4. 評価時期、および運動指導の実施手順

観察開始前の身体活動量を測定する期間として、全被験者にはLCを1-2週間装着することを指示した（図2）。その後、観察開始前評価として観察開始前の身体活動量と血管内皮機能を測定し、採血を実施した。4週間後、同様の評価を実施した。観察終了後の身体活動量を測定する期間は観察期間の4週間とし、その平均値を解析値として用いた。

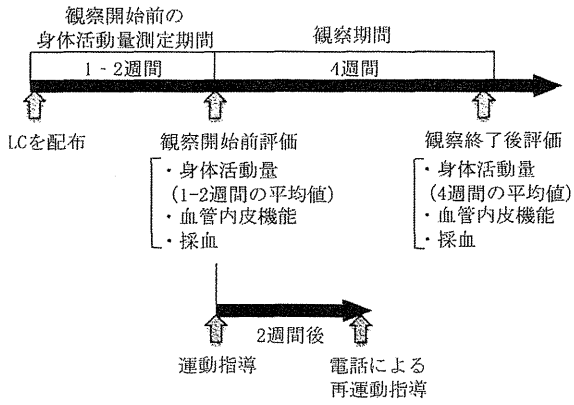

図2　評価時期、運動指導の実施手順
観察開始前の身体活動量測定期間（1-2週間）、および観察期間（4週間）の合計5-6週間を研究期間とした。全被験者に生活習慣記録機Lifecorder GS（LC）を配布して1-2週間経過した後、観察開始前評価、運動指導を行った。2週間後に電話による再運動指導、4週間後に観察終了後評価を実施した。

5. 統計学的解析

観察期間の前後における各測定項目の比較には、対応のあるt検定を用いて検討した。運動指導後における歩数と中強度の運動時間のガイドライン推奨値に対する達成度から被験者を3群に分けた場合、各測定項目の比較には分散分析を用いた。さらに、観察期間前後の各測定項目の差分から変化量（Δ）を算出し、ピアソンの積率相関係数を用いて相関関係を検討した。統計ソフトはSPSS12.0J for Windowsを用い、統計学的有意水準は5%未満とした。

III. 結　　果

対象者の臨床的背景因子、および観察期間前後の各測定項目の変化を表1に示す。表1のよう

表1　臨床的背景因子、観察期間前後の各測定項目の変化

測定項目	観察開始前	観察終了後	P値
年齢（歳）	68.8±7.8	—	—
性別（男/女）	23/4	—	—
冠危険因子保有数（個）	3.6±0.9	—	—
高血圧（%）	89	—	—
糖尿病（%）	52	—	—
脂質異常症（%）	93	—	—
喫煙（%）	4	—	—
肥満（%）	52	—	—
冠動脈疾患の既往（%）	67	—	—
降圧薬（%）	85	—	—
糖尿病治療薬（%）	15	—	—
高脂血症治療薬（%）	70	—	—
BMI（kg/m²）	24.8±2.6	24.9±2.7	0.146
腹囲（cm）	88.3±8.7	88.1±9.1	0.619
収縮期血圧（mmHg）	121.7±11.4	118.6±11.4	0.162
拡張期血圧（mmHg）	72.2±10.1	69.1±9.2	0.160
LDL-C（mg/dL）	102.6±28.5	106.0±26.8	0.233
HDL-C（mg/dL）	51.6± 13.8	51.2±14.4	0.706
中性脂肪（mg/dL）	134.3±69.7	116.2±55.2	0.025
空腹時血糖（mg/dL）	104.7±13.6	99.9±15.3	0.154
HbA1c（%）	5.7±0.5	5.7±0.5	0.866
CRP（mg/dL）	0.18±0.36	0.21±0.23	0.666
歩数（歩）	6987.9±2230.6	9117.4±±2673.1	<0.0001
運動量（kcal）	188.9±79.5	258.2±98.9	<0.0001
中強度の運動時間（分）	21.5±15.6	35.3±23.4	<0.0001

平均値±標準偏差、BMI：Body mass index、LDL-C：LDLコレステロール、HDL-C：HDLコレステロール
HbA1c：ヘモグロビンA1c、CRP：C-reactive protein

に、冠危険因子の重積状態の患者が多く、高血圧・糖尿病・脂質異常症の3つを合併している患者は48%だった。観察開始前において、各種ガイドラインで推奨されている歩数が10,000歩/日、中強度の運動時間が30分/日の目標値のうち、両方の目標値を達成している割合は7%（n=2）、いずれかの目標値を達成している割合は33%（n=9）、いずれの目標値も達成できていない割合は59%（n=16）であった。観察開始時の運動指導から2週間後、歩数と中強度の運動時間が両方とも目標値を達成している割合は30%（n=8）、いずれかの目標値を達成している割合は22%（n=6）であり、52%（n=14）の被験者は少なくとも1つ以上の目標値を達成できていた。一方、いずれの目標値も達成できていない割合は48%であった。観察開始から2週目の再運動指導時に歩数と中強度の運動時間の目標値を両方達成できた被験者を両方達成群（n=8）、いずれかの目標値を達成できた被験者を片方達成群（n=6）、いずれの目標値も達成できなかった被験者を両方未達成群（n=13）の3群に分類し、観察開始前の身体活動量について比較すると、分散分析の結果、両方非達成群は両方達成群に比べて観察開始前の歩数（5,416.8±1,907.1 vs. 8,733.3±1,330.1、P<0.0001）、中強度の運動時間（11.6±9.9 vs. 36.9±13.1、P<0.0001）はそれぞれ有意に低下していた。また、両方非達成群は片方達成群と比べても観察開始前の歩数は有意に低下していた（5,416.8±1,907.1 vs. 8,064.5±1,408.7、P=0.009）。

非達成群には再運動指導を行い、初回から4週間後には、歩数、運動量、中強度の運動時間は有意に増加した（すべてP<0.0001）。さらに観察終了後、歩数が10,000歩/日、中強度の運動時間が30分/日の目標値の両方を達成できた割合は26%（n=7）、いずれかの目標値を達成しているものを含めると59%（n=16）であった。一方で、いずれの目標値も達成できなかった割合は41%（n=11）であった。また、運動指導後、中性脂肪は有意に低下した（P=0.025）。

血管内皮機能の改善効果を図3に示す。血管内皮機能の指標であるRH-PAT indexは観察終了後に有意に改善し（1.7±0.6 to 2.0±0.8、P=0.028）、16%増加した。さらに、観察終了後における歩数、中強度の運動時間の達成度が血管内皮機能の改善に与える影響を検討するため、被験者を歩数、中強度の運動時間が両方ともガイドラインの目標値を達成できた両方達成群（n=7）、いずれかの目標値を達成できた片方達成群（n=9）、いずれの目標

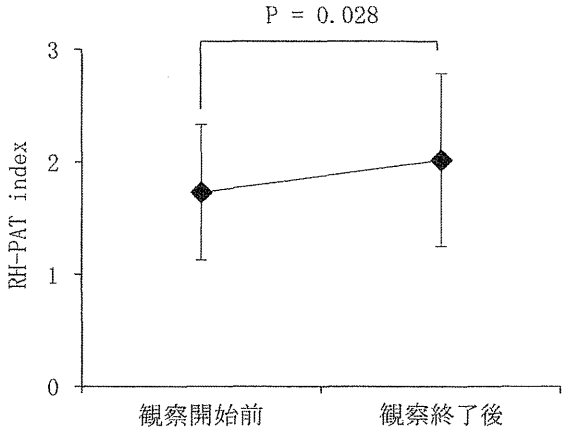

図3　観察期間前後の血管内皮機能の変化
観察終了後、血管内皮機能の指標であるReactive hyperemia-peripheral arterial tonometry (RH-PAT) indexは有意に増加した。

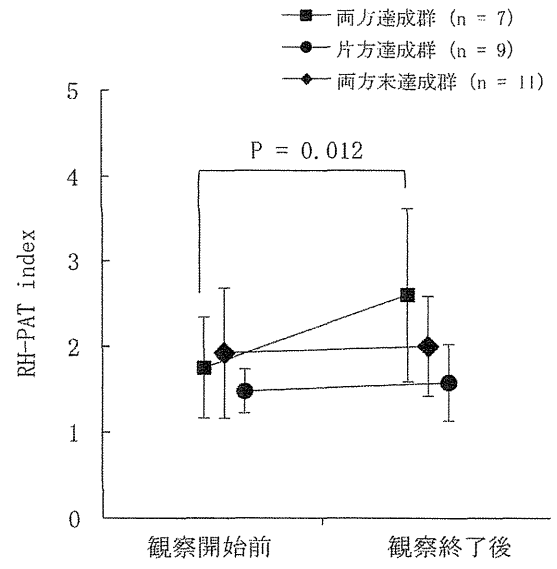

図4　歩数、中強度の運動時間の目標値に対する達成度別の血管内皮機能の変化
観察期間終了後、ガイドラインで推奨されている歩数が10,000歩/日、中強度の運動時間が30分/日の目標値に対する達成度から、被験者を両方達成群、片方達成群、両方未達成群の3群に分類した。両方達成群のみ、観察期間前後で血管内皮機能の指標であるReactive hyperemia-peripheral arterial tonometry (RH-PAT) indexは有意に改善し、交互作用を認めた。

値も達成できなかった両方未達成群（n=11）の3群に分類した。分散分析の結果、両方達成群のみRH-PAT indexは有意に改善して48%増加し、達成度と時間の要因に交互作用を認めた（1.8±0.6 to 2.6±1.0、F = 5.398、df（2,24）、P = 0.012）（図4）。

観察期間前後の血管内皮機能と理学的検査および血液生化学検査の変化量について相関関係を検討した。表2に示したように、ΔRH-PAT indexはΔ拡張期血圧と有意な負の相関を認めた（r=-0.539、P=0.004）。また、ΔRH-PAT indexはΔ歩数（r=0.531、P=0.004）（図5）、Δ運動量（r=0.555、P=0.003）（図6）と有意な正の相関を認めた。

表2　血管内皮機能の変化量と理学的検査および血液生化学検査の変化量の相関関係

測定項目の変化量	ΔRH-PAT index	
	r	P値
ΔBMI（kg/m²）	0.140	0.487
Δ腹囲（cm）	-0.042	0.836
Δ収縮期血圧（mmHg）	-0.248	0.213
Δ拡張期血圧（mmHg）	-0.539	0.004
ΔLDL-C（mg/dL）	-0.184	0.359
ΔHDL-C（mg/dL）	0.336	0.087
Δ中性脂肪（mg/dL）	-0.145	0.471
Δ空腹時血糖（mg/dL）	0.195	0.329
ΔHbA1c（%）	-0.038	0.851
ΔCRP（mg/dL）	-0.021	0.916

Δ:変化量、BMI:Body mass index、LDL-C:LDLコレステロール、HDL-C:HDLコレステロール、HbA1c:ヘモグロビンA1c、CRP:C-reactive protein、RH-PAT:reactive hyperemia peripheral arterial tonometry

IV. 考　　察

本研究の目的は、歩数計を用いたセルフモニタリングによる身体活動量の増加が血管内皮機能に与える影響を検討することであった。今回の結果から、生活習慣病患者における歩数計を利用した短期間の身体活動量の増加は血管内皮機能を改善し、身体活動量増加の程度に伴って血管内皮機能は改善することが示された。

身体活動量を高く維持することは心血管疾患の罹患率や死亡率に対して抑制的に作用する[6,7]ことから、日常的に身体活動量を高く保つことは非常に重要である。生活習慣病の予防、心血管疾患の発症・再発予防における各種ガイドラインでは10,000歩/日[1,2]、および中強度の運動時間を30分/日以上確保すること[4,5]が推奨されている。観察開始前、本研究の対象者の歩数、中強度の運動時間はこの推奨値に到達している割合は低く、身体活動量は低下している状況であった。初回の運動指導から2週目の再運動指導時には各種ガイドラインの推奨値を達成した割合は増加する様子が

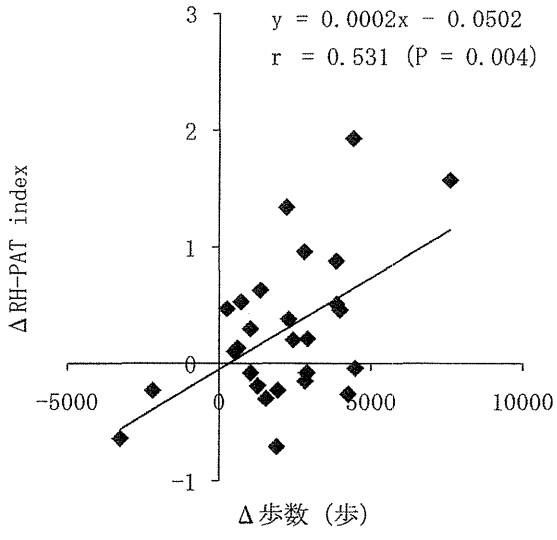

図5　血管内皮機能の変化量と歩数の変化量の相関関係
観察期間前後の血管内皮機能の変化量（ΔRH-PAT index）は、Δ歩数と有意な正の相関関係を認めた。

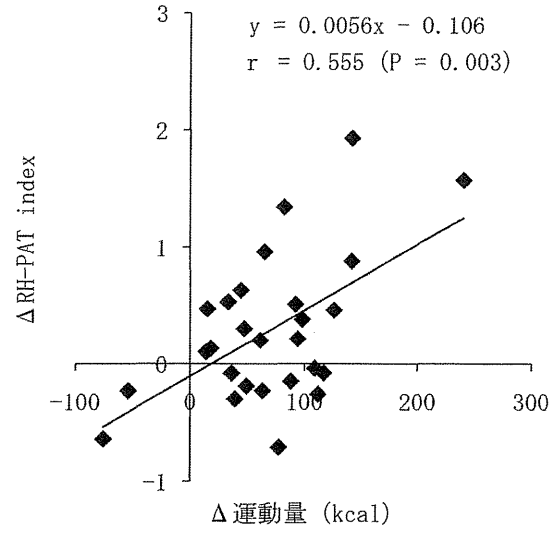

図6　血管内皮機能の変化量と運動量の変化量の相関関係
観察期間前後の血管内皮機能の変化量（ΔRH-PAT index）は、Δ運動量と有意な正の相関関係を認めた。

みられたが、目標値を達成できなかった割合も高く、歩数、および中強度の運動時間の両方の目標値を達成できなかった対象者の特徴として観察開始前の身体活動量が比較的低い様子がみられた。また、最終的な目標達成は、歩数と中強度の運動時間を両方達成できた割合は26%、いずれかの目標値を達成できた割合は33%であり、59%の被験者は歩数、中強度の運動時間のうち少なくとも1つ以上は目標値を達成しており、ガイドラインの推奨値を達成できた割合は増加した。しかし、いずれの目標値も達成できなかった割合は41%と比較的高い状況であった。歩数計を利用した身体活動量の増加には、明確な目標設定と日々の運動の実施状況をセルフモニタリングすることが重要であるとされている[14]。さらに、運動指導後に各対象者の運動に対するモチベーチョンを維持するためには、電話やはがきなどによるフォローアップの重要性が示唆されている[27]。したがって各対象者に対する明確な目標値の設定、およびセルフモニタリングの実施、さらに電話による運動指導後のフォローアップが身体活動量を増加させ、各種ガイドラインで推奨されている目標値の到達割合を増加させるのに有効であったと考えられた。しかし、今回は観察開始前の身体活動量の程度に関わらず、対象者全員に対して「1日10,000歩を目標に歩くこと」、および「中等度の運動強度である速歩をなるべく多く取り入れること」を指導しており、観察開始前に身体活動量が低い場合は目標を達成することが困難な対象者が多かったことが考えられた。目標値の設定には、対象者全体に同一の高い目標値を設定する場合と、各対象者に対して個別に達成可能な目標値を設定する場合とがある。Sidmanら[28]は非活動的な女性に対して、全員に「10,000歩/日」を指導した場合と、「1,000歩から3,000歩の間で達成可能な歩数」を個別に指導した場合とでは、両者ともに同程度に身体活動量は増加するが、全員に「10,000歩/日」を指導した場合の目標達成率は個別の目標設定をした場合に比べて、もともとの身体活動量が低い対象者の目標達成率は低かったと報告している。したがって、もともとの身体活動量が低い患者に対する運動指導は、到達可能な目標値を個別に設定することが目標達成には有効であると考えられた。

高血圧、糖尿病、脂質異常症、肥満などの冠危険因子は酸化ストレスの増加などから血管内皮機能障害を引き起こす[10]。血管内皮細胞には多様な働きがあり、中でもNOを産生して血管平滑筋を弛緩させる内皮依存性血管拡張反応は代表的な血管内皮機能として知られている。血管内皮機能障害とはこの内皮依存性血管拡張反応が低下していることを意味し、これには内皮型一酸化窒素合成酵素（eNOS）の発現低下やNO産生の低下、NOの不活性化が関与している[10]。RH-PAT検査は非侵襲的で再現性に優れた血管内皮機能の検査方法であり、RH-PAT indexはNO産生による血管内皮機能を反映すること[29]、Flow-mediated dilatation（FMD）と相関すること[30]が報告されている。また、RH-PAT indexが1.35未満では冠動脈の血管内皮機能が低下していること[31]や、1.49未満では心血管イベントの発生率が増加すること[13]が示されている。しかしながら、本研究における対象者は冠危険因子の重積者が多かったにも関わらず、観察開始前のRH-PAT indexは1.7であり、血管内皮機能は比較的良好であった。さらに、観察終了後に血管内皮機能は有意に改善する様子がみられた。従来より、降圧薬[32]や高脂血症治療薬[33]などの薬物療法はFMDによる血管内皮機能を改善することが知られている。対象者の中で、降圧薬と高脂血症治療薬を服薬している割合がそれぞれ85%、75%と高かったことから、観察開始前に積極的な薬物療法を施されていたことにより、観察開始前の血管内皮機能が比較的良好であったと考えられた。また、そのような疾病管理が十分に行われている患者に対しても、さらに運動指導を行うことが血管内皮機能の改善に有効であった。したがって、疾病管理されている患者に対する運動指導は、将来的な心血管疾患の発症を予防するうえで非常に重要であると考えられた。

運動療法による血管内皮機能の改善効果として、自転車エルゴメーターなどの機械を用いた有酸素運動では健常成人[16]、高血圧[17]、糖尿病[18]、脂質異常症[19]、冠動脈疾患患者[20]における血管内皮機能の改善が報告されている。また、血管内皮機能は従来の冠危険因子の改善とは独立して改善する

傾向があり、血管内皮機能の改善は他の変化よりも先行している可能性が示唆されている[21]。本研究では、4週間の観察期間前後において血管内皮機能は改善し、観察期間前後の血管内皮機能の変化量は、身体活動量の指標である歩数・運動量の変化量と相関関係を示した。慢性的なシェアストレスの増加はeNOSの遺伝子発現を増加させること[34]、最大酸素摂取量の50%である中等度の有酸素運動が血管内皮機能の改善に最も有効であること[16]が報告されており、血管内皮機能の改善には運動療法における運動の量、およびその強度設定による血管壁への適度なシェアストレスが重要であると推察される。また、LCにおける運動量は体重と運動強度の積から算出されるため、中強度の運動時間の増加は運動量の増加として反映される。したがって、今回は歩数の増加、および中強度の運動時間増加に伴う運動量の増加により、慢性的に血管壁に対して適度なシェアストレスが加わり続け、eNOSの遺伝子発現の増加、NO産生増加が起こり、その結果として身体活動量増加の程度に伴って血管内皮機能が改善した可能性が高い。しかしながら、血管内皮機能の改善効果を各種ガイドラインで推奨されている歩数と中強度の運動時間の目標値に対する達成度から検討すると、両方の目標値を達成できた場合に血管内皮機能の改善が認められ、改善率は48%であった。したがって、有効な血管内皮機能の改善を得るためには各種ガイドラインで推奨されている歩数、中強度の運動時間のうち、両方達成できればその改善効果は大きいと考えられた。以上より、生活習慣病患者における歩数計を利用した運動指導は容易に歩数と運動量を増加させ、血管内皮機能の改善に対して有効な方法であり、この血管内皮機能の改善は将来的な心血管疾患の罹患率や死亡率を抑制する可能性があることから予防医学的に意義の高いものであると考えられた。

しかし本研究の限界として、対照群を設けていないため、血管内皮機能の改善に身体活動量増加以外の交絡因子が関与している可能性を否定できない。さらに、本研究の対象者は外来通院されている中でも研究参加の同意が得られたことから、自身の健康や運動に対して比較的関心のある者であり、歩数計を使った運動指導が行い易かった特殊な対象である可能性がある。したがって、一般外来における歩数計を用いた運動指導は、自身の健康や運動に対して関心のある者に対して導入しやすいと思われた。そして、身体活動量の増加による血管内皮機能の改善効果を4週間という短期間でしか評価しておらず、身体活動量の増加により改善した血管内皮機能が4週間経過した後にどの程度維持されているのかは不明である。血管内皮機能は将来的な心血管疾患発症の予測因子である[12,13]が、短期的な身体活動量の増加により改善した血管内皮機能をその後長期にわたって維持することが心血管疾患の発症や死亡率の抑制には重要であり、身体活動量の増加が血管内皮機能に与える長期効果については今後の検討課題である。

V. 結　　論

歩数計を利用したセルフモニタリングによる運動指導はたとえ4週間という短期間であっても身体活動量を増加させ、血管内皮機能を改善することが明らかとなった。したがって、これらの運動指導は血管内皮機能の改善に有効であり、予防医学的に意義の高いものであると思われた。

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ABSTRACT

Pedometer-based exercise increases physical activity and improves endothelial function in patients at high risk of cardiovascular disease

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Background: High levels of physical activity (PA) have favorable effects on vascular endothelial function and prevent morbidity and mortality of cardiovascular disease. Although a pedometer is a useful tool to increase PA, few studies have examined the influence of pedometer-based exercise on endothelial function. The aim of this study was to clarify the effectiveness of pedometer-based exercise on endothelial function in patients at high risk of cardiovascular disease.

Methods: Twenty-seven patients at high risk of cardiovascular disease with at least 2 coronary risk factors were enrolled in the study. Participants wore a pedometer for 1 to 2 weeks for baseline assessment of the count of steps/day and moderate-intensity walking time/day before the intervention. Participants were instructed to walk more than 10,000 steps/day and increase walking time/day at moderate-intensity as much as possible for 4 weeks. Physical therapists gave instructions directly to each participant at the beginning of the intervention and 2 weeks later. Reactive hyperemia peripheral arterial tonometry (RH-PAT) index, which is used to assess vascular endothelial function, was measured by Endo-PAT®. RH-PAT index, steps/day, amount of energy consumption during exercise, and moderate-intensity walking time/day were measured before and after the 4-week intervention. Furthermore, we calculated the change (Δ) in each measurement and assessed the correlation with other parameters.

Results: RH-PAT index, steps/day, amount of energy consumption during exercise, and moderate-intensity walking time/day were significantly increased after the intervention ($P=0.028$, $P<0.0001$, $P<0.0001$, $P<0.0001$ respectively). ΔRH-PAT index was positively correlated to Δsteps/day ($r=0.531$, $P=0.004$), Δamount of energy consumption during exercise ($r=0.555$, $P=0.003$).

Conclusions: Increased PA by pedometer-oriented 4-week exercise improved endothelial function in patients at high risk of cardiovascular disease.

Key Words : *lifestyle related disease, pedometer, physical activity, endothelial function*

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Cardioprotective Effects of Sarcolemmal and Mitochondrial K-ATP Channel Openers in an Experimental Model of Autoimmune Myocarditis

Role of the Reduction in Calcium Overload During Acute Heart Failure

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SUMMARY

It has been reported that K-ATP channel openers have a cardioprotective effect in acute ischemia as a pharmacological preconditioning effect. In the present study, the chronic effects of clinical K-ATP channel openers, ie, nicorandil (Nic) and mexiletine (Mex), on cardiac function were evaluated in a rat model of experimental autoimmune myocarditis (EAM). Nicorandil (3 or 10 mg/kg/day) or Mex (10 or 25 mg/kg/day) was administered to the EAM rats, and the effects were compared with those in untreated EAM rats (control EAM) and sham rats without EAM on day 21 (acute phase) or day 60 (chronic phase). In the acute phase, the control EAM rats exhibited a reduced left ventricular ejection fraction (LVEF) and prolonged monophasic action potential duration (MAPD). Neither drug had an affect on the LVEF or degree of myocarditis, but Mex 25 mg suppressed the MAPD prolongation. In the chronic phase, EAM+Nic and EAM+Mex 25 mg exhibited a higher LVEF than the control EAM. Although the control EAM exhibited sustained MAPD prolongation, the other groups showed recovery of the MAPD in the chronic phase. The mitochondorial redox state was lower in the control EAM than in the sham, and EAM+Nic exhibited a similar level of the redox state as the sham in the chronic phase. Nicorandil exhibited a cardioprotective effect through the protection of mitochondrial function. Mexiletine exhibited a cardioprotective effect possibly through a reduction in the calcium overload by shortening the MAPD in the acute phase. (Int Heart J 2012; 53: 139-145)

Key words: K-ATP channel, Myocardial protection, Nicorandil, Mexiletine, Myocarditis

The K-ATP channel exists in the cell membranes of many organs and has been known to play various roles, such as triggering insulin secretion in the pancreas, or suppressing lymphocytes during inflammatory conditions.[1,2] In an ischemic myocardium, the sarcolemmal K-ATP channels open, corresponding to a reduction in the intracellular ATP, which prevents myocytes from an intracellular calcium overload by shortening the action potential duration. This response is considered to be an auto-cardioprotective effect during ischemic conditions, which is known to be enhanced by sarcolemmal K-ATP channel openers.[3-7] However, K-ATP channels are found not only in the sarcolemma of myocytes, but also in the mitochondrial membrane, and openers of these mitochondrial K-ATP channels, such as nicorandil, have been proven to play an important role in its cardioprotective effect by protecting mitochondrial function, especially in short-term acute ischemia.[8-10]

It has been reported that an increase in ventricular wall stress causes prolongation of the action potential duration, particularly in damaged myocardium with heart failure.[11] This prolongation of the action potential duration may increase transient calcium and may exaggerate intracellular calcium overload, resulting in the deterioration of myocardial dysfunction.[12] We documented that this electrophysiological change can also be observed in acute inflammatory conditions in an experimental autoimmune myocarditis (EAM) model in rats,[13,14] which is a model showing acute myocarditis in the acute phase followed by dilated cardiomyopathy in the chronic phase. In the acute phase of this model, there would be a worsening cycle between the intracellular calcium overload and prolonged action potential duration as a result of the myocardial injury, which may also adversely affect mitochondrial function. Based on these findings, we speculate that shortening of the action potential duration in this acute phase protects the myocardium by decreasing the intracellular calcium overload, and the protection of mitochondrial function prevents myocardial injury, based on similar mechanisms seen in ischemic conditions. In the present study, we evaluated the effects of two clinically available K-ATP channel openers, nicorandil (Nic: mainly a mitochondrial

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K-ATP channel opener) and mexiletine (Mex: a sarcolemmal K-ATP channel opener with a sodium channel blocking effect), on ventricular dysfunction in EAM.

Methods

Induction of autoimmune myocarditis: EAM was induced in 6-week-old male Lewis rats by immunization with purified porcine cardiac myosin as previously described (n = 110).[13,14] The sham rats received injections of the same amount of saline (0.25 mL) in the same manner (n = 20). EAM was induced in all 110 rats immunized, however, 7 rats died during the acute phase so 103 of 110 were used in the experiments. The present study was performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and Ethics Committee of the Kitasato University School of Medicine.

Grouping and administration of drugs: The rats were divided into 6 groups in accordance with the induction of EAM and drug administration as follows; 1) control EAM: rats with induction of EAM but without any additional drug administration (n = 28), 2) EAM+Nic 3 mg: rats with EAM and treatment with Nic 3 mg/kg/day (n = 25), 3) EAM+Nic 10 mg: rats with EAM and treatment with Nic 10 mg/kg/day (n = 24), 4) EAM+Mex 10 mg: rats with EAM and treatment with Mex 10 mg/kg/day (n = 14), 5) EAM+Mex 25 mg: rats with EAM and treatment with Mex 25 mg/kg/day (n = 12), and 6) sham: rats without induction of EAM and no additional drug administration (n = 20). A study drug was orally administered by adding it to the drinking water after the immunization to induce EAM. The dosage of the drug was calculated by monitoring the water consumption and body weight. The dosages used were determined based on previous reports,[15-17] ie, the lower and higher dosages represented the middle and highest doses of the therapeutic range, respectively.

Noninvasive echocardiography: The left ventricular dimension and function *in vivo* were assessed using transthoracic echocardiography (ProSound SSD-4000, Aloka, Tokyo) as previously described[13] and were performed in randomly selected rats at day 21 or day 60 after the initial immunization as summarized in Table I. The assessment was done noninvasively under light anesthesia with an intraperitoneal injection of 2,2,2-tribromoethanol (80-120 mg/kg). The left ventricular end-systolic and end-diastolic dimensions were measured from the M-mode echocardiogram to calculate the left ventricular ejection fraction (LVEF) and fraction shortening (LV%FS).

Open chest electrophysiological study: After the echocardiographical evaluation, an open chest electrophysiological study

Table I. Histological and Physiological Parameters

Day 21	Sham (n = 5)	Control EAM (n = 9)	EAM+Nic 3 mg (n = 7)	EAM+Nic 10 mg (n = 7)	EAM+Mex 10 mg (n = 7)	EAM+Mex 25 mg (n = 6)
Hw/Bw (g/kg)	3.5 ± 0.3	8.0 ± 0.6*	7.9 ± 0.7*	7.8 ± 0.8*	7.7 ± 1.0*	8.4 ± 1.1*
Area of cellular infiltration (%)	0.0 ± 0.0	37.6 ± 6.6*	35.2 ± 7.2*	31.6 ± 5.6*	35.4 ± 7.2*	36.1 ± 6.9*
Area of fibrosis (%)	-	-	-	-	-	-
Heart rate (bpm)	376 ± 36	382 ± 40	376 ± 42	386 ± 39	377 ± 42	386 ± 36
LVSP (mmHg)	116 ± 26	104 ± 25	108 ± 32	105 ± 22	106 ± 26	99 ± 32*
LVEDP (mmHg)	1.9 ± 0.8	12.1 ± 5.2*	11.9 ± 4.8*	12.4 ± 4.6*	11.6 ± 4.3*	13.4 ± 6.8*
LVEF (%)	76 ± 5	40 ± 6*	42 ± 5*	42 ± 8*	41 ± 5*	36 ± 8*
LV%FS	45 ± 4	21 ± 6*	22 ± 5*	23 ± 7*	22 ± 6*	19 ± 9*
ERP (BCL = 150 ms, ms)	70 ± 6	84 ± 9*	83 ± 7*	81 ± 6*	79 ± 8*	75 ± 6†
ERP (BCL = 120 ms, ms)	67 ± 5	81 ± 7*	79 ± 6*	80 ± 9*	75 ± 6*	72 ± 8†
$MAPD_{20}$ (BCL = 150 ms, ms)	13 ± 4	30 ± 4*	28 ± 6*	26 ± 5*	24 ± 5*	18 ± 7*†
$MAPD_{20}$ (BCL = 120 ms, ms)	14 ± 3	31 ± 6*	26 ± 7*	24 ± 5*	22 ± 4*	17 ± 7†
$MAPD_{90}$ (BCL = 150 ms, ms)	62 ± 5	108 ± 8*	102 ± 8*	104 ± 8*	95 ± 12*	78 ± 9*†
$MAPD_{90}$ (BCL = 120 ms, ms)	60 ± 6	104 ± 10*	104 ± 10*	99 ± 10*	96 ± 11*	74 ± 12*†
Day 60	**Sham (n = 5)**	**Control EAM (n = 9)**	**EAM+Nic 3 mg (n = 8)**	**EAM+Nic 10 mg (n = 7)**	**EAM+Mex 10 mg (n = 7)**	**EAM+Mex 25 mg (n = 6)**
Hw/Bw (g/kg)	3.5 ± 0.5	5.0 ± 0.6*	4.0 ± 0.5†	3.7 ± 0.4†	4.4 ± 0.7*	3.9 ± 0.8†
Area of cellular infiltration (%)	0.0 ± 0.0	2.6 ± 2.2	1.6 ± 1.5	1.4 ± 1.8	2.6 ± 1.4	1.6 ± 2.1
Area of fibrosis (%)	0.0 ± 0.0	29.6 ± 5.6*	18.6 ± 4.6*	8.2 ± 4.3†	16.6 ± 6.2*	14.4 ± 6.2*
Heart rate (bpm)	381 ± 46	368 ± 36	377 ± 42	379 ± 40	368 ± 36	359 ± 46
LVSP (mmHg)	122 ± 30	106 ± 28	110 ± 26	119 ± 26	116 ± 21	111 ± 24
LVEDP (mmHg)	2.0 ± 1.0	13.9 ± 8.2*	6.4 ± 3.4*	3.4 ± 2.9†	8.6 ± 4.2*	3.9 ± 3.0†
LVEF (%)	75 ± 6	37 ± 6*	62 ± 6†	72 ± 8†	46 ± 7*	67 ± 9†
LV%FS	43 ± 7	20 ± 5*	41 ± 3†	44 ± 6†	26 ± 7*	40 ± 6†
ERP (BCL = 150 ms, ms)	71 ± 6	74 ± 8	74 ± 4	70 ± 5	73 ± 4	68 ± 6
ERP (BCL = 120 ms, ms)	67 ± 5	70 ± 7	71 ± 6	68 ± 7	69 ± 5	65 ± 9
$MAPD_{20}$ (BCL = 150 ms, ms)	14 ± 3	24 ± 5*	20 ± 4*	16 ± 4	19 ± 3	16 ± 5
$MAPD_{20}$ (BCL = 120 ms, ms)	15 ± 3	21 ± 4*	19 ± 5	16 ± 4	17 ± 5	15 ± 4
$MAPD_{90}$ (BCL = 150 ms, ms)	65 ± 6	69 ± 8	66 ± 7	66 ± 9	67 ± 9	67 ± 7
$MAPD_{90}$ (BCL = 120 ms, ms)	62 ± 4	67 ± 8	65 ± 6	63 ± 7	65 ± 6	63 ± 9

* $P < 0.05$ versus sham, † $P < 0.05$ versus Control EAM. Hw/Bw indicates heart and body weight ratio; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LVEF, leftventricular ejection fraction; LV%FS, left ventricular percent fraction shortening; ERP, effective refractory period; BCL, basic cycle length, and MAPD, duration of the monophasic action potential.

was performed to evaluate the electrophysiological parameters on day 21 or day 60 as summarized in Table I. A median sternotomy was carried out under intraperitoneal anesthesia with 2,2,2-tribromoethanol (240 mg/kg). To avoid the influence of these macroscopic procedures on mitochondrial function, the rats used in the assessment of mitochondrial function were prepared separately from these rats for the electrophysiological measurements as shown in Table II. For the hemodynamic parameters, the left ventricular systolic pressure (LVSP) and left ventricular end-diastolic pressure (LVEDP) were monitored by a needle tip micromanometer (SPR477, Millar, USA) as previously described.[13,14] For the electrophysiological evaluation, a pair of platinum needle electrodes (φ 0.1 mm) was directly inserted into the left ventricular free wall, and used for electrical stimulation and recording. The analogue signals were converted into digital signals at a sampling frequency of 1000 Hz (Power Lab 8sp, Bio Research, Tokyo) and stored on a computer hard disk. The band pass filter was set at 50-300 Hz for a standard electrocardiogram recording and at an open-300 Hz for recording the monophasic action potential (MAP). To evaluate the ventricular effective refractory period (ERP), a 2 ms step shortening the coupling interval of the extrastimulus was employed in two basic cycle lengths of 150 and 120 ms. The MAP duration (MAPD) was determined as the interval from the onset of the MAP to 20% of the repolarization time ($MAPD_{20}$) or to its 90% ($MAPD_{90}$).[13,14]

Heart weight and histology: After the electrophysiological measurements, the heart was totally excised and the weight of the whole heart was measured to calculate the ratio of the heart to body weight (Hw/Bw). The heart was fixed with 10% formalin and embedded in paraffin. After the heart was sliced transversely, the tissue was stained with hematoxylin-eosin or Azan-Mallory for histological evaluation. The area infiltrated by inflammatory cells and/or fibrosis was evaluated using Mac SCOPE software (Mitani Co., Japan) and was expressed as the percentage of the whole ventricular area.[4,13]

Evaluation of mitochondrial function: In randomly selected rats, the contents of the metabolites in the ventricular myocardium were enzymatically measured to evaluate the mitochondrial function on day 21 or day 60 as summarized in Table II. Since Mex, a sarcolemmal K-ATP channel opener, rarely affects mitochondrial function, it was not assessed in the rats treated with Mex. The ventricular muscle was freeze clamped in liquid nitrogen immediately after the median sternotomy and stored at -80°C until measurement of the myocardial metabolites. The cardiac muscle was extracted with 3.6% perchloric acid and neutralized, followed by enzymatic measurement of adenosine-triphosphate (ATP), phosphocreatinine (P-Cr), creatinine (Cr), pyruvate (Pyr), lactate (Lac), dihydroxyacetone phosphate (DHAP), 3-phosphoglycerate (3-PG), α-ketoglutarate (α-KG), glutamate, and ammonia (NH_4) as previously described.[18,19] The myocardial contents of ADP and Pi, the [ATP]/[ADP] ratio, cytosolic phosphorylation potential (PP), ie, [ATP]/[ADP]/[Pi], mitochondrial redox state (mit [NAD^+]/

Table II. Metabolites in the Myocardium

Day 21	Sham (n = 5)	Control EAM (n = 5)	EAM+Nic 3 mg (n = 5)	EAM+Nic 10 mg (n = 5)
ATP (μmol/g wet tissue)	2.14 ± 0.08	1.65 ± 0.05**	1.65 ± 0.09*	1.68 ± 0.05*
P-Cr (μmol/g wet tissue)	6.31 ± 0.38	3.62 ± 0.36*	3.65 ± 0.33*	3.88 ± 0.28*
Pyruvate (μmol/g wet tissue)	0.051 ± 0.003	0.035 ± 0.003*	0.032 ± 0.002*	0.036 ± 0.003
Lactate (μmol/g wet tissue)	0.54 ± 0.02	0.65 ± 0.03	0.62 ± 0.03	0.65 ± 0.03
DHAP (μmol/g wet tissue)	0.040 ± 0.004	0.020 ± 0.002*	0.021 ± 0.003*	0.019 ± 0.003*
3PG (μmol/g wet tissue)	0.019 ± 0.001	0.014 ± 0.002	0.013 ± 0.002	0.013 ± 0.002
Glutamate (μmol/g wet tissue)	2.61 ± 0.09	2.07 ± 0.09*	2.23 ± 0.09	2.36 ± 0.09
α-KG (mmol/g wet tissue)	0.037 ± 0.003	0.057 ± 0.004	0.054 ± 0.004	0.051 ± 0.002
NH_4 (mmol/g wet tissue)	0.97 ± 0.005	1.53 ± 0.08*	1.51 ± 0.009*	1.33 ± 0.06*
ΔG ATP hy (kcal/mol)	15.7 ± 0.1	15.1 ± 0.1*	15.2 ± 0.1*	15.1 ± 0.1*
mito-[NAD^+]/[NADH]	8.14 ± 0.77	24.79 ± 1.44**	21.39 ± 1.36**	17.51 ± 1.73*
Eh-mito-[NAD^+]/[NADH] (mV)	292.3 ± 1.3	277.3 ± 0.9**	279.7 ± 1.0**	282.1 ± 1.4*
Day 60	**Sham (n = 5)**	**Control EAM (n = 5)**	**EAM+Nic 3 mg (n = 5)**	**EAM+Nic 10 mg (n = 5)**
ATP (μmol/g wet tissue)	2.24 ± 0.07	1.76 ± 0.06*	2.42 ± 0.06 ††	2.26 ± 0.07 †
P-Cr (μmol/g wet tissue)	6.33 ± 0.33	4.30 ± 0.35*	6.12 ± 0.32 †	6.20 ± 0.33 †
Pyruvate (μmol/g wet tissue)	0.050 ± 0.004	0.041 ± 0.004	0.050 ± 0.003	0.052 ± 0.004
Lactate (μmol/g wet tissue)	0.52 ± 0.03	0.59 ± 0.04	0.55 ± 0.03	0.52 ± 0.04
DHAP (μmol/g wet tissue)	0.045 ± 0.005	0.030 ± 0.003	0.044 ± 0.003	0.043 ± 0.002
3PG (μmol/g wet tissue)	0.025 ± 0.005	0.019 ± 0.001	0.027 ± 0.002	0.024 ± 0.002
Glutamate (μmol/g wet tissue)	2.56 ± 0.10	2.05 ± 0.11	2.36 ± 0.10	2.53 ± 0.12
α-KG (mmol/g wet tissue)	0.039 ± 0.003	0.043 ± 0.002	0.043 ± 0.003	0.038 ± 0.003
NH_4 (mmol/g wet tissue)	0.946 ± 0.051	1.212 ± 0.059	1.216 ± 0.055	0.979 ± 0.053
DG ATP hy (kcal/mol)	15.7 ± 0.1	15.3 ± 0.1*	15.5 ± 0.1	15.6 ± 0.1 †
mito-[NAD^+]/[NADH]	8.36 ± 0.22	15.30 ± 1.29*	13.58 ± 1.30	8.92 ± 0.77 †
Eh-mito-[NAD^+]/[NADH] (mV)	291.6 ± 0.4	284.0 ± 1.1*	285.7 ± 1.2	291.5 ± 1.4 †

* $P < 0.05$ and ** $P < 0.01$ versus sham, † $P < 0.05$ and †† $P < 0.01$ versus Control EAM. ATP indicates adenosine-triphosphate; P-Cr, phosphocreatinine; DHAP, dihydroxyacetone phosphate; 3PG, 3-phosphoglycerate; α-KG, α-ketoglutamate; NH_4, ammonia; ΔG ATP hy, G-ATP hydrolysis energy; mito [NAD+]/[NADH], mitochondrial redox state; and Eh-mito-[NAD+]/[NADH], mitochondrial redox state of [NAD+]/[NADH].

[NADH]), mitochondrial redox potential of [NAD^+]/[NADH] ($Eh_{NAD+/NADH}$), and $\Delta G_{ATP\ hydrolysis}$ energy ($\Delta G_{ATP\ hydr}$), which is the change in the Gibbs free energy of the ATP hydrolysis in the cytosol of the cells, were calculated as previously reported.[18,19] The myocardial metabolites are expressed as the content per 1g wet weight of myocardium.

Calculation formulae

$Cytosolic\ [ADP] = [ATP] \times [Cr] / [P\text{-}Cr] \times 1.27 \times 10^{-2}\ M$

$Cytosolic\ [ATP] / [ADP] = [P\text{-}Cr] / [Cr] \times 78.9$

$Cytosolic\ [Pi] = [3\text{-}PG] \times [Lac] \times [P\text{-}Cr] / [DHAP] / [Pyr] / [Cr] \times 1.45 \times 10^{-4}\ M$

$Mitochondrial\ [NAD^+] / [NADH] = [\alpha\text{-}KG] / [NH_4^+] \times [H^+] / [Glutamate] / K_{GLDH}$

$Eh_{NAD+/NADH} = E^o_{NAD+/NADH} + RT/nF \times ln([NAD^+] / [NADH]) \times 10^{-7} / [H^+]\ mV$

$Phosphorylation\ Potential = [DHAP] / [Pyr] / [3\text{-}PG] / [Lac] \times 5.45 \times 10^5\ M^{-1}$

$\Delta G_{ATP\ hydr} = \Delta G^o + RT \times ln([ADP] \times [Pi] / [ATP])\ kcal/mol$

(Pi: inorganic phosphate, NAD: nicotinamide adenine dinucleotide, NAD^+: the oxidized form of NAD, NADH: the reduced form of NAD, K: the equilibrium constant for the enzyme catalyzed reaction, K_{GLDH}: equilibrium constant of the glutamate dehydrogenase reaction)

Statistical analysis: All quantitative data are described as the mean ± SEM. The basic comparative statistics were performed using an unpaired Student's *t* test and a $P < 0.05$ was considered as statistically significant.

Results

Heart weight and histology: Figure 1 shows representative examples of the histological findings of the sliced ventricles of the rats in each group on days 21 and 60. Because the histological findings in EAM can be characterized by infiltration of inflammatory cells in the acute phase (day 21) and tissue fibrosis in the chronic phase (day 60), HE and Azan staining were chosen for the presentations of examples, respectively. On day 21, the rats with EAM exhibited a general enlargement due to tissue edema and inflammatory cellular infiltration in comparison with the sham. The treatment with Nic or Mex did not affect the appearance of these findings of acute myocarditis. On day 60, the rats with EAM now exhibited tissue fibrosis represented by a purple color in comparison with the sham. Similar to day 21, treatment with Nic or Mex did not strongly affect the degree of fibrosis, but the area of the fibrosis in the EAM+Nic 10 mg rats seemed to be somewhat smaller than that in the control EAM rats in this example.

Table I shows the histological and physiological data of the rats on day 21 and day 60. On day 21, the heart and body weight ratio (Hw/Bw) was higher in the groups with EAM than in the sham and there was no difference among the groups with EAM regardless of the treatment with Nic or Mex. In contrast, on day 60, the Hw/Bw was still higher in the control EAM and EAM+Mex 10 mg groups than in the sham, but it was significantly smaller in the EAM+Nic 3 mg, EAM+Nic 10 mg, and EAM+Mex 24 mg groups than in the control EAM group. In the histological findings, the area of the inflammatory cellular infiltration on day 21 was larger in the groups with EAM than in the sham and it was not affected by the treatment with Nic or Mex. On day 60, the cellular infiltration was already negligible in all groups, but the area of the tissue fibrosis was significantly larger in the groups with EAM than in the sham regardless of the treatment with Nic or Mex, with the exception of the group with EAM+Nic 10 mg.

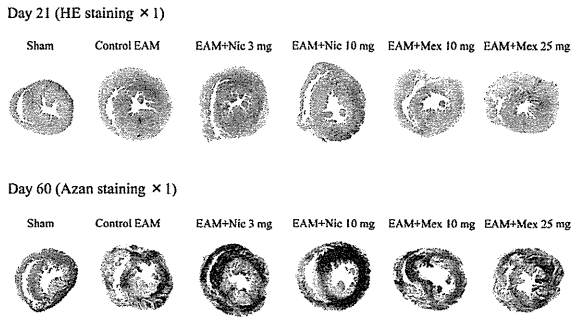

Figure 1. Representative examples of sliced ventricles on days 21 and 60. This figure shows representative examples of the histological findings of the sliced ventricles of rats in each group on days 21 and 60. HE staining was chosen for the presentation of day 21 to exhibit any inflammatory cellular infiltration and Azan staining for day 60 to exhibit any tissue fibrosis. On day 21, the rats with EAM exhibited typical findings of acute myocarditis and were not affected by treatment with Nic or Mex. On day 60, the rats with EAM exhibited tissue fibrosis and were not affected by treatment with Nic or Mex except in the EAM+Nic 10 mg rats. See text for details. HE indicates hematoxylin-eosin; EAM, experimental autoimmune myocarditis; Nic, nicorandil; and Mex, mexiletine.

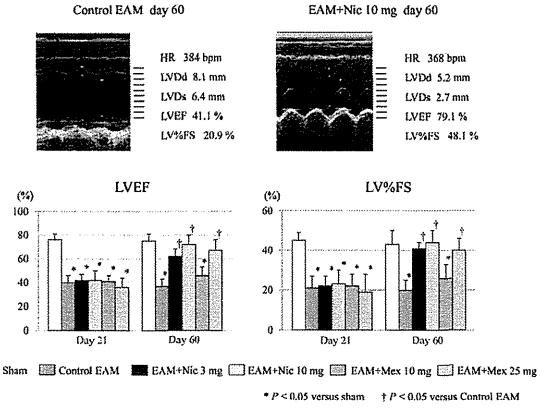

Figure 2. Echocardiographical findings. This figure exhibits the echocardiographical findings of each group on day 21 or day 60. The upper panels show representative examples of the recordings on day 60 in rats in the control EAM and EAM+Nic 10 mg groups. Although the control EAM rats (left panel) exhibited severe hypokinesia of the ventricular wall motion, the EAM+Nic 10 mg rats exhibited almost normal ventricular wall motion. The lower graphs exhibit the summary data of the LVEF and LV%FS in each group. On day 21, the groups with EAM exhibited a lower LVEF and LV%FS than the sham group regardless of the treatment with Nic or Mex. In contrast, on day 60, although the LVEF and LV%FS were still lower in the control EAM and EAM+Mex 10 mg groups than in the sham group, the EAM+Nic 3 mg, EAM+Nic 10 mg, and EAM+Mex 25 mg groups exhibited a higher LVEF and LV%FS than the control EAM group. See text for details. EAM indicates experimental autoimmune myocarditis; Nic, nicorandil; LVEF, left ventricular ejection fraction; LV%FS, left ventricular percentage of fraction shortening; and Mex, mexiletine.

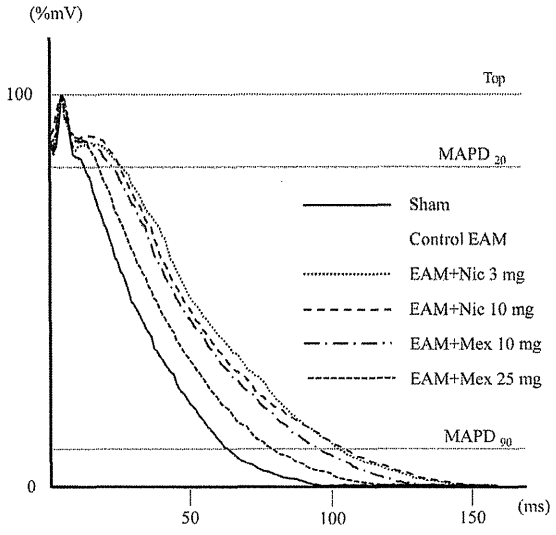

Figure 3. Representative examples of MAP traces on day 21. This figure exhibits representative examples of MAP traces of ventricular muscle in each group on day 21. The control EAM rats exhibited a more prolonged MAPD than the sham rats. The prolonged MAPD was significantly shortened by Mex 25 mg. See text for details. MAPD indicates duration of the monophasic action potential; EAM, experimental autoimmune myocarditis; Nic, nicorandil; and Mex, mexiletine.

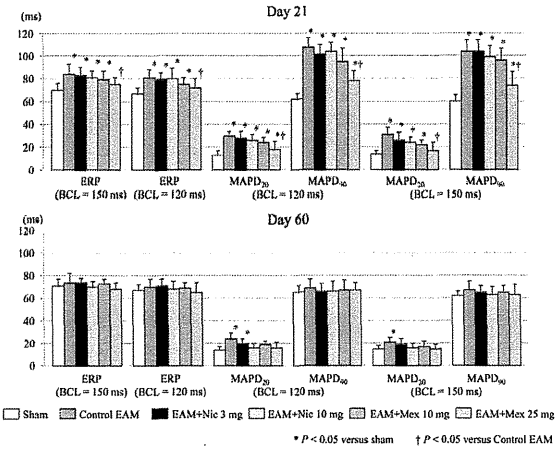

Figure 4. Summary of electrophysiological data. Summary of the ERP and MAPD data in all groups on days 21 and 60. On day 21, the groups with EAM exhibited a prolonged ERP and MAPD in comparison with the sham group, but the Mex treatment partly suppressed this prolongation and the EAM+Mex 25 mg group exhibited a significantly shorter ERP and MAPD than the control EAM group. On day 60, there was no significant difference in the ERP and $MAPD_{90}$ data among all groups, but the EAM+Nic 3 mg and EAM+Nic 10 mg groups exhibited sustained prolongation of the $MAPD_{20}$ even in this phase. See text for details. ERP indicates effective refractory period; BCL, basic cycle length; MAPD, duration of the monophasic action potential; Nic, nicorandil; and Mex, mexiletine.

Hemodynamic and echocardiographical findings: Figure 2 presents the echocardiographical findings of each group on day 21 or day 60. The upper panels show the representative examples of the recordings on day 60 in the rats in the control EAM and EAM+Nic 10 mg groups, and the lower graphs exhibit the summary data of the LVEF and LV%FS in each group. The numbers of rats and data from each group are also shown in Table I. On day 21, the groups with EAM exhibited a lower LVEF and LV%FS than the sham group regardless of the treatment with Nic or Mex and there was no difference among the 5 groups with EAM. In contrast, on day 60, although the LVEF and LV%FS were still lower in the control EAM and EAM+Mex 10 mg groups than in the sham group, the groups with EAM+Nic 3 mg, EAM+Nic 10 mg, and EAM+Mex 25 mg exhibited a higher LVEF and LV%FS than the control EAM group, and no significant differences were observed in these parameters even in comparisons with the sham group.

Among the hemodynamic parameters, the heart rate did not show any significant difference among any groups on days 21 and 60. The LVSP did not show any difference among the groups either on day 21 or day 60, except for in the EAM+Mex 25 mg group on day 21 in which the value was lower than that in the sham. The LVEDP was higher in the groups with EAM than in the sham on day 21 regardless of the treatment with Nic or Mex. On day 60, the LVEDP was still higher in the control EAM, EAM+Nic 3 mg, and EAM+Mex 10 mg groups than in the sham group, but it was lower in the EAM+Nic 10 mg and EAM+Mex 25 mg groups than in the control EAM (Table II).

Electrophysiological parameters: Figure 3 presents representative examples of MAP traces of the ventricular muscle in each group on day 21. The MAP trace in the sham rats exhibited a relatively short MAPD, but it was prolonged in the control EAM rats as a result of a "dome" like prolongation in phase 2 of the action potential as previously described.[14] We expected that the MAPD would be shortened by the administration of Nic or Mex because of their opening effect on sarcolemmal K-ATP channels, however, the administration of Nic did not result in any significant shortening of the MAPD even with a higher dose (ie, 10 mg/kg/day in this study). As a result, the treatment with a higher dose of Mex (ie, 25 mg/kg/day in this study) caused a significant shortening of the MAPD in comparison with the control EAM.

Figure 4 and Table I summarize the ERP and MAPD data in all groups on days 21 and 60. On day 21, the groups with EAM exhibited a prolonged ERP and MAPD in comparison with the sham group. However, this prolongation was partly suppressed by Mex treatment and the EAM+Mex 25 mg group exhibited a significantly shorter ERP and MAPD than the control EAM group. On day 60, there was no significant difference in the ERP and $MAPD_{90}$ data among the groups, but the EAM+Nic 3 mg and EAM+Nic 10 mg groups exhibited a sustained prolongation of the $MAPD_{20}$ even in this phase.

Mitochondrial function: Table II summarizes the myocardial contents of the metabolites and presents variables in the myocardial mitochondria in the sham, control EAM, EAM+Nic 3 mg, and EAM+Nic 10 mg groups. The number of rats in each group is shown in the table. On day 21, the levels of ATP, P-Cr, $\Delta G_{ATP\ hydr}$, and $Eh_{NAD+/NADH}$ decreased and the mit $[NAD^+]/[NADH]$ increased in all groups with EAM regardless of the treatment with Nic, in comparison with the sham group. In contrast, on day 60, although the control EAM group exhibited still lower levels of ATP and P-Cr than the sham group, their

levels in the EAM+Nic 3 mg and EAM+Nic 10 mg groups recovered and became significantly higher than in the control EAM group. $\Delta G_{ATP\ hydr}$ and $Eh_{NAD+/NADH}$ were lowest and the mit [NAD^+]/[NADH] was highest in the control EAM group on day 60, while those in the EAM+Nic 3 mg and EAM+Nic 10 mg groups were at similar levels to the sham group. Furthermore, $\Delta G_{ATP\ hydr}$ and $Eh_{NAD+/NADH}$ were significantly higher and mit [NAD^+]/[NADH] was significantly lower in the EAM+Nic 10 mg group than in the control EAM group.

Discussion

This study evaluated the effects of two K-ATP channel openers, nicorandil (Nic) and mexiletine (Mex), on left ventricular dysfunction in rats with EAM. To the best of our knowledge, this is the first systematic study evaluating the effect of the chronic use of K-ATP channel openers on myocarditis and long-lasting left ventricular dysfunction. Several interesting findings were observed. First, the treatment with Nic or Mex did not affect the degree of acute myocarditis during its acute phase (day 21), but the tissue fibrosis in the chronic phase (day 60) was suppressed by a higher dose of Nic (10 mg/kg/day). Second, the ventricular dysfunction was not suppressed by these treatments in the acute phase, but the left ventricular function recovered better with treatment with Nic at 3 and 10 mg, or Mex at 25 mg in the chronic phase. Third, the ventricular MAPD was prolonged in EAM in its acute phase as previously described, and this prolongation was suppressed by a higher dose of Mex (25 mg/kg/day). Finally, although the level of the ATP and P-Cr was decreased in the myocardial mitochondria in the acute phase of the EAM regardless of the treatment, the redox state recovered better in the chronic phase with Nic treatment.

Cardioprotection by the use of K-ATP channel openers: The cardioprotective effect of Nic is known to be associated with a pharmacological preconditioning effect in acute ischemia.[3,20,21] Although two possible mechanisms for this cardioprotective effect have been discussed, a reduction in the intracellular calcium overload by shortening the action potential duration through sarcolemmal K-ATP channel opening and the protection of mitochondrial function by opening the mitochondrial K-ATP channels, the latter has been more emphasized in recent reports because the effect of Nic on mitochondrial K-ATP channels is much stronger than that on sarcolemmal K-ATP channels.[21-23] Therefore, Nic is now mainly viewed as a mitochondrial K-ATP channel opener, at least at the clinical dose. In contrast, we previously documented the cardioprotective effect of Mex in an acute ischemia model.[4] Although Mex has sodium channel blocking effects as well as sarcolemmal K-ATP channel opening effects, we concluded that the cardioprotective effect of Mex depended on the sarcolemmal K-ATP channel opening effect because its cardioprotective effect was totally suppressed by HMR-1098, the selective sarcolemmal K-ATP channel blocker used in the study.[4]

The time span of the myocardial injury in acute myocarditis is basically different from acute ischemia, in other words, the former appears in days or weeks, but the latter appears in minutes or hours. However, from the point of view of the process of the appearance of the final ventricular dysfunction, they are similar to each other because myocardial injury in the earlier phase results in ventricular dysfunction in the later phase in both diseases. In the present study, we hypothesized that myocardial protection utilizing K-ATP channel openers in the acute phase of myocarditis would lead to less ventricular dysfunction in the chronic phase similar to the acute ischemic model, and the result was that both Nic and Mex, mitochondrial and sarcolemmal K-ATP channel openers, exhibited a cardioprotective effect in the EAM model. To the best of our knowledge, this is the first documentation of the possibility of K-ATP channel openers being used for cardioprotection even in subacute or chronic ventricular dysfunction.

Mechanisms of the cardioprotection of K-ATP channel openers: The myocardial damage in EAM is considered to be caused by tissue inflammation including the myocardium and intercellular matrix.[13,14] It has been reported that acute inflammation causes myocardial damage, apoptosis, or necrosis and results in an intracellular and mitochondrial calcium overload similar to acute ischemia.[7-10] Therefore, a cardioprotective effect would be expected with a treatment which would reduce the calcium overload in the myocardium or its mitochondria. In the present study, we used two different clinically available K-ATP channel openers. As described above, Nic is mainly a mitochondrial K-ATP channel opener and Mex is a sarcolemmal K-ATP channel opener. As a result, Mex did not have an affect on the myocarditis itself, but a higher dose of Mex shortened the ventricular MAPD in the acute phase probably due to sarcolemmal K-ATP channel opening, and it led to better ventricular function in the chronic phase. This result indicates that shortening the myocardial action potential duration in the acute phase of myocardial injury may result in later myocardial protection possibly through the reduction in the intracellular calcium overload.[4,20,21] In contrast, Nic affected neither the myocarditis nor MAPD in the acute phase, but the Nic treatment resulted in better ventricular function in the chronic phase. Because the redox state of the mitochondria improved in the EAM+Nic groups in comparison with the control EAM group, protection of the mitochondrial function during the acute phase seemed to be the mechanism of the cardioprotection by Nic, similar to the acute ischemia model.[3-6,22]

Clinical implications: The results of this study indicate the possibility of a reduction in myocardial or mitochondrial calcium overload as a cardioprotective therapy even in acute myocarditis as it is in acute ischemia. Therefore, this kind of therapeutic approach might result in better cardiac function in the later phase of clinical myocarditis. Additionally, because the action potential duration is reportedly prolonged in patients with heart failure of any cause, intracellular calcium overload may play a role in promoting chronic myocardial injury. Therefore, a similar therapeutic approach, that is, a reduction in the calcium overload, might be a supportive therapy to obtain better ventricular function in the later phase in patients with heart failure.

Limitations: There are several limitations to the present study. First, the influence of the additional effects of the drugs, ie, the sarcolemmal K-ATP channel opening effect of Nic and sodium channel blocking effect of Mex, were not separated. This point can be discussed more specifically when using more specific K-ATP channel openers, such as diazoxide (selective mitochondrial K-ATP channel opener) and P1075 (selective sarcolemmal K-ATP channel opener) but they are not available for continuous oral administration. Second, additional studies uti-

lizing specific blockers such as 5-HD (5-hydroxydecanoate, mitochondrial K-ATP channel blocker) or HMR 1098 (sarcolemmal K-ATP channel blocker) were not performed.[4] However, they too are not available for long-term continuous oral administration. Finally, the effects of the drugs on the inflammation itself were not evaluated.

Conclusions: The effects of two clinically available K-ATP channel openers, Nic and Mex, were evaluated in EAM rats. Although Nic and Mex treatments did not exhibit any beneficial effects in the acute phase, they resulted in better ventricular function in the chronic phase. Nic, a mitochondrial K-ATP channel opener, exhibited a cardioprotective effect in EAM possibly through the protection of the mitochondrial function. Mex, a sarcolemmal K-ATP channel opener, also exhibited a cardioprotective effect in EAM in the chronic phase and suppressed the MAPD prolongation in the acute phase. Treatments utilizing K-ATP channel openers might be useful as cardioprotective therapies even in chronic or subacute heart failure.

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Excessive Fall of Blood Pressure During Maintenance Hemodialysis in Patients With Chronic Renal Failure Is Induced by Vascular Malfunction and Imbalance of Autonomic Nervous Activity

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Abstract: Acute hypotension during maintenance hemodialysis (HD) is not only a critical complication, but also an independent risk factor for mortality in patients with chronic renal failure (CRF). This study was designed to clarify the mechanisms underlying excessive fall of blood pressure during HD. Fifty-six CRF patients with HD thrice a week were divided into two groups according to the intradialytic hypotension episodes after 4 weeks of the observation period; the hypotension group, showing four or more episodes of intradialytic hypotension, and the non-hypotension group, showing three episodes of intradialytic hypotension or less. The intradialytic hypotension was defined as a fall of ≥30 mm Hg in the systolic blood pressure during HD. The brachial-ankle pulse wave velocity (ba-PWV), serum high-sensitivity (hs)-CRP, reactive oxygen species (ROS) generation, and serum malondialdehyde-modified LDL (MDA-LDL) were measured before HD. The high- frequency (HF) and low-frequency components (LF) of the heart rate variability and entropy were analyzed by the maximal entropy method. The ba-PWV, hs-CRP, ROS generation, and MDA-LDL were significantly higher in the hypotension group than in the non-hypotension group. HF, LF/HF, and entropy during HD increased significantly in the non-hypotension group, while entropy during HD decreased significantly in the hypotension group as compared with the baseline. LF/HF and entropy during HD were significantly lower in the hypotension group than in the non-hypotension group. These findings suggest that the major factors causing excessive fall of blood pressure during HD in patients with CRF might be vascular malfunction and imbalance of autonomic nervous activity. **Key Words:** Arterial stiffness, Autonomic nervous activity, Hemodialysis, Hypotension, Oxidative stress.

Acute hypotension occurring during maintenance HD is not only a critical complication, but also an independent risk factor for mortality in patients with CRF (1). In addition, up to 30% of HD patients are estimated to require interruption of HD on account of development of HD-induced hypotension (2).

The main causes of HD-induced hypotension have been reported to be acute hypovolemia during ultrafiltration and inadequate compensatory mechanisms such as left ventricular dysfunction, and inappropriate plasma refilling (3). Impaired vasoconstriction induced by dysregulation of sympathetic nervous activity has also been implicated as one of the major factors in the excessive fall of blood pressure during HD (4,5). On the other hand, spectral analysis of the heart rate variability (HRV) determined by the R-R interval using the maximal entropy method (MEM) has been documented as being a clinically useful tool to assess the autonomic nervous activity in HD

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patients (6). Indeed, previous studies have shown a decreased HRV in patients with HD-induced hypotension (4,5,7). Furthermore, it has been reported that vascular malfunction due to elevated arterial stiffness, in addition to dysregulation of the sympathetic nervous activity, is involved in the HD-induced hypotension (8). It has also been reported that vascular endothelial dysfunction caused by oxidative stress increases arterial stiffness (9). The mechanisms of vascular malfunction underlying the excessive fall of blood pressure during HD are largely obscure, although several hypotheses have been proposed.

The present study was designed to clarify the mechanisms underlying the excessive fall of blood pressure during HD, focusing on the imbalance of autonomic nervous activity and arterial stiffness contributing to vascular malfunction.

PATIENTS AND METHODS

Patients

Patients with CRF who were on regular maintenance HD three times a week were enrolled in the present study. All the patients were able to walk into the HD center by themselves, none needing assistance for visiting the center. The study was approved by the Ethics Committee of Kitasato University, and written informed consent was obtained from each of the patients who participated in the study after providing them with a detailed explanation of the study protocol. The following patients were excluded from the study: patients with overt heart failure, patients receiving α- and/or β-blockers, patients in whom 5% or more of the body fluid volume was removed during HD. Furthermore, patients with any of the following complications were also excluded by the attending physician: uncontrolled hypertension, prior myocardial infarction and uncontrolled cardiac arrhythmias. Out of 68 patients recruited for the present study, 12 patients were withdrawn from the study for the following reason: four patients had the exclusion criteria concerning the rate of removed body fluid; five had been taking β-blocker drugs; three had a history of myocardial infarction. Consequently, 56 patients with CRF were eligible for the present study.

Study design

In the present study, intradialytic hypotension was defined as a fall of 30 mm Hg or more in systolic blood pressure (SBP) during HD as compared with the SBP before the start of HD (10). Furthermore, an observation period of 4 weeks was established to ensure constant HD conditions. Following the observation period, the patients were divided into two groups according to the intradialytic hypotensive episodes out of 12 HD sessions in this period; the hypotension group, showing four or more episodes of dialysis hypotension, and the non-hypotension group, showing three episodes of dialysis hypotension or less (10). Blood measurement and clinical assessments described in the present study were performed at the end of 4 weeks of the observation period.

Blood pressure and heart rate

The brachial BP and heart rate (HR) were monitored every 20 min by the cuff method using an automatic manometer throughout the HD, with special attention paid to the period during which the patients showed hemodynamic instability. We recorded the BP and HR just before the start and after the completion of HD, and also the time during the HD at which the minimum SBP was recorded (minimum SBP).

Clinical characteristics

The clinical characteristics recorded from the patients' medical records included the age, sex, body mass index, presence of underlying DM, the duration of HD, and the dry body weight. The left ventricular ejection fraction (LVEF) (by echocardiography) and the serum level of B-type natreuretic peptide (BNP) were measured before the start of the HD. The body fluid removal rate during the HD was assessed according to the following equation: Body fluid removal rate (%) = (predialysis body weight-postdialysis body weight)/postdialysis body weight × 100.

Renal function, glycolipid metabolism, and electrolyte balance

Blood samples for assessments of renal function, glycolipid metabolism, and electrolyte balance were obtained just before HD after the patients had fasted overnight.

Characteristic symptoms of HD patients

We investigated the characteristic symptoms of the HD patients using the Kidney Disease Quality of Life-Short Form (KDQOL-SF) (11), which consists of 12 items related to the characteristic symptoms of kidney disease, such as muscle cramps and dry skin. All the items were scored on a scale of 0 to 100, with higher scores indicating less characteristic symptoms on HD.

Arterial stiffness, vascular inflammation and endothelial function, and oxidative stress

Arterial stiffness was evaluated by measurement of the brachial-ankle pulse wave velocity (ba-PWV) (12); this parameter was measured before the HD with the patient lying in the supine position, using the vascular profile device (BP-203RPE, Omron Colin, Tokyo, Japan). We also measured the serum level of high-sensitivity (hs)-CRP as a parameter of vascular inflammation, serum levels of thrombomodulin and von Willebrand factor (vWF) as parameters of vascular endothelial function, and reactive oxygen species (ROS) generation and serum levels of malondialdehyde-modified LDL (MDA-LDL) and pentosidine as parameters reflective of the oxidative stress level before the HD.

Assessment of the autonomic nervous activity

A 24-h Holter ECG (FM-300, Fukuda Denshi, Tokyo, Japan) was recorded throughout HD to assess the autonomic nervous activity based on analysis of the HRV. The HRV of the R-R intervals was analyzed by the maximal entropy method (MEM) (MemCalc, Suwa Trust, Tokyo, Japan) to obtain the frequency domain power spectra for the low (0.04–0.15 Hz) and high (0.15–0.4 Hz) frequency components (LF and HF, respectively) and the entropy values. The HF component is known to reflect the parasympathetic nervous activity, and LF/HF indicates the dominance of sympathetic nervous activity over parasympathetic nervous activity (13). Furthermore, entropy is reported to be an indicator of adequate cardiovascular control (14).The measurement was conducted from 08:00 hours on the morning of the HD, in accordance with the circadian rhythm of autonomic nervous activity (15). All patients were instructed to rest for 20 min in the supine position before the start of HD, during which the average values of HF, LF/HF, and entropy were calculated as the baseline values. To compare the changes in HF, LF/HF, and entropy, the average values of HF, LF/HF, and entropy recorded during HD were also calculated.

Physical activity

The physical activity level, consisting of the magnitude and duration of physical activity, of the study participants was assessed over a week using an accelerometer (Lifecorder, SUZUKEN, Nagoya, Japan) (16). The accelerometer was tied to the waist by the patients throughout the week, except during bathing and sleeping, and the vertical acceleration of the body was recorded. Furthermore, the accelerometer determined the time spent in light, moderate, or vigorous physical activity, corresponding to metabolic equivalents (METs) (16). The physical activity level was expressed as the average energy expenditure per week.

Statistical analysis

The changes in BP, HR, HF, LF/HF, and entropy were analyzed by two-way ANOVA with repeated measures in each group. The unpaired *t*-test and χ^2 test were used to compare the differences between the two groups. All statistical analyses were performed using the SPSS, version 12.0J, statistical software program (SPSS Japan, Tokyo, Japan). All data were expressed as mean ± SD, and $P < 0.05$ was accepted as representing statistical significance.

RESULTS

The patients' baseline characteristics are summarized in Table 1. There were 30 patients in the hypotension group and 26 in the non-hypotension group. There were no significant differences in the clinical characteristics, including renal function, glycolipid metabolism, electrolyte balance, or frequency of characteristic symptoms of HD patients between the two groups.

The ba-PWV, parameters of vascular inflammation, vascular endothelial function and oxidative stress, and physical activity level are shown in Table 2. Significantly higher values of ba-PWV, hs-CRP, ROS generation and serum MDA-LDL were observed in the hypotension group as compared with those in the non-hypotension group ($P = 0.04$, $P = 0.04$, $P = 0.02$ and $P = 0.04$, respectively). The mean physical activity level was significantly lower in the hypotension group than in the non-hypotension group ($P = 0.004$).

The changes in the BP and HR throughout HD are shown in Figure 1. Although no significant changes of the SBP or DBP were observed during the HD in the non-hypotension group, significant decreases of both the SBP and DBP were observed during the HD in the hypotension group ($P < 0.01$ and $P < 0.01$, respectively). In addition, the mean SBP and DBP during the HD were significantly lower in the hypotension group than in the non-hypotension group ($P < 0.01$ and $P < 0.01$, respectively). In contrast, no significant changes of the HR were observed during the HD in either group.

The HF, LF/HF, and entropy at baseline and during HD are shown in Figure 2. In this study, we analyzed HF, HF/LF and entropy as parameters to assess the HRV. We observed several findings on the HRV. First, LF/HF during HD was significantly lower in the hypotension group than in the non-hypotension

TABLE 1. *Baseline characteristics of the patients*

	Non-hypotension group ($n = 26$)	Hypotension group ($n = 30$)	P-value
Age (years)	62 ± 12	61 ± 13	ns
Male/Female	12/14	12/18	ns
BMI (kg/m^2)	20.8 ± 2.6	20.9 ± 2.5	ns
LVEF (%)	69 ± 9	65 ± 7	ns
BNP (pg/mL)	376 ± 263	362 ± 238	ns
Duration of HD (years)	4.6 ± 5.3	5.1 ± 6.7	ns
Diabetes mellitus (+/–)	9/17	16/14	ns
Dry weight (kg)	53.2 ± 9.1	53.1 ± 14.2	ns
Removed body fluid rate (% body weight)	3.5 ± 1.2	3.4 ± 0.9	ns
Ht (%)	30.2 ± 11.6	31.1 ± 2.7	ns
Hb (g/dL)	9.5 ± 2.8	9.6 ± 0.9	ns
BUN (mg/dL)	66.1 ± 0.9	68.8 ± 11.0	ns
Cr (mg/dL)	10.1 ± 2.9	10.8 ± 2.2	ns
UA (mg/dL)	7.3 ± 0.6	7.1 ± 1.1	ns
Alb (g/dL)	3.8 ± 0.3	3.8 ± 0.2	ns
TP (g/dL)	6.5 ± 0.5	6.5 ± 0.5	ns
Glucose (mg/dL)	119.8 ± 29.1	116.4 ± 35.8	ns
HbA_{1c} (%)	4.9 ± 0.7	5.2 ± 0.8	ns
T-cho (mg/dL)	157.4 ± 35.3	166.1 ± 32.2	ns
TG (mg/dL)	93.2 ± 42.6	96.5 ± 50.4	ns
HDL-C (mg/dL)	56.1 ± 15.6	53.7 ± 14.9	ns
LDL-C (mg/dL)	83.2 ± 26.5	95.1 ± 30.5	ns
Na (mEq/dL)	140.9 ± 10.6	142.1 ± 10.4	ns
K (mEq/dL)	4.7 ± 0.8	5.0 ± 0.6	ns
Ca (mg/dL)	9.0 ± 0.7	8.8 ± 0.7	ns
IP (mg/dL)	5.4 ± 1.2	5.4 ± 1.4	ns
Mg (mEq/dL)	2.5 ± 0.4	2.7 ± 0.4	ns
KDQOL (score)	89.4 ± 10.6	87.3 ± 9.5	ns

Data are expressed as the mean ± SD. ns, not significant in the non-hypotension group vs the hypotension group. Alb, serum albumin; BNP, serum B-type natreuretic peptide; Ca, serum calcium; Cr, serum creatinine; Hb, serum hemoglobin; HbA_{1C}, serum glycated hemoglobin; HDL-C, serum high density lipoprotein cholesterol; IP, serum inorganic phosphate; K, serum potassium; KDQOL, Kidney Disease Quality of Life; LDL-C, serum low density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; Mg, serum magnesium; Na, serum sodium; T-cho, serum total cholesterol; TG, serum triglyceride; TP, serum total protein; UA, serum uric acid.

group ($P < 0.01$). A significant increase of LF/HF during HD was also observed as compared with the baseline in the non-hypotension group ($P < 0.01$). Second, HF was significantly increased during HD as compared with the baseline in the non-hypotension group ($P < 0.01$). Third, entropy was significantly increased during HD as compared with the baseline in the non-hypotension group ($P < 0.01$). In addition,

TABLE 2. *The ba-PWV, parameters of vascular inflammation, vascular endothelial function and oxidative stress, and physical activity*

	Non-hypotension group ($n = 26$)	Hypotension group ($n = 30$)	P-value
ba-PWV (cm/s)	1706 ± 494	2028 ± 465	0.04
hs-CRP (ng/mL)	648.4 ± 300.1	918.1 ± 512.0	0.04
Thrombomodulin (FU/mL)	12.2 ± 3.1	12.8 ± 2.8	ns
vWF (%)	165.5 ± 69.0	150.7 ± 56.1	ns
ROS generation (Units)	111.9 ± 35.5	136.5 ± 32.3	0.02
MDA-LDL (U/L)	73.1 ± 24.7	90.7 ± 30.9	0.04
Pentosidine (μg/mL)	0.38 ± 0.20	0.46 ± 0.18	ns
Physical activity (kcal/week)	892.2 ± 791.4	567.5 ± 819.2	0.004

Data are expressed as the mean ± SD. ns, not significant in the non-hypotension group vs the hypotension group. ba-PWV, brachial-ankle pulse wave velocity; hs-CRP, high-sensitivity C-reactive protein; MDA-LDL, malondialdehyde-modified low density lipoprotein; ROS, reactive oxygen species; vWF, von Willebrand factor.

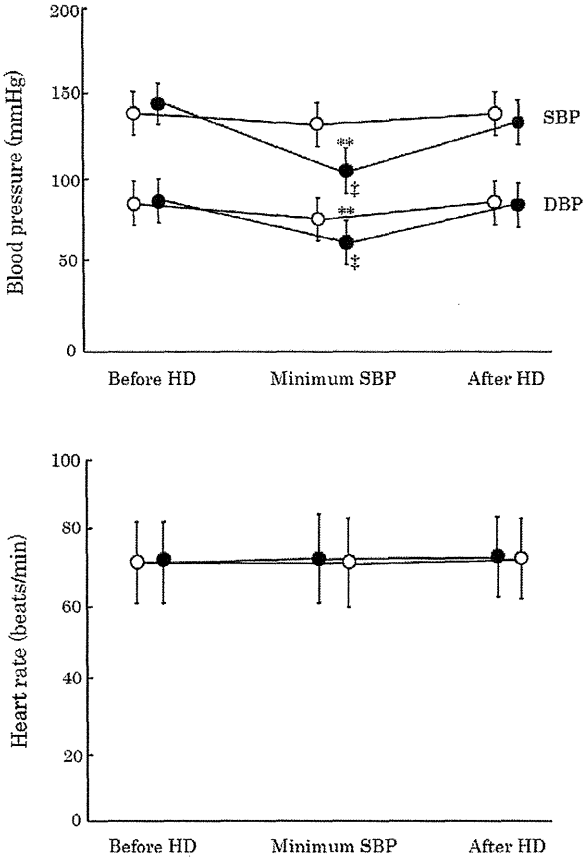

FIG. 1. Changes in blood pressure and heart rate during HD. Data are expressed as mean ± SD. Open circles: non-hypotension group, filled circles: hypotension group. **: $P < 0.01$ vs. before or after HD, ‡: $P < 0.01$ vs. non-hypotension group. DBP, diastolic blood pressure; SBP, systolic blood pressure.

the entropy during HD was significantly lower in the hypotension group than in the non-hypotension group ($P < 0.05$).

DISCUSSION

It is well documented that acute hypotension observed during HD develops as a result of hypovolemia and inadequate compensatory mechanisms to counter the reduced intravascular volume (3). In the present study, we focused on the imbalance of the autonomic nervous activity and increased arterial stiffness contributing to the vascular malfunction in CRF patients undergoing maintenance HD therapy. The present study showed significantly increased value of the ba-PWV in the hypotension group than in the non-hypotension group, and also significantly increased serum hs-CRP and MDA-LDL as well as of ROS generation in the hypotension group than in the non-hypotension group. The ba-PWV is the currently used clinical indicator of arterial stiffness (12). In addition, obvious evidence of arterial stiffness as one of the independent predictors of cardiovascular morbidity and mortality in patients with CRF, particularly those with end-stage renal disease, has been accumulated (17). Excessive vascular stress induced by hypertension, glucose intolerance, or oxidative

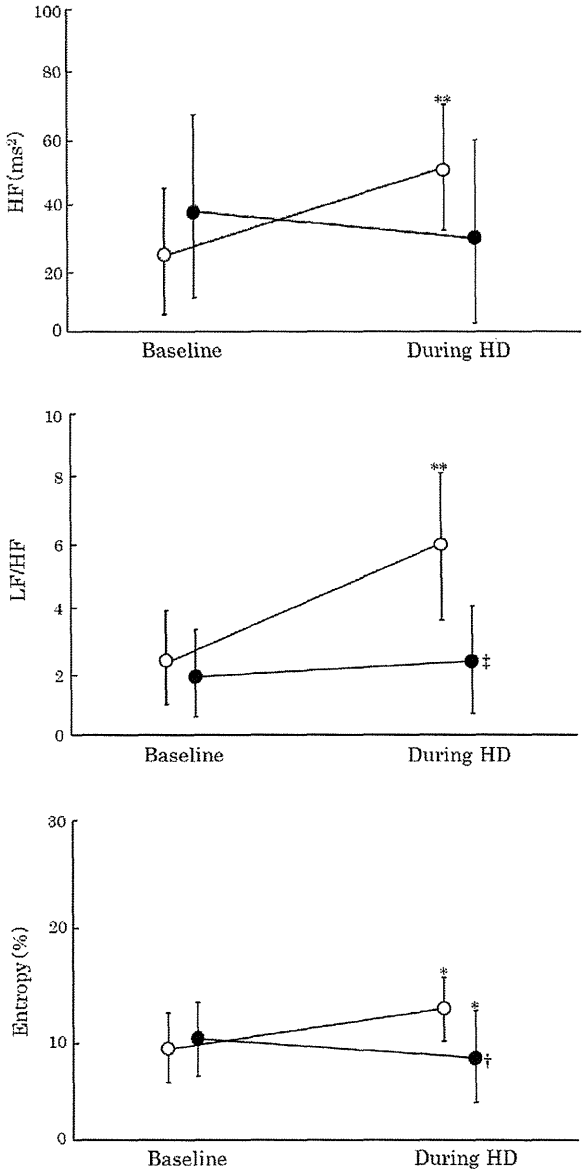

FIG. 2. Changes in HF, LF/HF, and entropy during HD. Data are expressed as mean ± SD. Open circles: non-hypotension group, filled circles: hypotension group. * and **: $P < 0.05$ and $P < 0.01$ vs. baseline, respectively, † and ‡: $P < 0.05$ and $P < 0.01$ vs. non-hypotension group, respectively, HF: high-frequency component, LF: low-frequency component.