生活習慣アンケート

記入方法：機械で読み取りますので、わくからはみ出さないように当てはまるところのみ1つずつ鉛筆でご記入ください。訂正の際には、消しゴムできれいに消してからご記入ください。

お名前　姓（カタカナ）　名（カタカナ）　性別 □男性 □女性　年齢 □□歳

このアンケートにお答えになった方　□ご本人　□ご家族　□その他

以下の質問で当てはまるものに○をご記入ください。

（補足）「他の人より食べる量が多い」と思われる場合には□の中に○印をつけてください。当てはまらない場合には何も印をつけないでください。

- □ 他の人より食べる量が多い
- □ 間食を日に3回以上する
- □ 甘い菓子（和菓子・洋菓子）を月に1回以上食べる
- □ めん類の汁を飲む
- □ 味のついたおかずや漬物にしょうゆやソースをかける
- □ 牛乳は濃厚なものをよく飲む
- □ 外食（社員食堂は除く、出前は含む）は月に1回以上する
- □ 出来合いのお惣菜、ご飯もの、弁当などを週1回以上食べる
- □ 揚げもの、炒めものを日に1回以上食べる
- □ 漬物や佃煮を週3回以上食べる
- □ 果物を日に1回以上食べる
- □ ばら肉、しもふり肉、ミンチ肉（ハンバーグを含む）を日に1回以上食べる
- □ ハム、ソーセージ、ベーコンを週に1回以上食べる
- □ 洋菓子（ケーキ、シュークリーム、クッキーなど）を月1回以上食べる
- □ 甘い飲料（砂糖を入れたコーヒー、紅茶を含む）を日に3回以上飲む
- □ 卵をほぼ毎日1個以上たべる
- □ アルコール飲料は10日に1回以上は飲む
- □ アルコール飲料を飲むときに、塩辛いおつまみをよく食べる

以下の食品をどのくらいの頻度で摂取していますか。最も適するものに○をつけてください。

	ほぼ毎日	週5～6回	週3～4回	週1～2回	週1回未満		ほぼ毎日	週5～6回	週3～4回	週1～2回	週1回未満
野菜	□	□	□	□	□	海草類	□	□	□	□	□
果物	□	□	□	□	□	卵	□	□	□	□	□
肉類	□	□	□	□	□	大豆製品	□	□	□	□	□
牛乳	□	□	□	□	□	魚介類	□	□	□	□	□
間食	□	□	□	□	□	チーズ ヨーグルト	□	□	□	□	□

食事をとるのがはやいですか	□はい	□いいえ	□どちらでもない
ビタミン剤を利用していますか	□はい	□ときどき	□いいえ
健康補助食品を利用していますか	□はい	□ときどき	□いいえ
タバコを吸いますか	□吸う	□吸わない	□禁煙した
お酒を飲みますか	□飲む	□飲まない	□禁酒した

一日のうちで、以下の状態は平均してどのくらいの時間ありますか。

・睡眠時間　□4時間未満　□4時間台　□5時間台　□6時間台　□7時間台　□8時間台　□9時間台　□10時間以上

・立位・歩行状態　□なし　□1時間未満　□1時間台　□2時間台　□3時間台　□4時間台　□5時間台　□6時間台　□7時間以上

・10キロ程度の重いものを持つ状態または持続できないような激しい作業状態

□なし　□数分程度　□10分台　□20分台　□30分台　□40分台　□50分台　□60分台　□70分台　□80分以上

短い距離（徒歩10分）でも車を利用しますか。

□利用する　□ときどき利用する　□あまり利用しない　□利用しない

裏に続きます。

資料－6

受診日 平成 15 年 7 月 12 日　9083703　1

お名前 国循 太郎 様 性別 F 年齢 55 歳

国立循環器病センター循環器病予防検診部

食事について

エネルギー摂取量

特に問題点はありません。

脂質摂取量

洋菓子は脂肪を多く含みますので、和菓子に変えましょう。

食塩摂取量

塩分の多い漬物や佃煮の摂取量が多いようです。

その他

そのほかの食生活で特に問題はありません。

身体活動について

睡眠について

睡眠時間がだいたい規則正しいです。今後とも規則正しい生活習慣を目指してください。

運動について

運動不足が考えられます。日常生活で身体を動かす工夫に心がけてください。日ごろ運動をあまりしていません。日常生活の中に運動を取り入れるようにしてください。

1日の身体活動量の合計

座りがち　普通　活動的

身体活動量はやや活動的です。

ストレスについて

ストレスを感じています。ストレスをあまりためすぎないように、運動や気分転換をはかるようにしてください。

喫煙について

喫煙をしていませんが、他人の煙は自分が吸っているのと同様に健康を害する恐れがありますのでご注意ください。

飲酒について

お酒は飲みません。

医療機関控え

受診日 平成 15 年 7 月 12 日　9083703　1

お名前 国循 太郎 様 性別 F 年齢 55 歳

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食生活について

今回、食生活のアンケートに答えていただくことにより、食事からのエネルギー、脂質、食塩において過剰摂取であるかどうかの判定ができるようになっています。

身体活動について

日常生活の中で便利なものが増え、身体を動かす機会が少なくなってきています。運動不足になりがちな日ごろの生活の中で、健康を維持させるためには、意識して身体を動かすことが重要です。適切な運動が多くの生活習慣病の予防と治療に有効なことは、これまでの多くの疫学研究により明らかになってきています。また、適度な運動はストレスの発散にもなります。

今回、身体をどの程度動かしているか、その強さと時間とを掛け合わせたものの合計が総身体活動量として計算されます。これより、１日どれだけ身体を動かしているか定量的に評価しております。

生活習慣アンケート結果報告書

今回、提出されたアンケートに基づいて解析された結果報告書です。検査結果とあわせて、今後の生活習慣の改善に役立ててください。

国立循環器病センター

この報告書は2つ折にし、健康手帳にはさんでご利用ください。
お問い合わせは、受診された医療機関でお聞きください。

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えんぴつで濃く、はっきりと枠からはみ出さないように黒太枠内のみご記入ください。

吹田市基本健康診査受診票　A票

〒
吹田市
フリガナを必ずお付けください
氏名　　様

シール貼付位置

電話番号
（　　）
性別　男性　女性

生年月日　明治　大正　昭和　　年　　月　　日　　年齢　　歳

市民の健康保持増進をはかることを目的に匿名化を行った後、健診情報をデータ化し、集団的特徴を把握し、保健事業に活用することがありますのでご了承ください。また、この健康診査の結果、吹田市立保健センターから保健指導もしくは健康教育・相談のご案内をすることがありますのでご了承ください。

最近の体調であてはまるものに○をつけてください。

- □ 手足のむくみ
- □ 手足の麻痺やしびれ感
- □ 胸がしめつけられる感じや胸痛
- □ 舌のもつれ
- □ のどがよく乾く
- □ 坂道を上がった時の動悸や息切れ
- □ めまい、耳鳴り、難聴
- その他の項目（　　）

右側もご記入ください

以下の欄は健診の結果を医療機関の医師に記入していただきますので、空欄のままにしておいてください。

結核検診・肺検診の受診希望：なし □　あり □ → 40歳以上の方で、喫煙者、血たんなどの症状のある方は □ 喀たん細胞診検査実施
喀たん細胞診検査票が必要な場合があります。（検査票は医療機関にあります）

計測
- 身長　　cm
- 体重　　kg

血圧
- 収縮期　　mmHg
- 拡張期　　mmHg

理学的所見

	整	不整
脈拍		
	順	不順
心音		
	あり	なし
呼吸音		
貧血		
浮腫		
肝腫大		
脾腫大		
その他		

聴力検査依頼　要　不要
聴力依頼医院名

眼底検査　要　不要
眼底依頼医院名

心電図　異常なし　異常あり
所見
依頼医院名

尿定性検査

	－	±	＋	＋＋	＋＋＋
糖					
タンパク					
潜血					

診査の種類（必ず1日目に記入してください）　来院　医師のみ訪問　看護師同行

健康手帳交付　あり　なし

平成	年	月	日

医療機関名
担当医師名

以下の質問にあてはまる場合、その枠内に○をご記入ください。（生活習慣指導に必要ですが、必須ではありません。）

- □ 他の人より食べる量が多い
- □ めん類の汁を半分以上飲む
- □ 牛乳は濃厚なものをよく飲む
- □ 揚げ物、炒め物を日に1回以上食べる
- □ 外食(出前も含む)は月に1回以上する
- □ 果物を日に1回以上食べる
- □ 漬物や佃煮を週3回以上食べる
- □ 卵をほぼ毎日1個以上食べる
- □ ハム、ソーセージ、ベーコンを週1回以上食べる
- □ 洋菓子（ケーキ、クッキーなど）を月1回以上食べる
- □ 味のついたおかずや漬物にしょうゆやソースをかける
- □ 出来合いのお惣菜、ご飯もの、弁当などを週1回以上食べる
- □ ばら肉、霜降り肉、ミンチ肉(ハンバーグなど)を日に1回以上食べる
- □ 甘い飲料（砂糖入りのコーヒー紅茶を含む）を日に3回以上飲む
- □ 間食（おやつなど三度の食事以外の食事）を日に3回以上する
- □ 上記のうち、ひとつも該当なし

夕食は何時頃とりますか	午後7時以前 □	午後7時台 □	午後8時台 □	午後9時以降 □			
食事をぬくことがありますか	はい □	ときどき □	いいえ □				
食事をとるのが早い(早食い)ですか	はい □	いいえ □	どちらでもない □				
たばこを吸いますか	吸わない □	禁煙した □	吸う □ →	1~9本/日 □	11~19本/日 □	21~39本/日 □	31本以上/日 □
お酒を定期的に飲みますか	飲まない □	禁酒した □	飲む □ →	週1~3日 □	週4~6日 □	ほぼ毎日 □	
くすりを飲んでいますか	飲まない □		飲む □ →	高血圧症 □	高脂血症 □	糖尿病 □	
			当てはまるもの全てに○をつけてください	脳卒中 □	狭心症・心筋梗塞 □	高尿酸血症 □	

1日のうち、以下の状態は平均どのくらいの時間ありますか。それぞれ1つずつ○をつけてください。

	4時間未満	4時間台	5	6	7	8	9	10時間以上
睡眠時間(昼寝を含む)	□	□	□	□	□	□	□	□
＊数字の単位はいずれも時間	1時間未満	1時間台	2	3	4	5	6	7時間以上
立位・歩行状態	□	□	□	□	□	□	□	□

息が切れるような肉体作業や活動の持続時間

なし	1~9分	10~29分	30~49分	50~69分	70~89分	90分以上
□	□	□	□	□	□	□

	いつもそうだ	だいたいそうだ	たまにそうだ	まったくそうでない
短い距離(徒歩10分)なら歩きますか	□	□	□	□
睡眠時間は規則正しいですか	□	□	□	□
現在ストレスを感じていますか	□	□	□	□

	2回以下/週	3回以上/週	していない
仕事以外で定期的に運動をしていますか	□	□	□

<本人用> 医療機関名:国立循環器病センター
循環器病予防検診部 受診番号 9999999

基本健康診査結果及び生活習慣アンケート結果

受診年月日: 平成 17 年 04 月 10 日 受診番号 2
お名前: 様 性別: 年齢: 歳
身長 160.5 cm 体重 54.2kg BMI: 21.0kg/m²
血圧:収縮期 142 mmHg 拡張期 94 mmHg 脈拍 94 /分
検尿: 尿糖:- 尿蛋白:- 尿潜血:±
心電図: 異常所見を認める
所見: 異常Q波 ST高度降下 ST上昇 T波陰性・平坦 房室伝導障害 心房細動 期外収縮頻発

眼底検査:
Scheie
高血圧性変化 1 度 Scott 3b
動脈硬化性変化 3 度 KW 2b
眼底所見: 網膜剥離 網膜変性症

その他の所見: 心雑音 呼吸音異常 浮腫 脾腫大

体調: 手足の麻痺・しびれ感 胸痛 舌のもつれ 動悸・息切れ

判定結果:
循環器 要指導 糖尿 要指導
貧血 異常を認めず 高脂血症 要医療
肝機能 異常を認めず その他 要医療

血液検査結果

	血液項目	結果	判定	基準値
血清脂質	総コレステロール	197 mg/d		150~200
	HDL-コレステロール	66 mg/d		>39
	中性脂肪	74 mg/d		<150
	LDL-コレステロール	116.2 mg/d		<140
肝機能	総蛋白	6.9 g/d		6.5~8.0
	A/G比	2.1	↑	1.1~1.8
	GOT(AST)	24 U/l		8~40
	GPT(ALT)	21 U/l		5~35
	γ-GTP	33 U/l		<60
	ZTT(クンケル)	3.9 U		3~12
腎機能	尿酸	5.4 mg/d		2.0~6.0
	クレアチニン	0.77 mg/d		<1.1
糖代謝	ヘモグロビンA1c	5.7 %		<5.9
	血糖	92 mg/d		<110
貧血	赤血球	4.21 x10⁶/μl		3.75~4.80
	白血球	5.8 x10³/μl		3.3~9.4
	ヘモグロビン	14.7 g/d		11.7~15.5
	ヘマトクリット	44.3 %		34.0~43.0
	血小板	283 x10³/μl		135~375

※↑、↓は要指導:↑↑、↓↓は要医療の目安です。
※LDLコレステロールは計算値で空腹時に有効な値です。

食事について

エネルギー摂取状況
エネルギー摂取量の過剰が考えられます。食事量が多くなっています。腹八分目を意識しましょう。甘い飲み物の摂取量が多いようです。お茶類に変えましょう。

脂肪摂取状況
脂質摂取量の過剰が考えられます。肉の脂身の摂取量が多くなっていますので、赤身を利用しましょう。卵の摂取量が多いようです。週に3個程度にしましょう。

食塩摂取状況
食塩の摂取量の過剰が考えられます。めん類の汁は半分以上残しましょう。外食は塩分の摂取が多くなりますので頻度を減らしましょう。

その他
欠食は栄養の偏りがおきやすいです。一日三食摂るようにしましょう。

身体活動について 印刷日 2005年6月7日
日ごろ運動をしたり身体を動かすように心がけております。今後も日常生活で身体を動かしてください。

身体活動量は座りがちです。

睡眠について
睡眠時間が短いようです。睡眠時間を十分にとり、疲れをためないようにしましょう。睡眠時間があまり規則正しくありません。規則正しい生活習慣がとれるように心がけてください。

ストレスについて
ストレスを感じています。ストレスをあまりためすぎないように、運動や気分転換をはかるようにしてください。

喫煙について
現在禁煙しています。喫煙は健康を損なう恐れがありますので、喫煙することのないようにしてください。

飲酒について
現在禁酒をしています。

<かかりつけ医殿用>

受診番号 9999999

基本健康診査結果及び生活習慣アンケート結果

受診年月日: 平成 17 年 04 月 10 日 受診番号 2
お名前: 様 性別: 年齢: 歳
身長 160.5 cm 体重 54.2kg BMI: 21.0kg/m²
血圧:収縮期 142 mmHg 拡張期 94 mmHg 脈拍 94 /分
検尿: 尿糖:- 尿蛋白:- 尿潜血:±
心電図: 異常所見を認める
所見: 異常Q波 ST高度降下 ST上昇 T波陰性・平坦 房室伝導障害 心房細動 期外収縮頻発

眼底検査:
Scheie
高血圧性変化 1 度 Scott 3b
動脈硬化性変化 3 度 KW 2b
眼底所見: 網膜剥離 網膜変性症

その他の所見: 心雑音 呼吸音異常 浮腫 脾腫大

体調: 手足の麻痺・しびれ感 胸痛 舌のもつれ 動悸・息切れ

判定結果:
循環器 要指導 糖尿 要指導
貧血 異常を認めず 高脂血症 要医療
肝機能 異常を認めず その他 要医療

血液検査結果

	血液項目	結果	判定	基準値
血清脂質	総コレステロール	197 mg/d		150~200
	HDL-コレステロール	66 mg/d		>39
	中性脂肪	74 mg/d		<150
	LDL-コレステロール	116.2 mg/d		<140
肝機能	総蛋白	6.9 g/d		6.5~8.0
	A/G比	2.1	↑	1.1~1.8
	GOT(AST)	24 U/l		8~40
	GPT(ALT)	21 U/l		5~35
	γ-GTP	33 U/l		<60
	ZTT(クンケル)	3.9 U		3~12
腎機能	尿酸	5.4 mg/d		2.0~6.0
	クレアチニン	0.77 mg/d		<1.1
糖代謝	ヘモグロビンA1c	5.7 %		<5.9
	血糖	92 mg/d		<110
貧血	赤血球	4.21 x10⁶/μl		3.75~4.80
	白血球	5.8 x10³/μl		3.3~9.4
	ヘモグロビン	14.7 g/d		11.7~15.5
	ヘマトクリット	44.3 %		34.0~43.0
	血小板	283 x10³/μl		135~375

※↑、↓は要指導:↑↑、↓↓は要医療の目安です。
※LDLコレステロールは計算値で空腹時に有効な値です。

食事について

エネルギー摂取状況
エネルギー摂取量の過剰が考えられます。食事量が多くなっています。腹八分目を意識しましょう。甘い飲み物の摂取量が多いようです。お茶類に変えましょう。

脂肪摂取状況
脂質摂取量の過剰が考えられます。肉の脂身の摂取量が多くなっていますので、赤身を利用しましょう。卵の摂取量が多いようです。週に3個程度にしましょう。

食塩摂取状況
食塩の摂取量の過剰が考えられます。めん類の汁は半分以上残しましょう。外食は塩分の摂取が多くなりますので頻度を減らしましょう。

その他
欠食は栄養の偏りがおきやすいです。一日三食摂るようにしましょう。

印刷日 2005年6月7日

身体活動について
日ごろ運動をしたり身体を動かすように心がけております。今後も日常生活で身体を動かしてください。

身体活動量は座りがちです。

睡眠について
睡眠時間が短いようです。睡眠時間を十分にとり、疲れをためないようにしましょう。睡眠時間があまり規則正しくありません。規則正しい生活習慣がとれるように心がけてください。

ストレスについて
ストレスを感じています。ストレスをあまりためすぎないように、運動や気分転換をはかるようにしてください。

喫煙について
現在禁煙しています。喫煙は健康を損なう恐れがありますので、喫煙することのないようにしてください。

飲酒について
現在禁酒をしています。

国立循環器病センター循環器病予防検診部

II. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表(平成 15 年～16 年度)

主任研究者:友池仁暢

発表者氏名	論 文 タ イ ト ル 名	発 表 誌 名	巻号	ページ	出版年
Sasayama S, Ishii N, Ishikura F, Kamijima G, Ogawa S, Kanmatsuse K, Kimoto Y, Sakuma I, Nonogi H, Matsumori A, Yamamoto Y	Men's health study －Epidemiology of erectile dysfunction and cardiovascular disease	Circ J	67	656-659	2003
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Asakura M, Takashima S, Asano Y, HonmaT, Sanada S, Asanuma H, Shintani Y, Liao Y, Kim J, Ogita H, Node K, Minamino T, Agai A, Yorikane R, Kitamura S, Tomoike H, Hori M, Kitakaze M	Canine DNA array as a potential tool for combining physiology and molecular biology	Circ J	67	788-792	2003
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Okamoto A, Sakata T, Mannami T, Baba S, Katayama Y, Matsuo H, Yasaka M, Minematsu K, Tomoike H, Miyata T	Population-based distribution of plasminogen activity and estimated prevalence and relevance to thrombotic diseases of plasminogen deficiency in the Japanese: The Suita Study	J. hromb. Haemost	1	2397-2403	2003
Iwanaga Y, Mannami T, Goto Y, Nonogi H, Iwai N	Association analyses between polymorphisms in the GJA4 gene cluster and myocardial infarction in Japanese	Thromb. Haemostat	90	1226-1227	2003
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Shioji K, Kokubo Y, Goto Y, Nonogi H, Iwai N,	An association analysis between genetic polymorphisms of matrix metalloproteinase-3 and methylenetetrahydrofolate reductase and myocardial infarction in Japanese	J Thromb Haemostasis	2	527-528	2004
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III. 研究成果の刊行物・別刷

Circ J 2003; **67**: 656–659

Men's Health Study
—— Epidemiology of Erectile Dysfunction and Cardiovascular Disease ——

Shigetake Sasayama, MD; Nobuhisa Ishii, MD*; Fuminobu Ishikura, MD**; Gombei Kamijima, MD†; Satoshi Ogawa, MD††; Katsuo Kanmatsuse, MD‡; Yasusuke Kimoto, MD‡‡; Ichiro Sakuma, MD§; Hiroshi Nonogi, MD§§; Akira Matsumori, MD¶; Yasuhiro Yamamoto, MD¶¶

The present study collected data about 6,112 Japanese male patients from 447 outpatient clinics. Of those who underwent medical examination by a general practitioner on an outpatient basis, up to 81% had some degree of erectile dysfunction (ED), ranging from mild to severe. ED was noted to be predominant among patients affected by cardiovascular disease (CVD) or diabetes mellitus (DM), and the presence of CVD increased the risk of ED. In an aging society, patients undergoing treatment for ED as part of their routine medical care are highly likely to have concomitant CVD. As shown in the present survey, clinicians need to be aware of the high incidence of ED among such patients, because ED represents a symptom originating from damage to the vascular endothelium. A total of 41% of ED patients are either willing to receive pharmacotherapy for ED or will consider treatment. Active treatment of ED with sildenafil is suitable for patients with CVD. (*Circ J* 2003; **67**: 656–659)

Key Words: Cardiovascular disease; Comorbidity; Endothelium; Erectile dysfunction; Risk factors

Hypertension, diabetes mellitus (DM), hyperlipidemia, and smoking all represent significant risk factors for cardiovascular disease (CVD) because they are all considered to induce vascular endothelial damage, resulting in vascular obstruction, plaque rupture, thrombosis, arterial sclerosis, and also erectile dysfunction (ED). More specifically, ED can be considered a symptom of damage to the vascular endothelium (Fig 1). Therefore, it can be expected that ED will be concomitant with CVD, and that the presence of ED suggests the existence of CVD. Integrated medical care may be necessary for these disease states. However, epidemiological data on ED and information regarding the current status of ED treatment among general practitioners in Japan is limited[1] possibly because there are no established criteria for the diagnosis of ED, as well as the societal and psychological obstacles to the patient consulting a clinician regarding this condition.

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Since its launch in March 1999, sildenafil citrate (Viagra® Tablets) has become widely used by numerous patients with CVD. Sildenafil selectively inhibits the action of intracellular phosphodiesterase 5 (PDE5) in the corpus cavernosum of the penis, and ameliorates the effects of ED by enhancing the action of cyclic guanosine monophosphate (cGMP) on vascular smooth muscle. The activity of PDE in the ventricle and saphenous veins of humans has been precisely measured, and the magnitude of the action of sildenafil on the cardiovascular system and its high affinity for PDE5 have been clearly demonstrated[2] In vitro examination of the effects of sildenafil on the activity of PDE isozymes originating from the human corpus cavernosum also suggests a high selectivity of sildenafil for PDE5 in that specific tissue[3]

Since sildenafil became available, public interest in ED has increased substantially and we therefore conducted a survey of general practitioners setting, because understanding the consultation status of ED treatment is important for the promotion of the optimal treatment of ED.

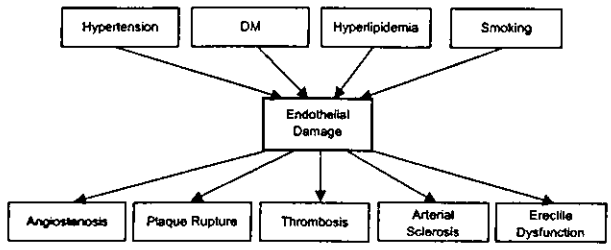
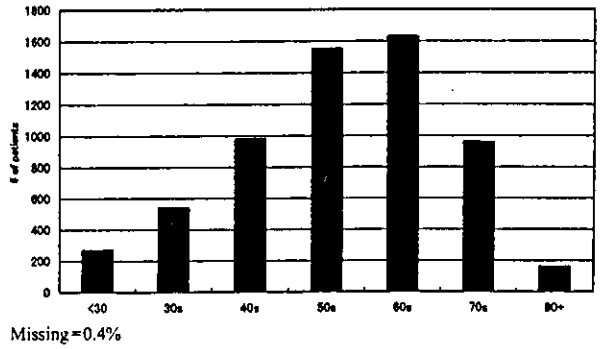


Fig 1. ED can be considered a symptom of damage to the vascular endothelium.



Missing=0.4%

Fig 2. Age distribution of the survey respondents. Missing data=0.4%

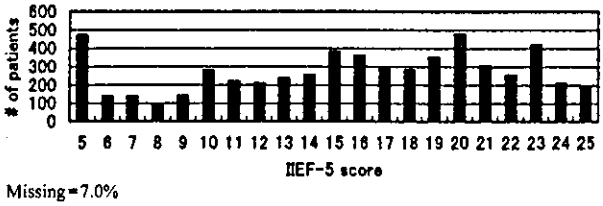
Study Objectives

To date, the Cardiovascular Issue Examining Committee of Viagra (organized by members affiliated to relevant academic societies in Japan, principally the Japanese Circulation Society) has issued 2 reports. The first reviewed the epidemiological studies of ED and existing statistics on CVD;[4] In as well as describing the mechanism of action of sildenafil and clinical experience. The second report described the precise indications for sildenafil and the management of adverse events relevant to both general practice and cardiology.[5] The present questionnaire survey of general practitioners was conducted as part of the activities of this committee, in order to investigate the current status of ED and its treatment in Japan.

Methods

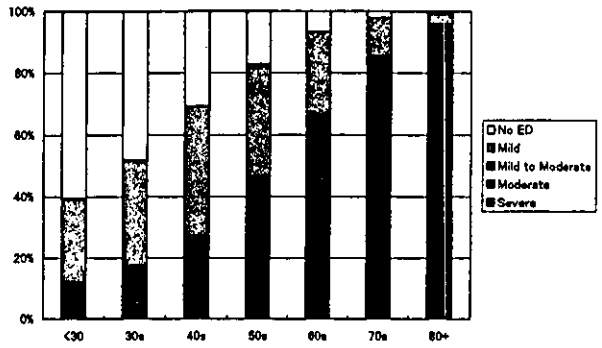
In the 4-month period from August to November 2001, a self-administered questionnaire survey was sent to general practitioners' offices and clinics all over Japan, because they represent the principal providers of medical care for lifestyle-related diseases. The level of patient satisfaction with their sex life was surveyed as a quality-of-life (QOL) profile, in addition to background information such as age, diseases under current medical treatment and health status, categorized into 5 levels. The level of satisfaction with sex life was evaluated by the question 'To what extent are you satisfied with your current sex life?' using 5 categories from 'very much' to 'very little'. The International Index of Erectile Function (IIEF)-5, which shortens the IIEF to 5 questions, was used to evaluate ED.[6] The score of the IIEF-5 ranges from 5 to 25 points. In general evaluations using the IIEF-5, subjects are considered to have ED if they have a score of 5–21 points and are considered normal with a score of 22–25 points. Subjective evaluation of erectile function scored using IIEF-5 has been verified to suitably reflect objective measures of erectile function as determined by the nocturnal penile tumescence (NPT) test using the RigiScan Plus.[7] In addition, psychological factors and urinary disturbance caused by prostate hyperplasia were evaluated using the Center for Epidemiologic Studies-Depression Scale (CES-D)[8] and the International Prostatic Symptoms Score (I-PSS),[9] respectively, as these are issues related to ED.[10,11] Medical consultation for these health conditions, and satisfaction with the outcome, were also examined.

With regard to the method of administering the questionnaire, letters of intent were obtained from physicians throughout Japan who agreed to the purpose of the present survey. The self-administered questionnaires were then sent to the physicians and the reply forms were mailed either individually by the patients themselves or collectively by the physician in charge. Data were entered on an anonymous basis.



Missing=7.0%

Fig 3. Severity of erectile dysfunction. Missing data=7.0%



Missing=7.3%

Fig 4. Severity of erectile dysfunction with age. Missing data=7.3%

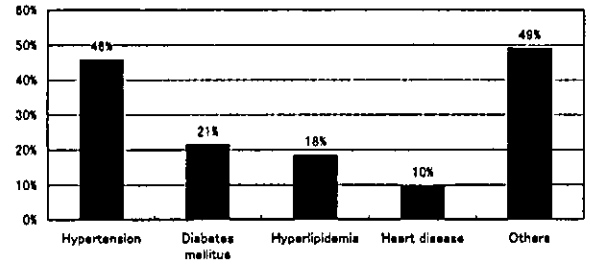
Statistical Analysis

The likelihood ratio test was conducted for concomitant CVD and severity of ED, and increased risk of ED with disease comorbidity. Furthermore, to calculate the risk of ED in the presence of any given disorder, subjects were categorized as either normal subjects or ED patients according to the IIEF-5 scores. A logistic model was then used to calculate the odds ratios (OR) and determine the magnitude of risk. Statistical significance was defined as $p<0.05$.

Results

Letters of interest were obtained from 700 outpatient clinics and 6,112 questionnaires were collected from 447 clinics. The mean (±SD) number of collected forms per medical institution was 14±9. The present survey was conducted in the outpatient setting of medical specialties including departments of Internal Medicine, Urology, Surgery, Orthopedics, Gastroenterology, Cardiology, and Radiology. Shin Joho Center, Inc collected and compiled the forms received from the participating medical institutions throughout Japan. Patients in the ages of 30–70 years accounted for 93% of the patients participating in the survey, with more than half (52.3%) of the total subject population represented by males aged in their 50s and 60s (Fig 2).

A total of 4,609 of the 5,683 patients who answered the IIEF-5 questions in the present survey had an IIEF-5 score ≤21 points, which was 81% of the respondents (Fig 3). According to the 5-level categorization of severity proposed by Rosen,[12] patients with ED in the categories of 'severe' to



Number of patients = 4,990

Fig 5. Patient background data. The category of 'others' includes prostatic disease, gastrointestinal ulceration, neurological disorder and/or psychiatric disorder. No. of patients = 4,990

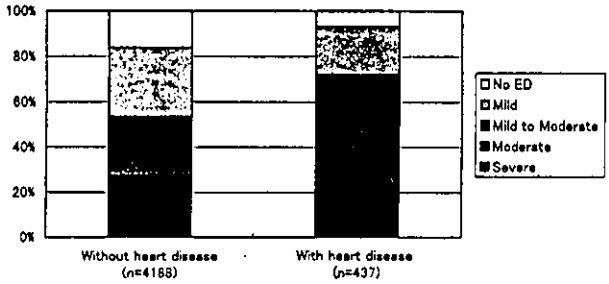


Fig 6. Severity of erectile dysfunction in patients with and without heart disease.

'mild to moderate' (IIEF-5 score: ≤16) accounted for 63.2% of all patients affected by ED (Fig 3). Severe ED was more pronounced among aged patients (Fig 4). Regarding the level of satisfaction with sex life, patients giving a reply of 'moderately dissatisfied' or 'very dissatisfied' comprised 30.2% of all replies.

CVD and ED

Subjects in the present survey displayed a mean of 1.45 concomitant diseases and the most frequently observed CVD was hypertension, accounting for 46% of valid replies (n=4,990) after excluding missing data (Fig 5). Furthermore, heart disease was observed in 10% of patients. In the present survey, patients over 70 years of age accounted for 40% of patients with heart disease who were undertaking medical treatment. These patients tended to be older than those with other conditions. ED was significantly more severe in the presence of heart disease ($p<0.0001$). Patients with severe or moderate ED comprised 46% of those with heart disease, but only 27% of those without (Fig 6).

Difference in Risk of ED According to Concomitant Disease

Among the concomitant lifestyle-related diseases, DM (21%) and hyperlipidemia (18%) predominated (Fig 5). Diabetes mellitus, heart disease, and hypertension displayed significant correlations with ED, with OR of 2.88, 2.82 and 1.79, respectively; however, the presence of hyperlipidemia did not affect the risk of ED (Table 1).

The risk of ED was calculated for an additional complication of an underlying disease. In the presence of heart disease, the risk of ED increased when DM was a complication ($p=0.0004$, p-value: likelihood ratio test) though it did not increase significantly when either hypertension ($p=0.2490$) or hyperlipidemia ($p=0.0917$) was the complication. Comparing the percentages of severe ED and moderate ED between patients with both heart disease and DM and those with only heart disease, the former was 65% and the latter was 42%.

Table 1 Concomitant Diseases and Correlation With Erectile Dysfunction

Estimated value of parameter	χ^2	*p value*	*OR*	*95% CI*
Intercept	*233.28*	*<0.0001*	–	–
Concomitant diseases				
Hypertension	*42.09*	*<0.0001*	*1.79*	*1.51–2.14*
Diabetes mellitus	*69.99*	*<0.0001*	*2.88*	*2.26–3.70*
Hyperlipidemia	*0.05*	*0.8313*	*0.98*	*0.80–1.20*
Heart disease	*27.66*	*<0.0001*	*2.82*	*1.95–4.23*

$R^2 = 0.0608$; Cutoff = 21/22 (IIEF-5); logistic. OR, odds ratio.

Willingness to Receive Pharmacotherapy for ED

A total of 41% of patients did not respond to the question regarding willingness to receive pharmacotherapy for ED if it was available; however, 22.3% of the patients who replied did expressed such a willingness. The percentage of patients considering treatment for ED and of those unwilling to undergo treatment was 18.8% and 58.8%, respectively. The percentage of patients willing to receive pharmacotherapy did not differ among patients with hypertension, heart disease, or hyperlipidemia. However, patients with DM showed a significantly higher willingness to receive treatment ($p<0.0001$). In addition, patients affected by heart disease expressed less satisfaction with their sex life, compared with patients without the disease ($p<0.0001$). Conversely, the lower the satisfaction with sex life, the higher the willingness to accept pharmacotherapy for ED. However, patients who had actually undergone some form of treatment for ED comprised less than 40% of those patients who were willing to receive medication for ED.

Discussion

The Men's Health Study used the IIEF-5, and the ED evaluation scale revealed that as many as 81% of patients aged in the 30s to 70s who underwent a medical examination by general practitioners on an outpatient basis had some degree of ED.

The prevalence of ED reportedly increases with age[1] and the prevalence of ED by age of patients with an IIEF-5 score ≤16 in the present study closely resembled that of patients categorized as displaying Complete or Moderate ED in the study conducted by Shirai[1] In addition, ED was more prevalent among patients who had concomitant CVD or DM, which is a comparable result to other epidemiological studies conducted in Japan. Marumo et al identified hypertension, DM, heart disease, and cerebral infarction as risk factors associated with ED in males between the ages of 40 and 79 years[13] Another nationwide epidemiological study also demonstrated that ED was predominant in patients affected by CVD or DM[14] Similar results were obtained in an epidemiological survey conducted among males between the ages of 40 and 70 years in Massachusetts, USA[15] Concomitant CVD and DM can therefore be considered to increase the risk of ED, although hyperlipidemia does not appear to represent a significant risk factor, as indicated by both the present results and the findings of Marumo et al[13]

Patients undergoing treatment for ED in the course of routine medical care are highly likely to have CVD. Cur-

rently, the first choice for ED treatment among general practitioners in daily clinical practice is sildenafil. Although the effects of sildenafil on the cardiovascular system were of concern in the early stages of its development,[6] experience has demonstrated that sildenafil exerts positive effects on vascular endothelial cells and the safety of the drug has been established in the clinical setting. Safety information on sildenafil collected from Japanese general practitioners was reported for 3,152 cases in the Drug Use Investigation Study on sildenafil.[7] According to that study, the proportion of adverse drug reactions was 5.27% (166/3,152), and no serious adverse reactions were reported. In a study that examined the effects of sildenafil on coronary flow reserve in patients with serious coronary disease, baseline and post-administration data were compared. Systemic arterial pressure and pulmonary arterial pressure were shown to decrease slightly (<10%), and no effects on pulmonary capillary wedge pressure, right atrial pressure, heart rate, or cardiac output were observed.[8] In addition, coronary flow reserve was shown to be significantly augmented. The acute inhibitory activity of sildenafil on PDE 5 is demonstrated by increases in endothelium-dependent, bloodstream-mediated vasodilation in patients with chronic heart failure.[9] Those findings indicate that the inhibition of PDE 5 by sildenafil can rapidly improve endothelium-dependent vasodilation in patients with chronic heart failure. Sildenafil represents a safe therapeutic option when used appropriately, and does not add to the risk of cardiovascular dysfunction.[4,5] However, as revealed in the present survey, ED treatment is not adequately meeting the needs of patients. Clinicians should be encouraged to actively question patients regarding ED and provide an environment in which patients can comfortably and easily consult with clinicians regarding such issues.

Conclusion

The Men's Health Study shows that middle-aged Japanese men with CVD, DM or hypertension are commonly affected by ED. Cardiovascular specialists need to pay attention to treatment of sexual dysfunction as a real component of their patients' QOL.

Patients who were either willing to receive pharmacotherapy for ED or were considering treatment accounted for 41% of the ED patients in the present study. Sildenafil is a practical therapy for cardiovascular specialists to administer in daily practice, and both pharmacological and clinical evidence has been accumulated regarding the safety of the drug on the cardiovascular system. Based on these factors, active treatment of ED using sildenafil is suitable for patients with CVD.

Acknowledgments

We express our thanks to those who kindly answered the questionnaire and to the doctors-in-charge for their cooperation with the administration of this survey.

This study was supported in part by grants from the Japan Heart Foundation.

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A calcium channel blocker activates both ecto-5′-nucleotidase and NO synthase in HUVEC

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Abstract

Since amlodipine, a long-acting Ca channel blocker, increases both NO and adenosine production in canine hearts, we investigated that amlodipine activates both ecto-5′-nucleotidase responsible for adenosine production and NO synthase (NOS) for NO production in human umbilical venous endothelial cells (HUVECs), and its cellular signaling. We measured activities of ecto-5′-nucleotidase and NOS in HUVECs in the condition with additions of xanthine (100 μM) + xanthine oxidase (1.6×10^{-3} U/ml) in the presence or absence of amlodipine (1×10^{-9}–1×10^{-6} M). Amlodipine increased both ecto-5′-nucleotidase and NOS activities. Xanthine + xanthine oxidase deactivated both NOS and ecto-5′-nucleotidase, and amlodipine increased both activities of NOS and ecto-5′-nucleotidase by 117 ± 33% and 48 ± 6%, respectively. Amlodipine phosphorylated p38MAP kinase and that an inhibitor of p38MAP kinase inhibited the amlodipine-induced activation of both NOS and ecto-5′-nucleotidase. Furthermore, amlodipine increased both adenosine and NO production in the canine ischemic hearts. We concluded that amlodipine activates both NOS and ecto-5′-nucleotidase via p38MAP kinase in vitro and enhances both NO and adenosine production in vivo.

Keywords: Ca channel blockers; Adenosine; NO; Endothelial cells; Canine hearts; p38MAP kinase

Ca channel blockers are often used for the treatment of ischemic heart diseases because of coronary vasodilation [1] via inhibition of Ca^{2+} entry into the smooth muscle cells [2]. Long-acting dihydropyridine Ca channel blockers were reported to protect the endothelial function of renal resistance arteries in hypertensive rats [3] and mesenteric arteries in circulatory shock rats [4]. Interestingly, amlodipine increases NO production in coronary arterial cells [5]; we have reported that long-acting Ca channel blockers have a potential to increase NO production in the hearts and this effect is enhanced in ischemic hearts relative to non-ischemic hearts [6,7]. Since adenosine is known to activate NO synthase (NOS), we hypothesized that amlodipine activates both ecto-5′-nucleotidase that is responsible for adenosine production and NOS.

We aimed to determine whether amlodipine activates either ecto-5′-nucleotidase or NOS in human umbilical venous endothelial cells (HUVEC) with or without oxidative stress. Furthermore, we investigated the cellular signaling pathways to activate either ecto-5′-nucleotidase or NOS.

Materials and methods

In the in vitro and in vivo studies, we used HUVEC and canine hearts, respectively. We used HBD (hybrid dogs mated with the Beagle, the American Fox Hound and the Labrador retriever, and bred for the laboratory use (Kitayama Labes, Yoshiki Farm, Gifu, Japan)). HBD (body mass, 15–21 kg) were anesthetized by an intravenous injection of sodium pentobarbital (30 mg/kg body mass), intubated, and

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ventilated with room air mixed with oxygen (100% O_2 at a flow rate of 1.0–1.5 L/min). The methods for the experimental set-up were described previously [8].

All studies conformed to the "Position of the American Heart Association on Research Animal Use" adopted by the Association in November 1984.

Experimental protocols

Protocol I: effects of amlodipine on ecto-5′-nucleotidase and NOS activities. In HUVEC with or without xanthine (1×10^{-4} M) and xanthine oxidase (1.6×10^{-3} U/ml), the time courses of the changes in ecto-5′-nucleotidase and NOS activities were observed following an exposure to amlodipine of 1×10^{-7} M for 60 min. We also observed the time courses of the changes in ecto-5′-nucleotidase and NOS activities following an exposure to xanthine and/or xanthine oxidase for 60 min.

Protocol II: activities of the dose–response relationship between amlodipine and ecto-5′-nucleotidase and NOS. Since we obtained amlodipine which maximized the activities of ecto-5′-nucleotidase and NOS at 30 min in Protocol I, we examined the dose–response relationship between amlodipine (0, 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, and 1×10^{-6} M) and the activity of the enzymes in the presence and absence of xanthine and xanthine oxidase. We also checked the phosphorylation of PKC or p38MAP kinase at the dose of, 1×10^{-6} or 1×10^{-7} M of amlodipine because either PKC or p38MAP kinase activates either ecto-5′-nucleotidase or NOS. Quantitative analysis was performed when we found the phosphorylation levels increased. Densitometry was performed on each sample and analyzed using NIH image software.

We examined whether SB203580 (1×10^{-6} M) attenuates the activation of these enzymes.

Protocol III: effects of amlodipine on both adenosine and NO release in canine hearts. In the open chest dogs, we sampled coronary arterial and venous blood for blood gas analysis and the measurements of lactate, nitrate + nitrite [9], and adenosine levels [8] by which we calculated coronary arteriovenous difference of nitrate + nitrite ($VAD(NO_x)$) or adenosine (VAD(Ado)). Lactate extraction ratio (LER) was obtained as the coronary arteriovenous difference of the lactate concentration multiplied by 100 and divided by the arterial lactate concentration.

We used 5 dogs in Protocol I. Hemodynamic parameters, i.e., systolic and diastolic aortic blood pressure as well as heart rate, were monitored. To examine whether amlodipine increases cardiac NO or adenosine levels in the ischemic hearts, after we reduced coronary perfusion pressure (CPP) so that coronary blood flow (CBF) decreased to 50% of the baseline for 5 min, we infused amlodipine (2 μg/kg/min, an infusion rate: 0.0167 ml/kg/min, a concentration of the solution: 0.12 mg/ml) for 60 min keeping CBF at the initial reduced value. In a preliminary study, the dose of amlodipine (2 μg/kg/min, an intracoronary infusion) was determined as the minimum dose that caused maximal coronary vasodilation.

Chemical analysis

The methods to measure plasma adenosine [8], NO_x [9], and lactate levels, and myocardial ecto-5′-nucleotidase [10], and NOS [11] activities have been reported previously.

For the measurement of NOS activity, we employed the Sigma's Fluorometric Cell Associated NOS Detection System (FCANOS-1, Sigma). Using the 5×10^5/ml HUVECs in the suspension buffer, we used 0.2 ml of cultured HUVECs for each well of 96-well plate (i.e., 1×10^5 HUVEC cells/well). In these wells with HUVEC and the reaction buffer, we added 1 μM diaminofluorescein-2 diacetate (DAF-2 DA). We added 1 mM arginine and 3.0 μM NADPH, which made the final volume of 0.2 ml. We measured the fluorescence using spectrofluorometer with excitation filter at 492 nM and an emission filter at 515 nM 2 h after the incubation. Since we used intact cell cultures, we did not add flavin mononucleotides, tetrahydrobiopterin or calmodulin. In fact, the addition of each component increased non-specific background or decreased the signals without alteration of the NOS activity. Since diphenylene iodonium chloride (DPI) did not affect NOS activity in HUVEC in the preliminary study, we did not use DPI in the present study.

Statistical analysis

Statistical analysis was performed using ANOVA [12] when data were compared among the groups. When ANOVA indicated a significant difference, we compared paired data using the Bonferroni test. Changes of the hemodynamic and metabolic parameters over time were compared by ANOVA for repeated measures. Results were expressed as means ± SEM, with $p < 0.05$ being considered significant.

Results and discussion

Ecto-5′-nucleotidase (control; 24.7 ± 2.2, 30 min; 32.6 ± 2.7 ($p < 0.05$), 60 min 33.8 ± 3.0 ($p < 0.05$) nmol/mg protein/min, $n = 6$ each) and NOS (control; 100%, 30 min; 136 ± 2% ($p < 0.01$), 60 min; 134 ± 3% ($p < 0.01$), $n = 6$ each) activities of HUVEC increased after the exposure to 1×10^{-7} M amlodipine. On the other hand, xanthine + xanthine oxidase decreased ecto-5′-nucleotidase (control; 25.2 ± 3.0, 15 min; 16.8 ± 3.0 ($p < 0.01$), 60 min; 16.1 ± 2.1 ($p < 0.01$) nmol/mg protein/min, $n = 6$ each) and NOS (control; 100%, 15 min; 81 ± 3% ($p < 0.01$), 60 min; 78 ± 6% ($p < 0.01$), $n = 6$ each) activities. Both enzyme activities became stable 30 min after the amlodipine exposure and 15 min after the exposure to xanthine + xanthine oxidase. Either xanthine or xanthine oxidase did not affect ecto-5′-nucleotidase activity (control; 23.9 ± 2.5, 60 min; 24.7 ± 1.9 or 25.2 ± 2.4 nmol/mg protein/min, $n = 6$ each) or NOS activity (control; 100%, 60 min; 102 ± 4% or 97 ± 3%, $n = 6$ each). Therefore, we examined the dose–response relationship at these time points. Table 1 shows that both ecto-5′-nucleotidase and NOS activities were activated by amlodipine in dose-dependent manner in the presence or absence of xanthine + xanthine oxidase. Table 1 further shows that (1) xanthine + xanthine oxidase deactivated both enzymes and (2) the concomitant administration of amlodipine in the presence of xanthine + xanthine oxidase restored both enzyme activities to the levels of the condition without xanthine + xanthine oxidase. Fig. 1 shows the phosphorylation of p38MAP kinase and several subtypes of PKC. Amlodipine phosphorylated p38MAP kinase, but it did not affect PKC phosphorylation. Quantitative analysis using densitometric analysis showed that amlodipine phosphorylated p38MAP kinase dose- and time-dependently compared with each control (10^{-7} M amlodipine; 30 min; 1.4 ± 0.2 (NS), 60 min; 1.6 ± 0.1 ($p < 0.01$), 60 min; 10^{-6} M amlodipine; 30 min; 2.0 ± 0.2 ($p < 0.01$), 60 min; 2.1 ± 0.3 ($p < 0.01$)). Table 1 shows that SB203580 blunts the amlodipine-induced activation of both ecto-5′-nucleotidase and NOS.

Table 1
The effects of amlodipine on the activities of ecto-5′-nucleotidase and NO synthase

		Control	Doses of amlodipine (M)			
			1×10^{-9}	1×10^{-8}	1×10^{-7}	1×10^{-6}
Without SB203580						
Ecto-5′-nucleotidase activity	Without X + XO	25.8 ± 3.5	25.5 ± 3.4	27.6 ± 3.2	30.6 ± 3.1*	31.0 ± 3.3*
(pmol/mg protein/min)	With X + XO	15.6 ± 3.1	17.2 ± 2.3	23.4 ± 3.2*	28.1 ± 3.5*	30.9 ± 3.5*
NOS activity	Without X + XO	100	108 ± 6	118 ± 4*	126 ± 6*	125 ± 5*
(% of control)	With X + XO	83 ± 4	88 ± 5	109 ± 3*	119 ± 3*	122 ± 4*
With SB203580						
Ecto-5′-nucleotidase activity	Without X + XO	25.1 ± 2.9	25.9 ± 3.1	24.8 ± 2.5	26.9 ± 2.2	26.5 ± 2.2
(pmol/mg protein/min)	With X + XO	18.7 ± 3.2	19.7 ± 2.1	17.3 ± 3.0	18.1 ± 2.9	17.1 ± 3.0
NOS activity	Without X + XO	100	97 ± 3	93 ± 3	101 ± 4	103 ± 4
(% of control)	With X + XO	91 ± 5	92 ± 4	90 ± 5	87 ± 4	90 ± 4

Values are means ± SEM, $n = 6$ each. *Abbreviations:* X, xanthine; XO, xanthine oxidase.
* $p < 0.01$ vs. the control value.

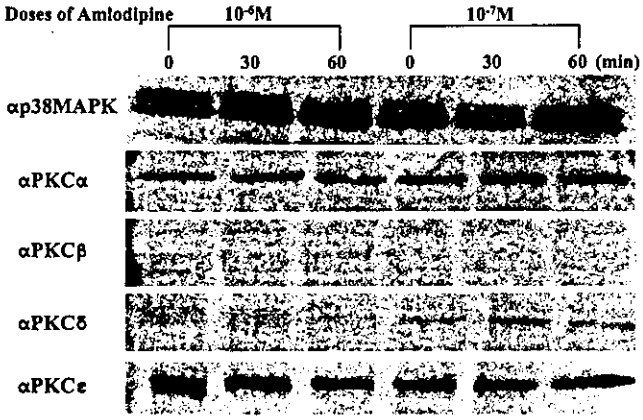

Fig. 1. Immunoblot of p38MAP kinase and several subtypes of PKC. P38MAP kinase is phosphorylated by amlodipine in 30–60 min. Immunoblot is performed using antibody of anti-p38MAPK or subtypes of PKC.

In the five ischemic canine hearts (CPP; 53 ± 2 from 103 ± 3 mm Hg, $n = 5$), we observed that an intracoronary administration of amlodipine increased VAD(Ado) (control, 16 ± 2; ischemia, 99 ± 7 ($p < 0.01$ vs. a control); and ischemia + amlodipine, 311 ± 21 ($p < 0.01$ vs. ischemia) pmol/ml, $n = 5$) and VAD(NO_x) (control, 4.2 ± 0.3; ischemia, 9.1 ± 1.1 ($p < 0.05$ vs. a control); and ischemia + amlodipine, 16.7 ± 2.0 ($p < 0.05$ vs. ischemia) nmol/ml, $n = 5$) despite controlled low CBF (control, 91 ± 1; ischemia, 46 ± 2 ($p < 0.01$ vs. a control); and ischemia + amlodipine, 46 ± 2 (NS vs. ischemia) ml/100 g/min, $n = 5$) and LER (control, 25.9 ± 2.3; ischemia, −47.4 ± 3.2 ($p < 0.01$ vs. a control); and ischemia + amlodipine, −43.9 ± 2.2 (NS vs. ischemia) ml/100 g/min, $n = 5$). Amlodipine deceased CPP (53 ± 2 from 46 ± 1 mm Hg, $p < 0.05$, $n = 5$), which indicates that amlodipine decreases coronary vascular resistance. Either systemic blood pressure (106 ± 2 from 102 ± 4 mm Hg, $n = 5$) or heart rate (139 ± 3 from 137 ± 3/min, $n = 5$) was not altered by amlodipine administration.

The present study revealed that amlodipine, a long-acting Ca channel blocker, activated both ecto-5′-nucleotidase and NOS in HUVEC via p38MAP kinase-dependent pathway. The effects of amlodipine on ecto-5′-nucleotidase and NOS were enhanced in the condition with oxidative stress. Furthermore, the effects of amlodipine on both adenosine and NO production were also seen in the in vivo canine hearts.

We have revealed novel aspects of amlodipine on ecto-5′-nucleotidase and NO synthase. First, as for the mechanisms of these novel aspects, although amlodipine is related to the inhibition of the Ca^{2+} inward, since HUVEC lacks voltage-dependent Ca^{2+} channels, we speculate Ca^{2+}-dependent pathways may not be likely to explain the present phenomenon. Since either NOS or ecto-5′-nucleotIdase is activated by PKC, we tested whether amlodipine activates PKC and we observed the negative results for PKC. On the other hand, we found that amlodipine directly affects p38MAP kinase and that SB203580, an inhibitor of p38MAP kinase, blunted the activation of both NOS and ecto-5′-nucleotidase. Since amlodipine is reported to enter the lipid bilayer of the cell membrane, it is likely that amlodipine affects the intracellular signal transduction pathway of p38MAP kinase, and the activation of p38MAP kinase activates both ecto-5′-nucleotidase and NOS. PKC, which can activates both enzymes are not related to the present observation.

Second, since oxygen-derived free radicals attenuate both ecto-5′-nucleotidase [13] and NOS, the elimination of oxidative stress may restore the reduction of these two enzymatic activities. Since amlodipine is reported to reduce oxidative stress [14], the reduction of oxidative stress due to amlodipine may explain the enhanced effects of amlodipine on the activation of ecto-5′-nucleotidase and NOS. We also excluded the possibility that the non-specific effects of either xanthine or xanthine oxidase alter ecto-5′-nucleotidase and NOS activities in HUVEC. Interestingly, this in vitro phenomenon is

completely reproduced in the in vivo canine hearts. Amlodipine increased both adenosine and NO production in the canine hearts.

Although amlodipine increased both adenosine and NO production, and thus decreased coronary resistance, the cellular signal transduction for coronary vasodilation due to either adenosine or NO is completely different. Indeed, both adenosine and NO elevate cyclic AMP and GMP levels, respectively, which independently cause coronary vasodilation. The former mainly causes detachments of actin–myosin interaction and the latter mainly causes the re-uptake of Ca^{2+} into sarcoplasmic reticulum. Therefore, the increased adenosine and NO levels independently cause coronary vasodilation. Since both adenosine and NO are believed to attenuate the severity of myocardial ischemia [15], both adenosine and NO increase CBF, attenuate myocardial anaerobic metabolism, inhibit platelet aggregation and leukocyte activation, and attenuate the activation of sympathetic nerve activity in an ischemic heart.

The present study suggests that amlodipine contributes to the activation of both ecto-5′-nucleotidase and NOS, and contributed to the elevation of the cardiac adenosine/NO levels, coronary vasodilation, and the attenuation of the severity of myocardial ischemia. Therefore, it is likely that the beneficial effects of amlodipine on the ischemic hearts seen in the present study are attributable to combination of the anti-oxidant effects, and adenosine-/NO-induced effects and Ca channel blocking effects of amlodipine.

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Activation of Adenosine A_1 Receptor Attenuates Cardiac Hypertrophy and Prevents Heart Failure in Murine Left Ventricular Pressure-Overload Model

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Abstract—Sympathomimetic stimulation, angiotensin II, or endothelin-1 is considered to be an essential stimulus mediating ventricular hypertrophy. Adenosine is known to protect the heart from excessive catecholamine exposure, reduce production of endothelin-1, and attenuate the activation of the renin-angiotensin system. These findings suggest that adenosine may also attenuate myocardial hypertrophy. To verify this hypothesis, we examined whether activation of adenosine receptors can attenuate cardiac hypertrophy and reduce the risk of heart failure. Our in vitro study of neonatal rat cardiomyocytes showed that 2-chloroadenosine (CADO), a stable adenosine analogue, inhibits protein synthesis of cardiomyocytes induced by phenylephrine, endothelin-1, angiotensin II, or isoproterenol, which were mimicked by the stimulation of adenosine A_1 receptors. For our in vivo study, cardiac hypertrophy was induced by transverse aortic constriction (TAC) in C57BL/6 male mice. Four weeks after TAC, both heart to body weight ratio (6.80 ± 0.18 versus 8.34 ± 0.33 mg/g, $P<0.0001$) as well as lung to body weight ratio (6.23 ± 0.27 versus 10.03 ± 0.85 mg/g, $P<0.0001$) became significantly lower in CADO-treated mice than in the TAC group. Left ventricular fractional shortening and left ventricular dP/dt_{max} were improved significantly by CADO treatment. Similar results were obtained using the selective adenosine A_1 agonist N^6-cyclopentyladenosine (CPA). A nonselective adenosine antagonist, 8-(*p*-sulfophenyl)-theophylline, and a selective adenosine A_1 antagonist, 8-cyclopentyl-1,3-dipropylxanthine, eliminated the antihypertrophic effect of CADO and CPA, respectively. The plasma norepinephrine level was decreased and myocardial expression of regulator of G protein signaling 4 was upregulated in CADO-treated mice. These results indicate that the stimulation of adenosine receptors attenuates both the cardiac hypertrophy and myocardial dysfunction via adenosine A_1 receptor–mediated mechanisms. (*Circ Res.* **2003;93:759-766.**)

Key Words: adenosine ■ cardiomyopathy ■ echocardiography ■ heart failure ■ myocytes

Patients with pressure-overload diseases such as systemic hypertension exhibit left ventricular hypertrophy (LVH), a major determinant of mortality and morbidity in cardiovascular diseases. It is well-known that many neurohumoral factors such as angiotensin II (Ang II),[1,2] endothelin-1 (ET-1),[3] catecholamines,[2,4] growth factors,[5,6] and tumor necrosis factor-α (TNF-α)[7] cause LVH via the activation of intracellular signal transduction mediated by calcineurin[8,9] or mitogen-activated protein kinases.[10,11]

Adenosine, a nucleoside abundantly produced by cardiac cells, is known to inhibit norepinephrine release from presynaptic vesicles,[12] reduce production of ET-1,[13] attenuate the activation of the renin-angiotensin system,[14] and counteract TNF-α.[15] Because norepinephrine, ET-1, Ang II, and TNF-α are believed to be involved in cardiac hypertrophy and remodeling,[1–4] we hypothesized that adenosine may reduce cardiac hypertrophy and improve subsequent cardiac dysfunction. Indeed, myocardial concentration of adenosine was found to markedly increase in the hypertrophied heart,[16] whereas exogenous or endogenous adenosine has been shown to inhibit the growth of rat cardiac fibroblasts in vitro.[17] We also demonstrated that the plasma concentration of adenosine increased in patients with chronic congestive heart failure (CHF)[18] and that an increase in plasma adenosine levels ameliorated CHF.[19] The enhancement of adenosine metabolism is therefore thought to improve the pathology of cardiac hypertrophy and subsequent heart failure.

Taking these findings into consideration, we postulated that sustained stimulation of adenosine receptors would be beneficial for attenuation of LVH and improvement of heart

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function. As far as we know, however, the role of adenosine on myocardial hypertrophy and heart function in pressure-overload state remains poorly understood. The study presented here was therefore undertaken to determine whether administration of 2-chloroadenosine (CADO), a stable analogue of adenosine, would have beneficial effects on the LV structure and heart function in a murine model of transverse aortic constriction (TAC) and, if so, to clarify the potential underlying mechanisms involved.

Materials and Methods

Agents

CADO, 8-sulfophenyltheophylline (8-SPT), phenylephrine (PE), ET-1, Ang II, isoproterenol (Iso), forskolin, N^6-cyclopentyladenosine (CPA), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamino adenosine hydrochloride (CGS21680), 5-ethylcarboxamidoadenosine (NECA), and N^6-(3-iodobenzyl)-5′-*N*-methylcarbamoyladenosine (IB-MECA) were purchased from Sigma Chemical Company.

Cell Culture for the In Vitro Study

Neonatal rat ventricular myocytes were isolated as described previously.[20] Cardiac myocytes were cultured in DMEM (Sigma) supplemented with 10% FBS (Equitech-Bio Inc). Culture media were changed to serum-free at 72 hours. Cardiomyocytes were cultured in serum-free conditions for 48 hours before experiments. Protein synthesis in cultured cells was evaluated by analysis of [^{3}H]leucine incorporation as described.[6] For cell surface area measurement, cardiomyocytes were stained with rhodamine-phalloidin and 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI); confocal microscopic images (×400) were captured and surface area was measured using Scion image software (Scion Corporation).

Surgical Procedures for the In Vivo Study

Mice (C57BL/6, male, 8 to 9 weeks old, weight 18 to 25 g) were anesthetized with a mixture of pentobarbital (50 mg/kg IP) and ketamine (25 mg/kg IP). The animal model of pressure overload was created, and invasive measurement of trans-stenosis pressure gradient and left ventricular dP/dt_{max} was performed as described previously.[21–23]

Experimental Protocols I (In Vitro Study)

Cardiomyocytes were exposed to PE (10^{-4} mol/L), ET-1 (10^{-8} mol/L), Ang II (10^{-7} mol/L), Iso (10^{-5} mol/L), or forskolin (10^{-5} mol/L) for 30 hours in the presence or absence of CADO (10^{-6} mol/L), and the extent of increase in [^{3}H]leucine uptake was examined. We studied the effects of A_1 (CPA), A_{2A} (CGS21680), and A_3 (IB-MECA) receptor selective agonists and the nonselective agonist (NECA) for A_1, A_{2A}, and A_{2B} on cardiac myocyte hypertrophy.

Experimental Protocol II (In Vivo Study)

Determination of the Dosage of the Agents

Preliminary experiments were performed to determine the dosage of agents used in vivo studies. All of the agents were delivered by minipump infusion for 4 weeks (Alzet micro-osmotic pump model 1002, replaced at 2 weeks).

Roles of Stimulation of Adenosine Receptors

We treated mice with saline (TAC group), CADO alone (2 mg/kg per day), CADO (2 mg/kg per day) plus 8-SPT (10 mg/kg per day), 8-SPT alone (10 mg/kg per day), CPA (5 mg/kg per day), or CPA (5 mg/kg per day) plus DPCPX (5 mg/kg per day), respectively. Tail-cuff BP and HR measurements (BP-98A, Softron) were done at 1, 2, and 4 weeks, and the echocardiographic assessments were performed at 4 weeks after TAC. Mice were euthanized to obtain the organs for morphometric analysis. All procedures were performed in accordance with the guiding principles of Osaka University Graduate School of Medicine with regard to animal care.

Measurements of 5′-Nucleotidase Activity and the Levels of Norepinephrine and Renin

To examine whether the enzyme to produce adenosine via AMP is activated in the myocardial hypertrophic mice, we measured the myocardial 5′-nucleotidase (5′-ND) activity[24] in a time course. Plasma norepinephrine and renin levels were determined as described.[25,26]

Determination of the Expression of B Natriuretic Peptide and Regulator of G Protein Signaling 4 Using Quantitative Polymerase Chain Reaction

Total RNA was extracted from whole heart by using TRIzol reagent (GIBCO/BRL) as described by the manufacturer. Primers for quantitative polymerase chain reaction (PCR) were designed using Gene Express software (Applied Biosystems). Expression levels of natriuretic peptide precursor type B (BNP) and regulator of G protein signaling 4 (RGS-4) were determined using Quantitect SYBR Green RT-PCR kit (QIAGEN) according to the manufacturer's instruction.

Results

Chloroadenosine Inhibits Myocyte Hypertrophy Induced by the Agonists of G-Protein–Coupled Receptor

Treatment with CADO alone did not affect the basal [^{3}H]leucine uptake of myocytes when the concentration of CADO was not higher than 10^{-5} mol/L, but CADO decreased [^{3}H]leucine uptake at concentrations higher than 10^{-5} mol/L (Figure 1A). Thus, we used CADO at the concentrations of $\leq 10^{-5}$ mol/L to assess its effects on myocyte hypertrophy. Figure 1B showed that CADO inhibited PE-induced cardiomyocyte hypertrophy in a concentration-dependent fashion. Myocyte cross-sectional area was also decreased by CADO (Figures 1C and 1D). In addition, the exposure to ET-1 or Ang II induced cardiomyocyte hypertrophy, as was gauged by changes in [^{3}H]leucine incorporation, and cotreatment with CADO (10^{-6} mol/L) inhibited these G-protein–coupled receptor agonist–induced increase in [^{3}H]leucine uptake (Figure 1E).

Chloroadenosine Also Blocks Protein Kinase A–Dependent Hypertrophic Signal Pathway

Treatment of cardiomyocytes with Iso (10^{-5} mol/L) increased protein synthesis, and cotreatment with CADO dose-dependently inhibited the increase of [^{3}H]leucine uptake (Figure 2A). Cellular enlargement induced by Iso was also attenuated in CADO-treated myocytes (Figures 2B and 2C). Furthermore, treatment with forskolin, a stimulator of adenylate cyclase, also increased [^{3}H]leucine uptake, which was abolished completely by CADO at the concentration of 10^{-5} to 10^{-6} mol/L (Figure 2A).

Antihypertrophic Effect of Chloroadenosine Is Mediated by the Stimulation of Adenosine A_1 Receptors

CPA, an A_1 selective agonist, and NECA, a nonselective agonist for A_1, A_{2a}, and A_{2b} receptors, significantly inhibited the PE-induced increase of cardiac myocyte protein synthesis, but neither CGS21680, an A_{2a} receptor agonist, nor IB-MECA, an A_3 selective receptor agonist, affected the PE-

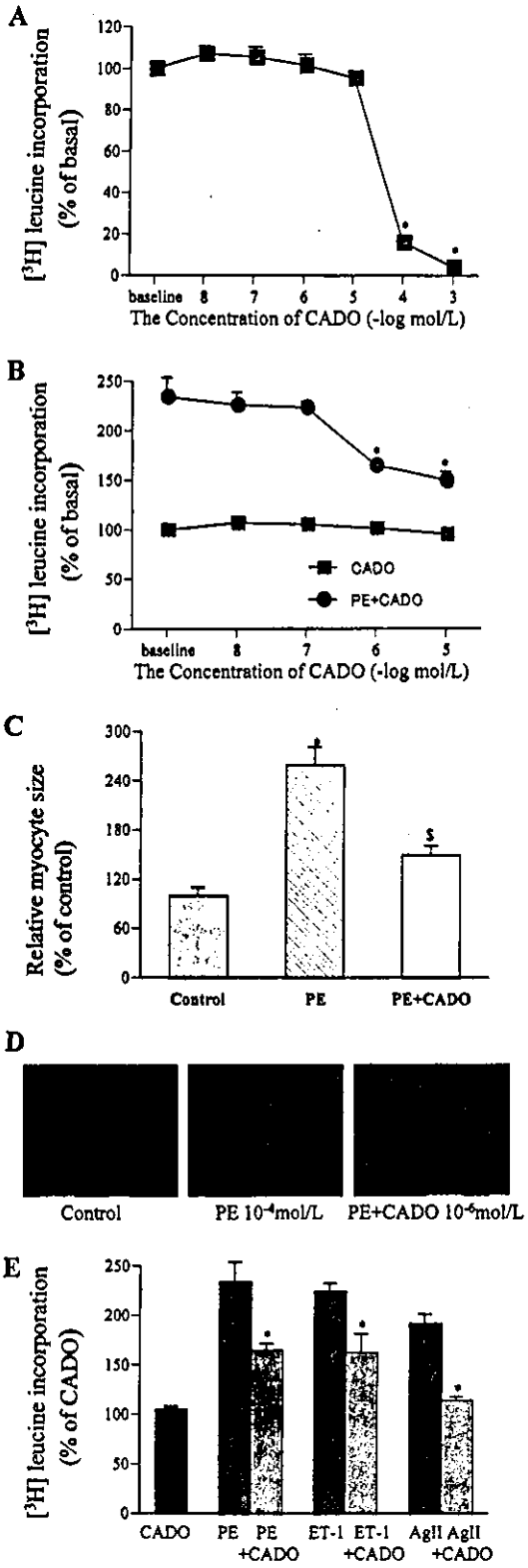

Figure 1. Inhibition of protein synthesis of myocytes by CADO. A, Concentration-dependent effects of CADO on [^{3}H]leucine incorporation in myocytes without adding stimulators. *P<0.01 compared with the value at baseline. B, Concentration-dependent effects of CADO on [^{3}H]leucine incorporation induced by PE (10^{-4} mol/L). *P<0.01 compared with the value at baseline. C, Enlargement of myocyte cross-sectional area induced by PE (10^{-4} mol/L) was decreased in the presence of CADO (10^{-6} mol/L), *P<0.01 compared with the value at control, $$P$<0.01 vs PE (n=200 cells in every group). D, Representative confocal microscopic images of myocytes with rhodamine-phalloidin staining of actin and DAPI staining of the nucleus; CADO reduced PE (10^{-4} mol/L)-induced enlargement of myocyte cross-sectional area. E, Effects of CADO (10^{-6} mol/L) on protein synthesis stimulated by PE (10^{-4} mol/L), ET-1 (10^{-8} mol/L), and Ang II (10^{-7} mol/L). *P<0.01 vs the corresponding stimulator alone. All values are expressed as mean±SEM. Every experiment was repeated at least 3 times.

induced increase of [^{3}H]leucine uptake (Figure 3A). Similar results were obtained in forskolin-induced cardiac myocyte hypertrophy (Figure 3B). Therefore, we conclude that it is A_1, not A_{2a} or A_3 receptors, that mediates the antihypertrophic effect.

Activation of Adenosine A_1 Receptors Attenuates Myocardial Hypertrophy In Vivo

Myocardial 5′-ND activity in TAC mice increased from 2 weeks after surgery and achieved significant difference at 4 weeks compared with sham-operated mice (Figure 4A). Treatment with CADO in TAC mice and plasma concentrations of norepinephrine, renin, and a molecular marker of hypertrophy BNP were significantly reduced, whereas gene expression of RGS-4, an inhibitory factor of hypertrophy, was markedly upregulated (Figures 4B and 4C). The question is whether these changes are associated with attenuated cardiac hypertrophy. Interestingly, our preliminary study showed a dose-response attenuation of cardiac hypertrophy by 1 week of treatment with CADO (Figure 5A). Along with this preliminarily study, we determined that CADO of 2 mg/kg per day is the minimal dose that exerts the maximal effects. In 4-week chronic studies, the degree of cardiac hypertrophy in CADO-treated mice was significantly lower than in TAC mice receiving vehicle treatment (P<0.0001; Figures 5B through 5F), whereas TAC led to a 74% increase in heart weight at 4 weeks after the surgery. CADO attenuated the heart weight to body weight ratio by 41% and decreased the left ventricular posterior wall thickness by 52% (Table). No significant difference was found on body weight between CADO-treated and vehicle-treated TAC mice (Table). CADO also reduced myocardial (Figure 5G) and perivascular fibrosis (Figure 5H). Meanwhile, a selective adenosine A_1 receptor agonist CPA markedly attenuated cardiac hypertrophy, and this effect was abolished by a selective A_1 receptors antagonist DPCPX (Figures 5B and 5C). Treatment with 8-SPT alone did not additionally increase cardiac hypertrophy, but cotreatment with CADO abrogated the effects of CADO on attenuating cardiac hypertrophy, as determined by the heart weight to body weight ratio and the left ventricular posterior wall thickness (Figures 5B and 5C and Table). Similarly, 8-SPT alone did not deteriorate the heart function of TAC mice, but it reversed the effects of CADO on the improvement of heart function (Figure 6A and Table).

One, two, and four weeks after the pharmaceutical treatment, systolic blood pressure and heart rate were not significantly different among all the groups, except that systolic blood pressure was slightly higher in sham group. These