts

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Abstract

The efficacy of 2,3-butanedione monoxime (BDM) as additive to St. Thomas Hospital II solution (STH) as compared to initial BDM reperfusion with regard to myocardial ischaemia/reperfusion injury was investigated in isolated guinea pig hearts.

Isolated guinea pig hearts were perfused with Krebs-Henseleit buffer in the Langendorff technique at constant pressure of 55 mmHg. After cardioplegic arrest with STH solution, global ischaemia was induced for 50 min and recovery of myocardial function was monitored during 30 min reperfusion. Control hearts ($n = 5$) received no further treatment. In BDM_{CP} hearts ($n = 8$), 20 mM BDM were added to STH only. BDM_{REP} hearts ($n = 8$) were treated with 20 mM BDM during the initial 20 min of reperfusion. $BDM_{CP/REP}$ hearts ($n = 8$) received BDM during cardioplegic arrest as well as during initial reperfusion.

Left ventricular systolic function as assessed by developed pressure (L VDP) was depressed to $47 \pm 3\%$ of pre-ischaemic baseline in control. Only initial BDM reperfusion (BDM_{REP}) resulted in improved recovery of L VDP to $66 \pm 5\%$. Similar data were obtained for dP/dt_{max} and dP/dt_{min} . Reperfusion contracture was attenuated in both groups receiving initial BDM reperfusion (BDM_{REP} and $BDM_{CP/REP}$). BDM in STH did not protect hearts from cellular injury as assessed by release of lactate dehydrogenase (LDH) during reperfusion. In contrast, no increase in LDH release occurred during initial BDM reperfusion in BDM_{REP} and $BDM_{CP/REP}$ hearts, followed by a mild rebound after washout of the drug.

Addition of BDM to the cardioplegic STH solution did not protect isolated hearts from cellular injury or depression of post-ischaemic function. In contrast, initial BDM reperfusion alone attenuated reperfusion contracture, prevented LDH release, and improved recovery of systolic and diastolic myocardial function. The combination of BDM treatment during cardioplegic arrest with initial BDM reperfusion provides no additional protection from reperfusion injury.

Ann Acad Med Singapore 1999; 28:72-8

Key words: Calcium, Controlled reperfusion, Guinea pig, Hypercontracture, Myocardial reperfusion injury

Introduction

Despite the recent developments of minimally invasive surgery, most cardiac surgery procedures still include ischaemic cardioplegic arrest and cardiopulmonary bypass. For decades the efforts of many researchers aimed at improvement of cardioplegic solutions to minimise ischaemic injury of the myocardium. However, myocardial damage occurs not only during the ischaemic period, but also during reperfusion of the heart and may result in decreased myocardial contractility (stunning).¹⁻³ A number of mechanisms have been identified to contribute to myocardial reperfusion injury: these include generation of reactive oxygen species, release of proinflammatory mediators such as endothelin, TXA₂,

or platelet activating factor, capillary plugging or venular adhesion and subsequent emigration of leukocytes, and intracellular calcium accumulation.

The mechanisms of calcium entry into cells during ischaemia and reperfusion have been analysed in detail.⁴ Depolarisation of the sarcolemma due to ischaemia or the cardioplegic solution opens slow, L-type Ca⁺⁺ channels. The resulting influx of Ca⁺⁺ triggers Ca⁺⁺ release from the sarcoplasmic reticulum. Re-uptake of Ca⁺⁺ via Ca⁺⁺-ATPase is inhibited by the lack of energy. Sodium channels are also opened by depolarisation and Na⁺ accumulates in the cell, while the lack of ATP prevents elimination by Na⁺/K⁺-ATPase. Another entry mechanism for Na⁺ involves the H⁺/Na⁺-Antiport, which

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eliminates H^+ from the increasingly acidic cells in exchange for Na^+ . Intracellular accumulation of Na^+ then causes the Na^+/Ca^{++} -antiport to change its direction of transport and Na^+ is eliminated in exchange for even more Ca^{++} . While these exchange mechanisms are limited during ischaemia by the resulting alleviation of intra- and extracellular ion concentrations, the rapid normalisation of extracellular pH and Ca^{++} with the onset of reperfusion accelerates these processes, resulting in another steep increase in intracellular Ca^{++} levels. The most prominent effect of increased cytosolic Ca^{++} is contracture of the myocardium. The time-course of myocardial contracture correlates well with the time-course of cytosolic Ca^{++} increase. Cardiac contracture commences after some delay following induction of myocardial ischaemia and usually reaches a steady state, probably due to the described alleviation of ion gradients as well as the lack of energy for further contractile activity. With the onset of reperfusion, however, another steep increase in contracture is observed which may result in irreversible hypercontracture. In addition to the high energy demand for the contracture, it may damage the cell by disrupting myofibrills, the cytoskeleton and the sarcolemma.

2,3 butanedione monoxime (BDM) has the potential to antagonise the detrimental effects of increased intracellular Ca^{++} by decreasing the sensitivity of contractile proteins to Ca^{++} , or by directly inhibiting actin-myosin cross-bridge cycling.⁵⁻¹⁰ BDM thus uncouples the activity of the contractile apparatus from the cytosolic Ca^{++} concentration and has been shown to protect the myocardium during ischaemia or cardioplegic arrest¹¹⁻¹³ as well as during reperfusion.¹⁴⁻¹⁶

In a previous study we demonstrated that initial reperfusion with BDM for 5 min was more effective than initial hyperkalaemic reperfusion in improving left ventricular (LV) function and reducing reperfusion arrhythmia in isolated, Langendorff perfused guinea pig hearts.¹⁶ In a follow up study, 20 min of initial BDM reperfusion proved to be superior to shorter (5 min) or longer (40 min) therapy intervals.¹⁷

Since protective effects of BDM have been demonstrated not only during reperfusion but also during ischaemia,^{12,18} we now added BDM to the widely used St. Thomas Hospital II (STH) cardioplegic solution with or without subsequent initial BDM reperfusion and compared the results with the previously obtained data of control hearts and hearts receiving only initial BDM reperfusion.¹⁷

Materials and Methods

Following Animal Studies Committee approval, guinea pigs (250 to 350 g, Charles River-Savo, Kisslegg, Germany) were injected intraperitoneally with 20 mg of ketamine and 1000 units of heparin, and were decapi-

tated when unresponsive to noxious stimuli. The investigation conforms with the Guide for the Care and Use of Laboratory Animals.¹⁹ Our methods have been published in detail.^{3,17} After thoracotomy, the aorta was cannulated distal to the aortic valve and the venae cavae and pulmonary artery were cut. Each heart was immediately perfused through the aortic canula and excised. All hearts were perfused at a controlled perfusion pressure of 55 mmHg, measured at the aortic root. The perfusate, a modified Krebs'-Ringer solution, was filtered in-line (5 μ m pore size; Minisart, Sartorius, Göttingen, Germany) and contained (in mM): Na^+ , 137; K^+ , 4.5; Mg^{++} , 1.2; Ca^{++} , 2.5; Cl^- , 134; HCO_3^- , 15.5; $H_2PO_4^-$, 1.2; glucose, 11.5; pyruvate, 2; mannitol, 16; ethylene-diaminetetraacetic acid (EDTA), 0.05; and insulin, 5 units/L. Perfusate and bath temperatures were maintained at 37°C using a thermostatically controlled water circulator (RM 3, MGW Lauda, Lauda-Königshafen, Germany). Both solutions were equilibrated with a gas mixture of 97% O_2 and 3% CO_2 , which resulted in a pH of 7.4. Left ventricular pressure (LVP) was measured isovolumetrically with a transducer (Viggo-Spectramed DT-XX, Ohmeda, Erlangen, Germany) connected to a flexible, saline-filled latex balloon (Hugo Sachs Elektronik, KG, March-Hugstetten, Germany) inserted into the left ventricle through a cut in the left atrium via the mitral valve. Balloon volume was adjusted to maintain an end-diastolic LVP of approximately 5 mmHg and left ventricular developed pressure (LVDP) was calculated as $LVP_{systolic} - LVP_{end-diastolic}$. dP/dt was obtained electronically with an analogue differentiator (Recomed 216 080 02, Hellige, Freiburg, Germany). Heart rate was determined electronically using the positive peaks of the dP/dt curve as input signal (Recomed 236 045 06, Hellige).

Coronary flow was measured on-line with an electromagnetic flow probe (Recomed 236 046 01, Hellige). Coronary sinus effluat was collected by a cannula placed in the right ventricle via the pulmonary artery after ligating the venae cavae. All directly measured electronic signals were displayed on a six-channel recorder (Recomed 330-P, Hellige), and stored on a personal computer via a 16 channel AD-converter (ME 26, Meilhaus Electronic, Puchheim, Germany) for offline analysis.

In collected coronary sinus effluat, lactate concentration and the activity of LDH were measured with standard enzymatic test kits (Boehringer, Mannheim, Germany, measuring temperature: 25°C).

Protocol and Statistical Analysis

Four groups of hearts (each $n = 8$) were studied:

- 1) Control hearts undergoing cardioplegic arrest and 30 min reperfusion without any treatment;
- 2) in BDM_{CP} hearts 20 mM BDM was added to the

cardioplegic solution only;

- 3) in BDM_{REP} hearts 20 mM BDM was added to the initial perfusate for 20 min followed by 30 min of drug washout; and
- 4) in BDM_{CP/REP} hearts 20 mM BDM was added to both, the cardioplegic solution and the initial perfusate.

Following completion of the preparation and a 30 min period of stabilisation, coronary sinus effluante was collected, baseline values of all measured variables were obtained and cardioplegic arrest was induced by 20 ml of St. Thomas Hospital II solution (55 mmHg, 37°C). The hearts remained unperfused and submerged in the cardioplegic solution at 37°C for 50 min. Hearts were immediately reperfused with normal Krebs' solution (C and BDM_{CP}) for 30 min or with 20 mM BDM in Krebs' solution for 20 min before the perfusate was switched to normal Krebs for another 30 min (BDM_{REP}, BDM_{CP/REP}). The LV-balloon remained inflated during cardioplegic arrest and initial reperfusion to record the effect of contracture, but balloon volume was adjusted to yield again 5 mmHg end-diastolic pressure after 10 min of perfusion with normal Krebs'. At this time all hearts were also bolus infused with lidocaine (0.05 ml of 1%) to convert any hearts not already in sinus rhythm. Recordings of coronary flow and sampling of coronary sinus effluante were performed at baseline, at the peak of reactive hyperemic flow, i.e. at about 1 min of reperfusion, at the end of initial BDM perfusion, and at 5, 10, 15, and 30 mins of reperfusion with normal Krebs'. Post-ischaemic recovery of all other variables was determined after 30 mins of reperfusion.

All data are expressed as means $\pm$ standard error of the mean (SEM). After testing for normal distribution of data, statistical differences among groups were obtained by Student's t-test, and for within group comparisons by paired t-test. *P* values were corrected for multiple comparisons according to Bonferroni-Holm²⁰ as appropriate. Differences were considered significant at *P* < 0.05.

Results

Heart Rate

During pre-ischaemic baseline, mean heart rate was 219, 224, 229, and 232 bpm in C (Control), BDM_{CP}, BDM_{REP}, and BDM_{CP/REP} groups, respectively, and recovered to near baseline values until the end of the reperfusion period in all groups.

LV Systolic and Diastolic Function

Left ventricular developed pressure (LVDP) was significantly depressed to only $47 \pm 3\%$ of baseline in control (C) hearts following cardioplegic arrest and reperfusion. While addition of BDM to the cardioplegic solution (BDM_{CP}) had no effect on recovery of LVDP, initial reperfusion with BDM for 20 min (BDM_{REP}) improved recovery of LVDP from as early as 5 min of drug

washout until the end of the reperfusion period ($66 \pm 5\%$). In contrast, the combination of BDM supplementation in cardioplegic solution as well as in the initial perfusate (BDM_{CP/REP}) failed to improve recovery of LVDP versus C hearts (Fig. 1). Baseline values: 105 ± 4 , 104 ± 4 , 106 ± 3 , and 93 ± 3 mmHg in C, BDM_{CP}, BDM_{REP}, and BDM_{CP/REP} hearts, respectively.

In the isolated Langendorff perfused heart, $dP/dt_{\max}$ is a direct indicator of myocardial contractility. Fifty minutes of cardioplegia at 37°C considerably depressed $dP/dt_{\max}$ during reperfusion in control hearts to $50 \pm 3\%$ after 30 min reperfusion. Among the treatment groups only BDM_{REP} hearts exhibited significantly improved recovery of $dP/dt_{\max}$ to $70 \pm 6\%$ (Fig. 2). Baseline values: 1753 ± 61 , 1823 ± 75 , 1882 ± 122 , and 1837 ± 76 mmHg/s in C, BDM_{CP}, BDM_{REP}, and BDM_{CP/REP} hearts, respectively.

LV relaxation during early diastole was assessed by $dP/dt_{\min}$, which was significantly reduced after cardioplegic arrest and 30 min reperfusion in C hearts ($48 \pm 3\%$). Again only initial reperfusion with BDM (BDM_{REP}) improved recovery of $dP/dt_{\min}$ to $64 \pm 5\%$ while no beneficial effect was observed in BDM_{CP} and BDM_{CP/REP} groups (Fig. 3). Baseline values: -1459 ± 40 , -1505 ± 62 , -1533 ± 74 , and -1414 ± 37 mmHg/s in C, BDM_{CP}, BDM_{REP}, and BDM_{CP/REP} hearts, respectively.

During cardioplegic arrest and initial reperfusion LV diastolic pressure at constant balloon volume was monitored as an indicator of myocardial contracture. In all hearts balloon pressure increased moderately during cardioplegic arrest (Fig. 4). With the onset of reperfusion diastolic balloon pressure rapidly increased further and reached its peak after about 5 min. The presence of BDM in the cardioplegic solution had no effect on balloon

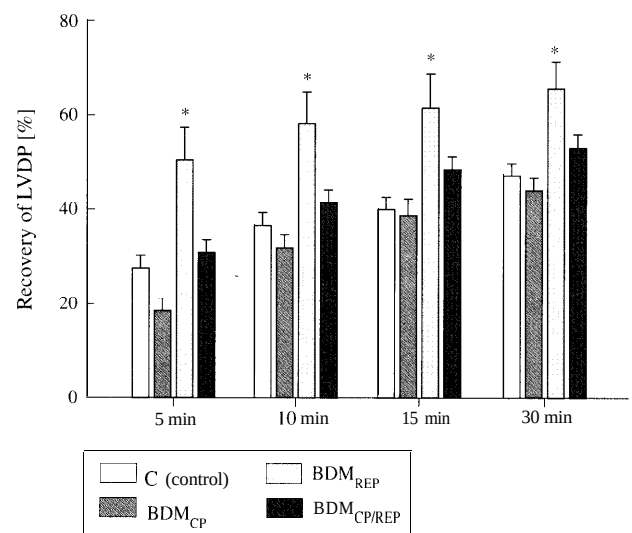


Fig. 1. Recovery of left ventricular developed pressure (LVDP) after 5, 10, 15, and 30 min of reperfusion with normal Krebs' solution is given in percent of pre-ischaemic baseline values. BDM_{REP} hearts presented with the best recovery of LVDP among all groups throughout the entire reperfusion period. Mean $\pm$ SEM, **P* < 0.05 versus C.

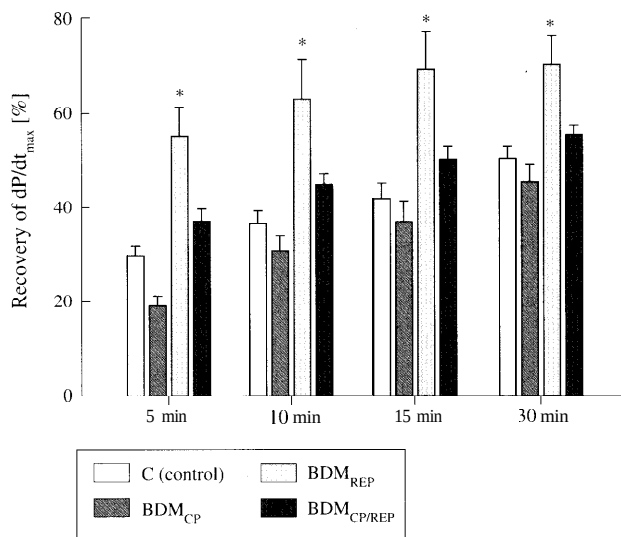


Fig. 2. Recovery of dP/dt_{max} after 5, 10, 15, and 30 min of reperfusion with normal Krebs' solution is given in percent of pre-ischaemic baseline values. Only hearts initially reperfused with BDM for 20 min after cardioplegic arrest, i.e. from the BDM_{REP} group, presented with improved recovery of myocardial contractility throughout the entire reperfusion period. Mean $\pm$ SEM, * $P < 0.05$ versus C.

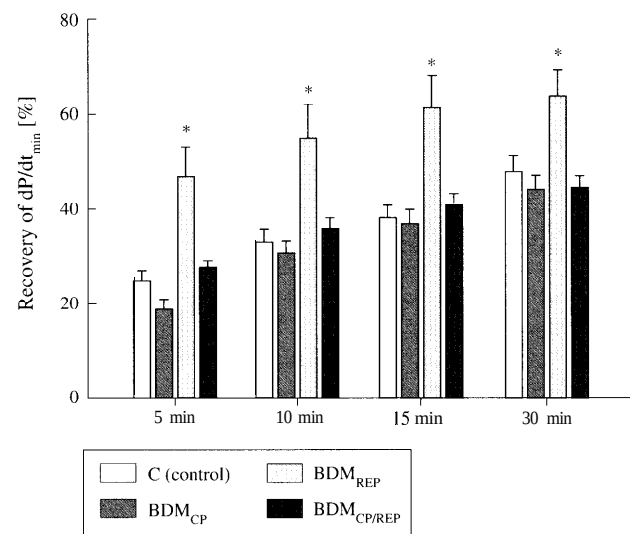


Fig. 3. Recovery of dP/dt_{min} after 5, 10, 15, and 30 min of reperfusion with normal Krebs' solution is given in percent of pre-ischaemic baseline values. Similar to systolic function, recovery of myocardial relaxation was improved versus control hearts only in the BDM_{REP} group. Mean $\pm$ SEM, * $P < 0.05$ versus C.

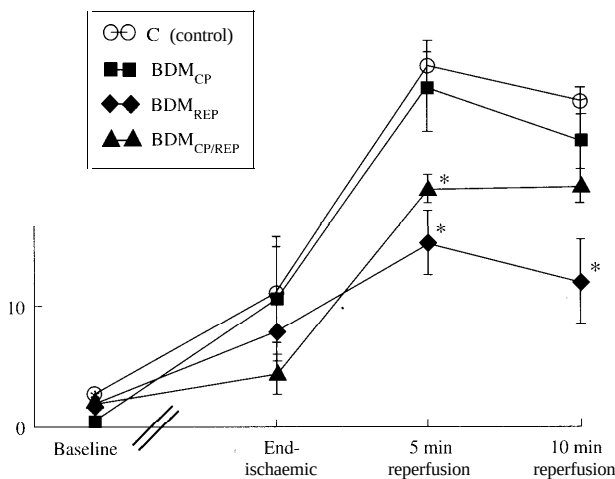


Fig. 4. Left ventricular end-diastolic balloon pressure at an unchanged volume is given for pre-ischaemic baseline (BL), the end of 50 min STH cardioplegia (End-Isch) and after 5 or 10 min of reperfusion. During cardioplegic arrest only mild contracture was observed, which was not influenced by the presence of BDM in the cardioplegic solution. In contrast, the steep increase in balloon pressure at the onset of reperfusion was significantly attenuated by the presence of BDM in the initial reperfusate. Mean $\pm$ SEM, * $P < 0.05$ versus C.

pressure. In contrast, addition of BDM to the initial reperfusate significantly attenuated the early increase of balloon pressure in BDM_{REP} as well as BDM_{CP/REP} hearts even after washout of the drug. However, a sustained beneficial effect on end-diastolic LV-balloon pressure until 10 min of BDM washout was only obtained in BDM_{REP} hearts (Fig. 4).

Coronary Flow

In all groups, coronary flow increased by 25% to 30%

over pre-ischaemic baseline during reactive hyperemic reflow within the first min of reperfusion. Within 5 min of reperfusion (C and BDM_{REP}) or within 5 min of BDM washout (BDM_{REP} and BDM_{CP/REP}), coronary flow decreased in all groups to about 80% to 90% of baseline and remained at this level until the end of the reperfusion period.

Lactate Washout

In all groups considerable amounts of lactate were washed out from the ischaemic myocardium with the onset of reperfusion. Lactate concentration was maximal at 1 min of reperfusion and quickly decreased as early as 5 min, reaching pre-ischaemic baseline values after 10 min of reperfusion. Addition of BDM to the cardioplegic solution or to the initial reperfusate had no effect on the total amount of lactate released from hearts or on the washout kinetics (Fig. 5).

LDH Release

LDH release from control hearts commenced immediately with the onset of reperfusion, peaked after 5 min (75 ± 19 U/L) and then declined to baseline values after 30 min. BDM in the cardioplegic solution protected hearts from LDH release only during the initial few min of reperfusion. Five minutes after the onset of reperfusion and thus washout of BDM the peak of LDH release reached 121 ± 23 U/L. In contrast, LDH release was completely suppressed during the initial 20 min of BDM reperfusion in BDM_{REP} and BDM_{CP/REP} groups. Even after washout of the drugs only a slight rebound of LDH release was observed with maximal activities at 10 min of 10 ± 2 and 14 ± 2 U/L in BDM_{REP} and BDM_{CP/REP} hearts, respectively (Fig. 6).

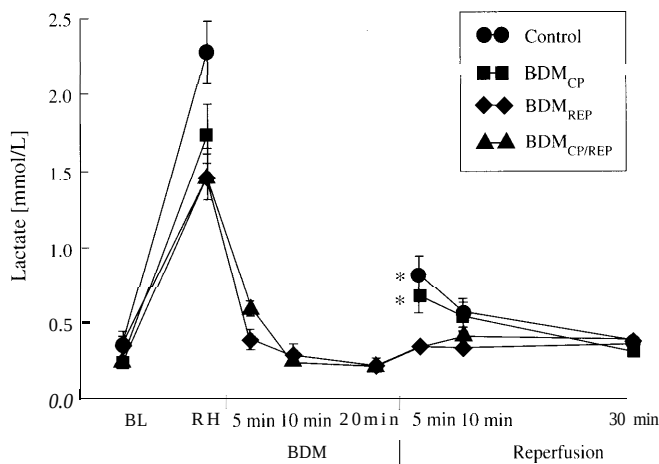


Fig. 5 Lactate concentration in the coronary effluite was determined during baseline (BL), at the peak of reactive hyperemic reflow (RH) which occurred at about 1 min of reperfusion, at 5, 10, and 20 min of initial BDM reperfusion (in BDM_{REP} and $BDM_{CP/REP}$ hearts), and at 5, 10, and 30 min of reperfusion with normal Krebs'. The steep increase in lactate washout at RH, and the quick decline during further reperfusion to baseline levels after 10 min indicate that all hearts switched to aerobic metabolism early during reperfusion and the observed release is due to washout of lactate previously accumulated in the tissue during ischaemic arrest.

Discussion

BDM uncouples muscle contraction from excitation and the cytosolic Ca^{++} concentration by decreasing the Ca^{++} sensitivity of contractile proteins,⁵⁻⁸ or by directly affecting actin-myosin crossbridge kinetics.^{9,10} In concentrations above 5 to 10 mM (20 mM in the present study) BDM also alters intracellular Ca^{++} kinetics and transsarcolemmal Ca^{++} transport,^{7,9,21-23} lowers heart rate and increases AV conduction time.^{6,18} Thus BDM reduces the intracellular Ca^{++} load as well as the Ca^{++} induced hypercontracture during early reperfusion and may attenuate the contracture induced structural cellular injury.¹³

In the present study contracture was estimated by recording diastolic balloon pressure at a constant volume during cardioplegia and early reperfusion. In all groups balloon pressure increased only moderately during cardioplegia, the presence of BDM in the cardioplegic STH solution had no effect on ischaemic contracture in our model. Possibly contracture was already sufficiently inhibited even in control hearts either by the lack of energy during ischaemic arrest and/or, by the protective properties of the STH solution. The high magnesium level in STH of 16 mM may provide sufficient Ca^{++} -antagonistic activity to limit Ca^{++} influx into cardiomyocytes and Ca^{++} -induced contracture.^{24,25} With the onset of reperfusion and washout of the cardioplegic solution, however, balloon pressure rapidly and markedly increased in the control as well as BDM_{CP} hearts resulting in the typical and damaging hypercontracture during early reperfusion. This indicates that both the protective STH solution and BDM are washed out quickly

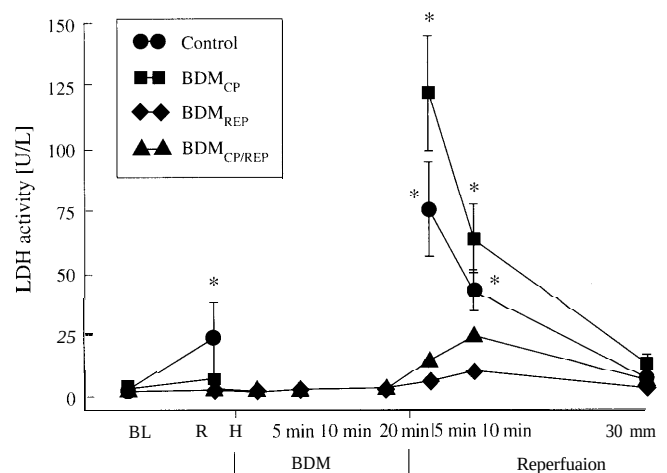


Fig. 6 LDH concentration in the coronary effluite was determined during baseline (BL), at the peak of reactive hyperemic reflow (RH) which occurred at about 1 min of reperfusion, at 5, 10, and 20 min of initial BDM reperfusion (in BDM_{REP} and $BDM_{CP/REP}$ hearts), and at 5, 10, and 30 min of reperfusion with normal Krebs'. The presence of BDM in the cardioplegic solution in BDM_{CP} hearts protected from LDH release due to cellular injury only during the first min of reperfusion. The peak of LDH release at 5 min was even higher than in control hearts. In contrast, no LDH was released during initial BDM reperfusion and even after washout of the drug only a mild rebound of LDH release occurred in BDM_{REP} and $BDM_{CP/REP}$ groups.

from perfused myocardium and provide no protection from contracture during reperfusion. As expected, the presence of BDM in the reperfusate attenuated this increase in balloon pressure in both, BDM_{REP} and $BDM_{CP/REP}$ hearts.

The quick washout of lactate accumulated in the tissue during ischaemia with coronary effluite concentrations reaching baseline after 10 min of reperfusion indicates that hearts from all groups switched to fully aerobic metabolism of glucose early after onset of reperfusion.

In our previous study,¹⁷ the hypothesis that BDM improved functional recovery of the hearts by attenuating hypercontracture induced cellular damage during initial reperfusion was supported by the electron microscopy studies: severe ultrastructural damage including markedly shortened sarcomers with widened and fuzzy Z-lines was observed in control hearts but not in hearts treated with 20 min of initial BDM reperfusion.

In the present study LDH release from isolated hearts during the reperfusion period was measured to monitor cellular injury. In accordance with previous studies^{4,17} and the concept that reduced contracture should go along with less cell damage, virtually no LDH release occurred during the initial BDM reperfusion period of 20 min in BDM_{REP} and $BDM_{CP/REP}$ groups. Even after washout of BDM, LDH release increased only very little which is compatible with the moderate increase in diastolic balloon pressure in these groups.

According to our hypothesis, reduced contracture and cellular injury during initial reperfusion should also result in improved functional recovery of the hearts.

However, only hearts treated with BDM exclusively during the initial reperfusion period exhibited an increased recovery of systolic (developed pressure, dP/dt_{max}) and diastolic (dP/dt_{min}) function, while the combination therapy with BDM present during cardioplegic arrest as well as during initial reperfusion did not improve recovery as compared to untreated controls. Possibly the extended duration of BDM exposure (50 min cardioplegic arrest + 20 min initial reperfusion) allowed for toxic side effects of BDM to outweigh the protective action seen after only 20 min BDM reperfusion. BDM may exert a directly damaging effect on cell membranes and intracellular structures, e.g. through its phosphatase like enzymatic activity.²⁶ A similar observation was made in our previous study where extension of 20 min initial BDM reperfusion to 40 min resulted in a complete loss of the functional improvement and partial loss of ultrastructural preservation.¹⁷ The lack of effectiveness of BDM as adjunct to STH was surprising because Stringham et al¹² had observed markedly improved functional recovery of isolated rabbit hearts when BDM and calcium were added to UW solution during cold cardioplegic arrest. The high magnesium concentration in STH (16 mM) as compared to UW (5 mM) solution, calcium paradox after storage in calcium free UW solution, or the different temperatures during cardioplegic arrest might account for this discrepancy.

Critique of the Methods

Following preliminary experiments, the duration of cardioplegic arrest was set at 50 min at 37°C to achieve pronounced depression of post-ischaemic function as to allow for monitoring of treatment effects. A reperfusion period in control hearts and a BDM washout period in treated hearts of 30 min each were selected, because no further recovery of myocardial function was observed beyond this time. Also, the kinetics of LDH release indicated no further cellular injury after this reperfusion/washout period, because in all groups LDH concentrations in coronary effluate were at baseline levels at the end of the experiments. Thus the different total periods of reperfusion among groups did not appear to influence the data on recovery of myocardial function or the estimation of cell injury by monitoring of LDH release. We also preferred to adjust end-diastolic balloon pressure to 5 mmHg in all hearts after reperfusion, because control experiments demonstrated that systolic developed pressure was maximal in our heart preparation at this preload in control as well as post-ischaemic hearts.

In conclusion, our data showed that addition of 20 mM BDM to the cardioplegic St Thomas Hospital II solution with or without initial BDM reperfusion had no effect on post-ischaemic functional recovery in our isolated heart model. In contrast initial reperfusion with BDM after cardioplegic ischaemic arrest, has the potential to at-

tenuate hypercontracture, to markedly reduce cellular injury and to improve functional recovery of the heart.

Thus, contraction uncoupling during initial reperfusion by BDM or similarly acting drugs may prove a viable and possibly superior alternative to warm cardioplegic reperfusion in patients undergoing open heart surgery.

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