Acta Med. Nagasaki 43 : 44−49

Acebutolol and Diltiazem Improves Cardiac Function and Survival of Dogs Exposed to Hyperthermia

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Whole body hyperthermia against malignant neoplasms has a limited temperature because of the clinical critical thermal maximum. This study was carried out to determine whether acebutolol or diltiazem could raise a critical temperature of dogs. Twentyfour male mongrel dogs were anesthetized with a large dose of morphine (2mg/kg), and randomly divided into 3 groups. Control (group C : n = 8) received only whole body hyperthermia (WBH). Group A (n = 8) received WBH and acebutolol, and Group D (n = 8) received WBH and diltiazem. The heart rate in control dogs increased by 53% at 43℃ from base line, whereas it increased by only 7% and 9% in the presence of acebutolol and diltiazem, respectively. Similarly, both acebutolol and diltiazem significantly improved the myocardial oxygen extraction ratio and myocardial lactate extraction ratio so well as endocardial electrocardiogram ST segment elevation. Consequently, cardiac output and LVdp/dt max at 43℃ were significantly improved by both acebutolol and diltiazem. Seventy-five percent and 87.5% of dogs administered acebutolol and diltiazem survived respectively, whereas none of control dogs survived at 44℃. In conclusion, acebutolol or diltiazem improves the myocardial oxygen demand-supply balance during hyperthermia. This effect would prevent circulatory collapse, and thus might raise CTMc above 42℃.

Key words : acebutolol : diltiazem : cardiac function : induced hyperthermia

Introduction

Therapeutic whole body hyperthermia (WBH) is clinically used as a therapy for cancer[1]. Irreversible biochemical damage occurs in malignant tumor cells exposed to a temperature of 42-44℃[2]. However, the clinical critical thermal maximum (CTMc), above which organ injuries occur, has been reported to be 41.8-42.5℃ in humans[3,4] and about 42℃ in dogs[5]. Because of this limitation of the applicable temperature in WBH, the technique of local hyperthermia are becoming a mainstream of therapeutic hyperthermia. However, WBH would be still beneficial for therapy of multiple micrometastases. Mechanisms involved in various organ injuries during WBH would include an impairment of enzyme systems, a dysfunction of the sympathetic nervous system, protein denaturation due to heat itself, exacerbation of dehydration due to an increase in vessel permeability[6], and a decreased tissue perfusion resulting from heart failure.

We hypothesized that the heart failure would be caused by the abnormality of myocardial oxygen supply-demand relationship resulting from hyperdynamic ventricular function. Thus, this study was designed to determine if the suppression of ventricular hyperdynamics could be effective to maintain cardiovascular function and could improve the outcome during WBH. Coronary and systemic hemodynamics were analyzed in the presence and absence of acebutolol or diltiazem.

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Methods

The experiments were approved under the Guidelines for Animal Experimentation at Nagasaki University and performed at Laboratory Animal Center for Biomedical Research, Nagasaki University school of medicine. Twentyfour male mongrel dogs weighing 13-20 kg (16.5 ± 2.2 kg) and with Hb of 14 g/dl or higher (16.0 ± 1.8 g/dl) were given kanamycin, 2.0 g, orally on the day before the experiment. Anesthesia was induced with pentobarbital, 20 mg/kg i.v., and tracheal intubation was facilitated with 0.15 mg/kg pancuronium. Morphine, 2 mg/kg i.v., was added to maintain anesthesia. The lungs were mechanically ventilated with a volume ventilator (R60 ; AIKA, Japan), and PaO2 and PaCO2 were adjusted to 100-150 torr and 35-40 torr, respectively. The surgical procedure was carried out aseptically, and each animal received cefotiam 2HCl, 0.5 g, before starting experiment. A7-Fr polyethylene catheter was inserted via the left femoral artery into the abdominal aorta for pressure monitoring and blood sampling. The same type of catheter was inserted into the left femoral vein, through which an equivalent mixture of

lactated Ringer's and 5% dextran solutions was administered to maintain diastolic pulmonary artery pressure within 80-100% of baseline throughout the experiment. A7-Fr pulmonary artery catheter (American Edwards Laboratories) was inserted from the internal jugular vein. Thoracotomy was performed in the fifth intercostal space, and a 20 gauge polyethylene catheter was inserted via the external jugular vein into the coronary sinus for blood sampling. Both aortic blood flow and left coronary artery blood flow were measured by electromagnetic flowmeters (MFX-2100 ; Nihon Kohden Co., Tokyo, Japan) placed at the aortic root and the origin of the left coronary artery, respectively. A catheter-tip transducer (PT-157 ; Goodman Co., Nagoya, Japan) was inserted into the left ventricle through the left atrium to measure the left ventricular pressure continuously, and the peak rate of increase in left ventricular (LV) pressure (LVdp/dt max) was calculated. An endocardial electrode insulated except the 1 mm at the tip was fixed under the left ventricular endocardium, which recorded ST segment changes in the endocardial ECG. The pulmonary artery temperature was measured at the tip of the pulmonary artery catheter, and the body temperature was measured with a temperature probe (TERUMO Finer CTM303 ; TERUMO, Japan) fixed under the liver with minilaparotomy. After the stabilization period, the dogs were randomly divided into 3 groups. Control (group C : n = 8) received only WBH. Group A (n = 8) received WBH and acebutolol, and Group D (n = 8) received WBH and diltiazem. In Group A, acebutolol was continuously administered starting at a dose of $1\mu g \cdot kg^{-1} \cdot min^{-1}$, and finally at a dose to maintain HR within 110% of baseline. In Group D, diltiazem was administered starting at a dose of $0.5\mu g \cdot kg^{-1} \cdot min^{-1}$ and finally at a dose to maintain HR within 110% of baseline. The dog was wrapped with a double layer polyethylene sheet and was heated by immersion in warm water 4-8℃ higher than the body temperature. The body temperature was increased at a rate of 0.5℃/30 min, maintained at 43℃ for 30 min, and increased further to 44℃. The measured parameters were heart rate (HR), systolic and diastolic blood pressures (SBP, DBP), pulmonary artery pressure (PAP), pulmonary capillary wedge pressure (PCWP), ascending aortic blood flow = cardiac output (CO), left ventricular dp/dt max (LVdp/dt max), left main trunks coronary blood flow (LCBF), endocardial electrocardiogram ST segment changes (Endo. ECG ST change), arterial blood gases (ABG), coronary sinus blood gases (CBG), aortic blood serum lactate, coronary sinus blood serum lactate, aortic blood serum creatine phosphokinase myocardial band (CPK-MB), coronary sinus blood serum creatine phosphokinase myocardial band, aortic blood serum potassium, and coronary sinus blood serum potassium. Left main trunks coronary vessel resistance (LCVR, unit) was calculated as (diastolic aortic pressure-left ventricular end-diastolic pressure)/left main trunks coronary blood flow. Myocardial oxygen extraction ratio was calculated as arteriocoronary sinus oxygen content difference x 100/arterial oxygen content. Myocardial lactate extraction ratio was calculated as arteriocoronary sinus lactate content difference x 100/arterial lactate content.

Statistical analysis was performed by paired and non-paired t-test, chi-square test, and Fisher's exact probability test. A P value less than 0.05 was considered significant.

Table 1. Systemic Hemodynamic Parameters

	℃	38	41	42	42.5	43
HR (bpm)	C	149±3	174±11¶	202±10¶	218±7¶	230±11¶
	A	153±3	160±2*	161±3*	158±3*	163±4*
	D	148±5	161±8	165±5*	168±7*	172±11*
mABP (mmHg)	C	90±9	108±9	102±8	98±7	51±5
	A	102±8	98±5	94±7	90±6	88±7*
	D	105±6	110±6	108±6	109±7	110±7*
CI (L/min/m²)	C	1.9±0.3	2.5±0.3	2.7±0.4	2.7±0.3	1.2±0.2§
	A	2.2±0.2	2.8±0.6	3.2±0.6	3.2±0.5	3.5±0.3*
	D	2.5±0.3	2.7±0.5	3.5±0.3	3.6±0.4	3.7±0.4*
SVI (ml/b/m)	C	17.3±2.5	15.8±3.0	14.1±3.3	12.3±2.4	7.1±1.2¶
	A	14.9±1.2	18.2±2.0	21.1±2.6	21.0±2.7	22.4±2.5*
	D	17.5±2.0	18.2±1.9	20.3±2.0	20.7±1.9	20.2±3.2*
PCWP (mmHg)	C	8.2±1.5	8.9±2.2	8.3±2.3	6.7±1.7	5.0±1.6
	A	5.9±0.8	6.8±1.0	6.6±1.2	6.1±0.8	5.9±0.7
	D	7.2±1.1	6.7±0.7	6.9±0.8	6.9±1.8	6.8±0.9

Data are mean±SEM.(n=8 for each value). C : control group A : acebutolol group D : diltiazem group
HR : heart rate. mABP : mean atrial blood pressure. CI : cardiac index. SVI : stroke volume index.
PCWP : pulmonary capillary wedge pressure
*$p<0.05$ vs control. ¶$p<0.05$ vs 38℃. §$p<0.05$ vs 42.5℃.

Table 2. Coronary Hemodynamic Parameters

	℃	38	41	42	42.5	43
LVdp/dt max	C	2.41±0.25	2.85±0.35	3.38±0.41¶	3.62±0.21¶	2.42±0.24§
(x10³mmHg/s)	A	2.15±0.14	2.35±0.30	2.55±0.22	2.65±0.25*	2.86±0.24¶
	D	1.88±0.26	2.36±0.18	2.83±0.32	3.08±0.42¶	3.22±0.22¶
LCBF(ml/s)	C	63.6±7.2	73.2±8.2	90.6±9.4	97.3±9.9¶	94.1±8.6¶
	A	69.5±4.8	75.1±5.8	80.5±11.0	88.3±9.5	91.8±11.2¶
	D	73.5±5.5	80.2±7.8	85.0±9.1	88.2±11.2	88.7±11.0
LCVR(dynexs/cm²)	C	1.22±0.11	1.11±0.98	0.91±0.18	0.72±0.11¶	0.65±0.09¶
dABP-LVEDP	A	1.27±0.18	1.21±0.14	1.02±0.16	0.88±0.09	0.85±0.19
LCBF	D	1.19±0.08	1.10±0.12	1.04±0.16	1.03±0.17	1.01±0.18

Values are mean±SEM.(n=8 for each value). C, A, D : Refer to table 1.
LVdp/dt max=left ventricular dp/dt max. LCBF=Left Coronary Artery Blood Flow.
LCVR=Left Coronary Vessel Resistance. *p<0.05 vs control. ¶p<0.05 vs 38℃. § p<0.05 vs 42.5℃.

Table 3. Coronary Sinus Blood Serum CPK-MB and Potassium

	℃	38	41	42	42.5	43
CPK-MB	C	3.8±2.0	4.4±2.8	5.5±3.1	6.8±2.4	18.0±3.6¶
	A	2.8±1.9	3.8±1.4	4.5±2.6	5.5±2.8	5.2±3.1*
	D	4.1±2.7	4.8±2.6	5.0±3.0	5.0±2.9	6.2±3.0*
Potassium	C	3.7±0.4	4.0±0.3	4.3±0.4	4.5±0.5	4.7±0.6
	A	3.9±0.5	4.0±0.3	4.0±0.3	4.1±0.3	4.3±0.4
	D	3.7±0.2	4.1±0.2	4.2±0.3	4.2±0.4	4.4±0.5

Values are mean±SEM.(n=8 for each value). C, A, D : Refer to table 1.
CPK-MB=creatine phosphokinase myocaldial band
*p<0.05 vs control. ¶p<0.05 vs 38℃. § p<0.05 vs 42.5℃.

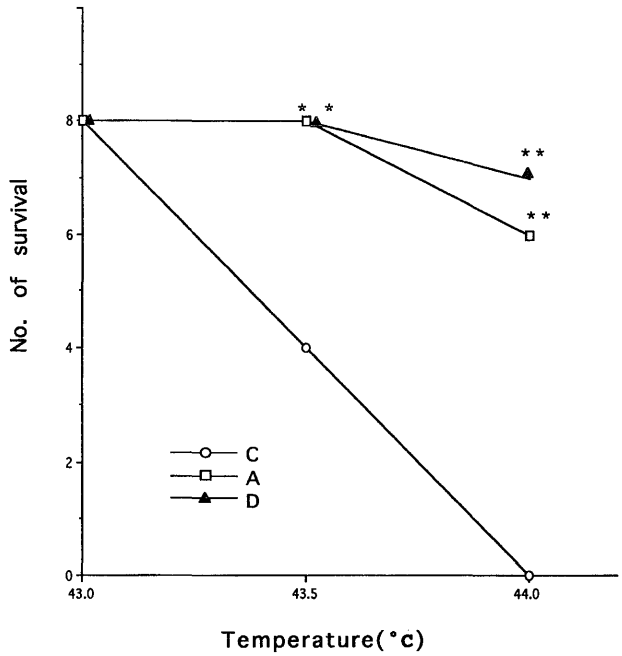

Fig. 1. The effects of acebutolol and diltiazem on the survival during hyperthermia above 43℃.

C : control group.
A : acebutolol group.
D : diltiazem group.
*p<0.05 **p<0.01 vs control.

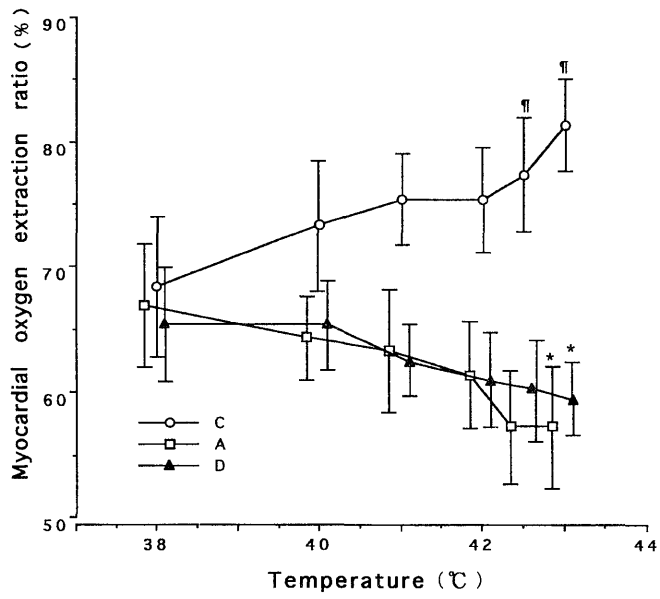

Fig. 2. The effects of acebutolol and diltiazem on myocardial oxygen extraction ratio during hyperthermia (mean±SEM ; n = 8 for each value).

C, A, D : Refer to Fig. 1.
*p<0.05 vs control. ¶ p<0.05 vs 38℃.

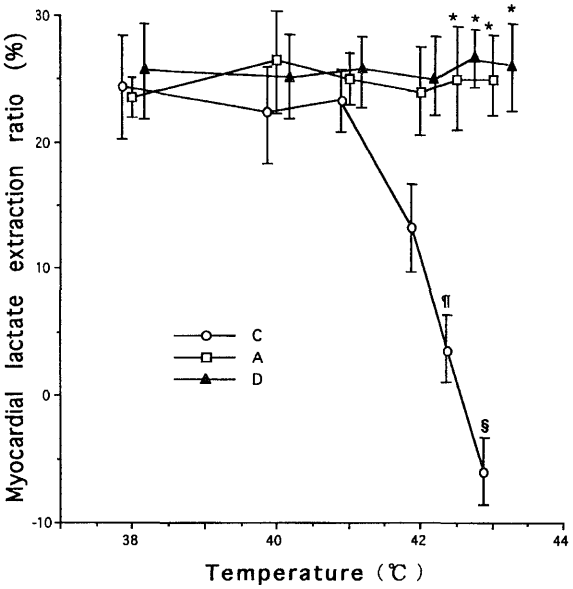

Fig. 3. The effects of acebutolol or diltiazem on myocardial lactate extraction ratio during hyperthermia (mean±SEM ; n = 8 for each value).

C, A, D : Refer to Fig. 1.
*p<0.05 vs control. ¶p<0.05 and §p<0.01 vs 38℃.

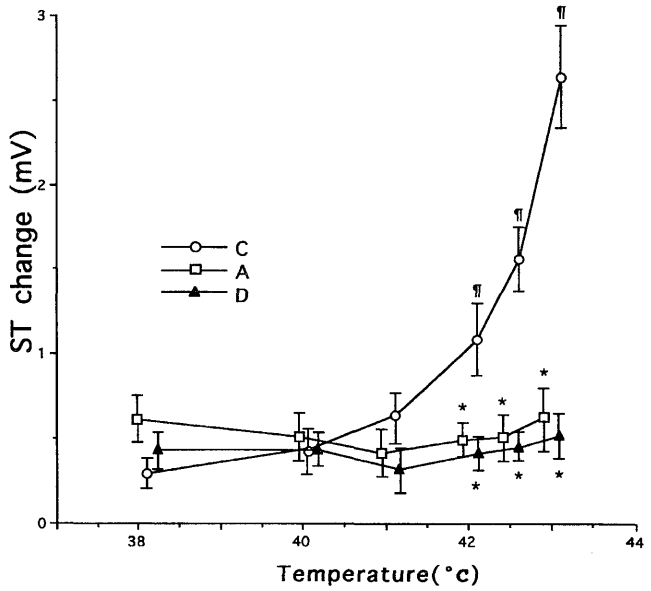

Fig. 4. The effects of acebutolol or diltiazem on endocardial ECG ST segment change during hyperthermia (mean±SEM ; n = 8 for each value).

C, A, D : Refer to Fig. 1.
*p<0.05 vs control. ¶p<0.05 vs 38℃ .

Results

No significant difference was observed in body weight, or Hb among the three groups.

In Group C, 4 of 8 animals survived at 43.5℃, and none of them survived at 44℃. In Group A, all animals survived at 43.5℃, and 6 of the animals survived at 44℃. The survival rate was significantly higher in Group A than in Group C. In Group D, all of 8 animals survived at 43.5℃, and 7 of the animals survived at 44℃. The survival rate was significantly higher in Group D than in Group C (Fig. 1).

The effects of acebutolol and diltiazem on systemic hemodynamics during WBH are shown in Table 1. In Group C, HR increased by 52% from 149 beats/min at 38℃ to 230 beats/min at 43℃, and MAP decreased to 51±5 mmHg at 43℃. In Groups A and D, HR and MAP were kept at the baseline level. CI was maintained in Group A (3.5±0.3 L/min/m^2) and Group D (3.7±0.4 L/min/m^2), and decreased in Group C to 1.2±0.2 L/min/m^2 at 43℃.

The effects of acebutolol and diltiazem on coronary hemodynamics during WBH are shown in Table 2. In Group C, LVdp/dt max increased as temperature increased up to 42.5℃, while it decreased markedly at 43℃. In Groups A and D, LVdp/dt max continued to increase up to 43℃. In Group C, LCBF significantly increased at 42.5℃ and 43℃ compared with that at 38℃ ($p<0.05$). LCVR significantly decreased at 42.5℃ and 43℃ compared with that at 38℃. The value of serum CPK-MB was abnormal only in Group C at 43℃ (18±3.6 U/ml, reference intervals 0-8 U/ml) (Table 3). The myocardial oxygen extraction ratio increased in Group C by 82.2±3.9% at 43℃. No changes were observed in Group A or Group D (Fig. 2). The myocardial lactate extraction ratio decreased in Group C to 4.0±3.6% at 42.5℃ and −5.0±4.1% at 43℃, with significant differences compared with the value at 38℃ (Fig. 3).

ST segment in endocardial ECG continued to increase in Group C and became 2.5 mV or above at 43℃. ST segment showed no significant change in Group A or D at a temperature up to 43℃ (Fig. 4).

Discussion

The present results show that either acebutolol or diltiazem could improve the coronary and systemic hemodynamics during induced hyperthermia resulting in a significant raise in CTMc in dogs. As compared with 38℃, HR increased by 53% at 43℃ in control dogs. In contrast the increase in HR was markedly inhibited by either acebutolol or diltiazem, i.e., 7% increase with acebutolol and 9% with diltiazem. As a result, CI and LVdp/dt max in Group C at 43℃ were significantly reduced as compared with those in Groups A and D, indicating a marked reduction in the contraction force. No changes in the contraction force were observed in Group A or Group C. In

Group C, the myocardial oxygen extraction ratio exceeded 80% (43℃). The myocardial lactate extraction ratio became 0 at 42.5℃ and negative at 43℃, suggesting that anaerobic metabolism was dominant at this temperature[7,8]. In Groups A and D, no significant difference was observed in the myocardial oxygen extraction ratio or the myocardial lactate extraction ratio as compared with the levels at 38℃, suggesting that aerobic metabolism remained dominant even during hyperthermia. ST segment in the endocardial electrocardiogram (Endo. ECG ST change) in Group C increased by 1 mV or more at 42℃ and by 2.5 mV or more at 43℃, indicating hypoxia at least in the subendocardial region. CPK-MB of the blood in the coronary sinus increased significantly only in Group C at 43℃, suggesting bleb formation due to hypoxia, collapse of the blebs that followed, and the consequent release of cardiac enzymes[9]. Since there were no significant differences in the serum potassium level in any group, the structure of the cell wall itself is might be intact.

The use of acebutolol or diltiazem during hyperthermia is considered to be advantageous in the following three respects. (1) They restrict the myocardial oxygen demand by preventing increases in the heart rate and contraction force of the heart and improve the demand-supply balance of oxygen. (2) β-blockers[10] and non-dihydropyridine Ca-blockers[11,12,13] themselves have protective effects on the myocardium against ischemia. (3) The ventricular filling time is obtained by restriction of the heart rate. Because of these advantages, the contraction force of the myocardium is maintained during hyperthermia, and the oxygen supply to various organs is thus sustained. Moreover, there were no significant difference between the group administered acebutolol and the group administered diltiazem, although the mechanisms of action of the two drugs are totally different. Therefore, this increased tolerance to hyperthermia is undoubtedly ascribed to improvements in the myocardial oxygen demand-supply balance.

With these regimens, it is considered possible that CTMc would be raised above 42℃ and that WBH could be conducted at a higher temperature. The anti-tumor effect of hyperthermia would increase by several times per 0.2℃ at a temperature of 43℃ or above[14]. Therefore, even slight increase in CTMc would have a great significance. In addition, widening of the difference between the effective temperature and CTMc would increase the safety of therapeutic hyperthermia.

Many experiments have been done regarding the hemodynamics during hyperthermia[15], but their results are complicated by accompanied factors such as the anesthetics, fluid infusion, and infection. In this study, to eliminate the effects of anesthesia on the myocardium and the coronary blood flow as much as possible, we used barbiturate only in the induction period[16] and avoided the use of gaseous anesthetics throughout the experiment[17,18] and anesthetized the animals with a large dose of morphine[19,20,21]. Moreover, to eliminate the effects of infection during hyperthermia (translocation and endotoxemia)[22,23], kanamycin was administered orally 1 day before the experiment, a second generation cefem antibiotic was administered at the beginning of the experiment, and the experiment was carried out by an aseptic procedure.

In conclusion, acebutolol or diltiazem improves the myocardial oxygen demand-supply balance during hyperthermia. This effect would prevent circulatory collapse, and thus might raise CTMc above 42℃.

Acknowledgements

The auther thanks Drs. K. Sumikawa and H. Nakamura for their continuing support, and Mr. H. Uresino for technical assistance.

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