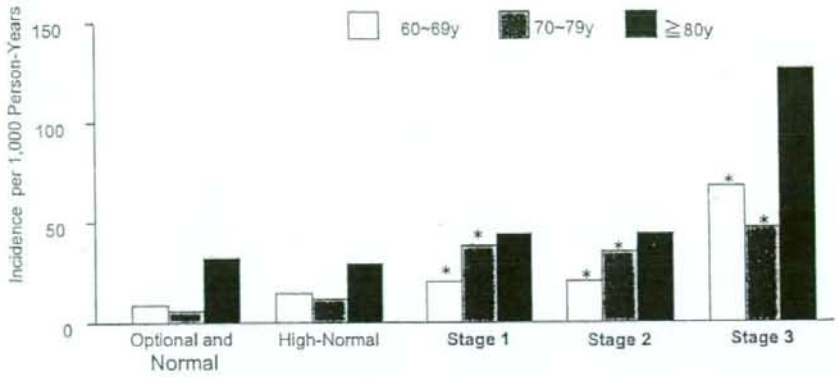

血圧区分（JNC-VI）

	SBP		DBP
Optional	＜120	かつ	＜80
normal	＜130	かつ	＜85
High-normal	130~139	または	85~90
Stage 1	140~159	または	90~99
Stage 2	160 ~179	または	100~109
Stage 3	＞180	または	＞110

図1　年齢階層別・血圧階層別の心血管疾患発症率（久山町研究）（文献1）より引用）

たとえば，60歳以上の収縮期高血圧患者だけを対象としたSHEP研究（1991年）は，約4,700例を治療群（利尿薬，効果不十分の場合はβ遮断薬追加）とプラセボ群に分け，4.5年にわたって追跡した．その結果，治療群で脳卒中，心筋梗塞，全血管疾患の発症率がいずれも有意に抑制され，老年者の収縮期高血圧に対する治療の有効性が立証された．

さらに，より高齢の高血圧患者（70~84歳）を対象としたSTOP-Hypertension（1991年）でも，治療群（β遮断薬，必要に応じて利尿薬追加）で心血管イベントの発生が有意に減少することが示された．続くMRC-old，STONEでもほぼ同様の成績が得られ，高齢者高血圧に対する降圧治療のエビデンスが確立された．

一方，EWPHE（European Working Party on High Blood Pressure in the Elderly Trial，1984年）やHYVETなどの超高齢者に対する介入試験の結果は，降圧による治療効果，すなわち，脳心血管事故の発症を，期待するほどの抑制はみられない．EWPHEでは80歳以上になると，プラセボ群と実薬群でほとんど違いがみられなくなる．一方，HYVET（Hypertension in the Very Elderly Trial）パイロット試験では，対象が85歳という高齢群であるにもかかわらず，降圧療法によって脳卒中だけが，降圧療法のベネフィットがあるという結果であった．欧米に比べて脳卒中が多い日本人において降圧療法により，脳卒中の発症がある程度抑制できるならば，介護予防の観点からも，高齢者に対する降圧療法は積極的に勧められるべきであると考えられる．

さらに，最近の日本人のデータであるNIPPON DATA90の解析[7]では，60歳以上の住民を対象とした降圧薬服用による血圧レベルと心血管疾患死亡のリスクとの関連を検討した報告がある（図2）．それによれば，降圧薬を服用していて，正常血圧レベルに達していない群のリスクがもっとも高く，降圧薬を服用し正常血圧にコントロールされている群では，降圧薬を服用せず正常血圧である健康群とほとんどリスクが変わらないことを示している．

疾患の発症と死亡との違いは臨床的には，大きな問題であり，死亡が減っても発症が増えることは，それだけ要介護高齢者を生み出すことにほかならない．老年医学的見地から，高齢者に対する降圧療法は，脳卒中などの死亡とともに発症を抑制することにも，力点が置かれなければ，「健康寿命」の延伸につながらないと思われる．

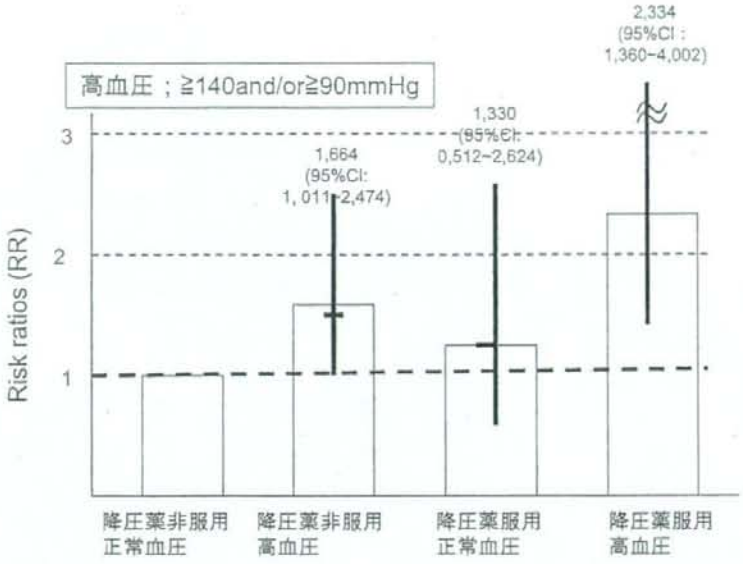

図2　60歳以上の住民における降圧薬服用状況と血圧レベルごとの心血管疾患死亡リスク（NIPPON DATA90）（文献2）より引用）

表1　10年後のADL低下（要介護状況発現）の危険因子
香北町1991年集団（1488，男：647，女：841）

	オッズ比	95% 信頼区間	p
年齢	1.163	1.120-1.207	< 0.0001
女性	1.741	1.026-2.954	0.04
情報関連機能（視聴覚）低下	1.950	1.293-2.941	0.0015
脳卒中	4.901	1.024-23.453	0.0466
飲酒	0.601	0.375-0.962	0.0339

（文献3）より引用）

高齢者高血圧に対する今後の課題：「高知県香北町健康長寿計画」から

高齢者の健康で自立した期間（健康寿命）をいかにして長くするか，健康寿命の延伸を目指した取り組みは，多くの全国自治体で行われてきた．高知県香北町の取り組みは，全人口の40%近くが65歳以上の高齢者で，高齢者政策に悩んでいた自治体と，寝たきりになる前の予防的介入の必要性が重要と考えてきた大学との利害が一致し，1990年から高知医科大学との自治体・大学共同事業がはじまった．これまで，高齢者一人当たりの医療費ののびの抑制や，85歳以上の自立高齢者割合の改善をはじめ，多くの老年医学的成果をあげ，当初の目標は達成できた．しかし，後期高齢者が高齢者の50%以上となり，さらに90歳以上の高齢者が高齢者人口の5%を超えるようになると，自立して生活を営める高齢者の割合がしだいに鈍化しはじめた．もちろん，同様のシステムで後追いした自治体の状況と比べれば，自立高齢者割合は85%以上ともっとも高い水準で推移しているのであるが，介護予防を目的とした老年医学的介入のこれからの課題が明らかになってきた．

松林らは10年後の要介護状況発現の要因について検討した（表1）[3]．この中で，年齢，性，生活機能（ADL）以外で脳卒中の発症が独立した要因として残り，介入し予防できる要因として重要であった．

われわれは，この点に着目し，脳卒中予防が，健康長寿のために大きな役割をきたすと考え，血圧と日常活動度の低下，すなわち，自立喪失との関連について検討した．

高齢者の血圧は変動しやすく，短時間の血圧変動が大きいばかりでなく，白衣高血圧や仮面高血圧といった比較的長期の血圧変動も来たしやすい．高齢者の場合，健

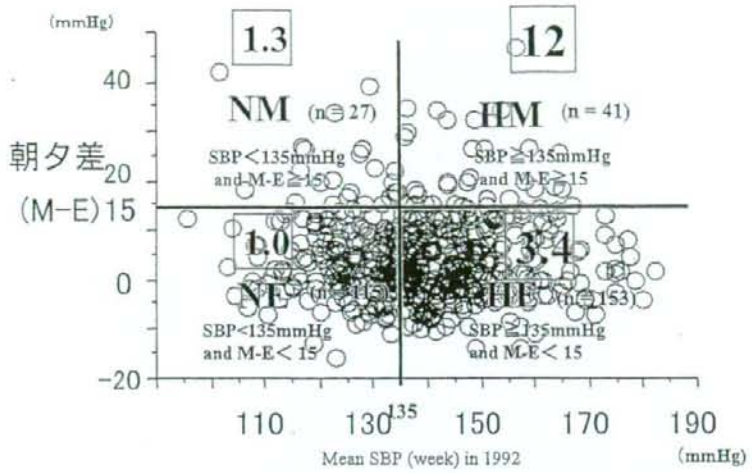

図3　家庭収縮期血圧における要介護オッズ比（文献4）より引用）

診時の血圧値が予後を十分に反映しないことも示されており，家庭血圧値が高齢者の血圧コントロールの指標として重要な役割を果たすと考えられている．

しかし，一方では高齢者，特に後期高齢者や80歳以降の高齢者の降圧には慎重な意見もあり，家庭血圧レベルでも十分な血圧コントロールがなされていないのが現状である．

私たちの検討でも，75歳以上の地域在住高齢者の連続5日間朝・夕の家庭血圧の平均値で収縮期血圧が135 mmHg以上の高齢者は約半数に認めた．

さらに，朝の血圧が，夕方より高い高齢者高血圧群では要介護になる可能性が，十分血圧のコントロールされている群に比べて，12倍も高いこと（図3）から，降圧可能な高齢者では，十分な降圧が，要介護予防の観点から重要であると考えられる[4]．

超高齢社会に突入しつつあるわが国においては，降圧療法による死亡数の減少や脳卒中発症の減少はもちろん重要であるが，介護予防の観点からも，高齢者高血圧における降圧療法の意義を捉えなおすことも重要であると思われる．

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第48回日本老年医学会学術集会記録
〈ミニレビュー〉

老年症候群；わずかな視・聴覚機能低下が生活機能やQOL低下に与える影響

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〈ミニレビュー〉

老年症候群；わずかな視・聴覚機能低下が生活機能やQOL低下に与える影響

西永　正典[1)]　池　成基[2)]　上総　百合[1)]　高田　淳[1)]　土居　義典[1)]

要　約　高齢者にとって視・聴覚機能の低下は，高齢者生活機能の自立維持の重要な阻害因子であるにもかかわらず，老化現象のひとつと考えられ治療可能であっても放置されることが多い．しかし，今回の検討では，ごく軽度の視・聴力機能の低下であっても，ADLばかりでなく，うつ尺度，QOL（主観的幸福度）に大きく影響するため，視・聴力機能評価は高齢機能評価（チェックリスト）に不可欠と考えられる．

Key words：視力障害，聴力障害，要介護，QOL（生活の質），ADL（日常生活活動度）

（日老医誌　2007；44：302-304）

はじめに

高齢者にとって，視覚および聴覚機能の低下は，本人や家族，そして医療・介護関係者にもさほど異常とは思われない加齢現象の一つとして捉えられており，治療可能にもかかわらず，「高齢であるから」という理由で放置されることが少なくない．さらに，平成18年度の介護保険改正により「介護予防」に重点が置かれるようになったが，要介護ハイリスクグループ（特定高齢者）の選定の一つの判断基準となる25項目のチェックリストの中に，視・聴覚機能低下に関する問いはみられず，わずかな視・聴覚機能の低下が，一見健常と思われる高齢者にどのような影響を与えるかについての検討は少ない[1)~3)]．

そこで，本ミニレビューでは，わずかな視・聴覚機能低下と地域在住高齢者の日常活動度，QOLとの関連について検討した．

対象・方法

高知県K町在住65歳以上の高齢者に2000年時に自記式アンケートを実施し，1,874人（男813，女1,061，平均年齢76±9歳）より，視・聴覚機能に関する項目，すなわち，視覚機能：問題なし（日常生活に支障がない），軽度支障あり（新聞の小さな文字は読めないが中等度以上は読める），中等度支障あり（新聞を読むことができない），重度支障あり（明暗しかわからない），聴覚機能：問題なし（日常生活に支障なし（補聴器使用可）），軽度支障あり（正常より大声が必要），中等度支障あり（耳のそばで大声でゆっくり反復が必要），重度支障あり（ほとんど，まったく聞こえない）のおのおの4段階の回答を得た（表1）．身体機能は基本的ADL（歩行，階段昇降，食事，更衣，排便排尿，入浴，整容：21点満点），高次ADL（老研式生活能力活動指標）のうち，公共交通機関の利用の項目を除いた12点満点，うつ尺度はGeriatric Depression Scale（GDS15），QOLはVisual Analogue Scale（VAS）を用いて評価した．

なお，本研究は，高知大学医学部倫理委員会において，住民調査に関する承認を得た．加えて，毎年，対象者アンケート調査実施時に文書による同意取得を行った．

結　果

（1）65歳以上の高齢者では，視覚支障なし1,610（85.9%），軽度障害210（11.2%），中等度障害　43（2.3%），重度障害11（0.6%）であった．一方，聴覚支障なし1,564

表1　視・聴覚自記式アンケート

（1）視覚機能アンケート（4段階）
- 支障なし：日常生活に支障なし（眼鏡使用可）
- 軽度支障あり：新聞の小さな字は読めないが、中等度以上の文字は読める
- 中等度支障あり：新聞は読むことができない
- 重度支障あり：明暗しかわからない

（2）聴覚機能アンケート（4段階）
- 支障なし：日常生活に支障なし（補聴器使用可）
- 軽度支障あり：正常より大声が必要
- 中等度支障あり：耳のそばで大声でゆっくり反復が必要
- 重度支障あり：ほとんど，まったく聞こえない

1）M. Nishinaga, Y. Kazusa, J. Takata, Y. Doi：高知大学医学部老年病・循環器・神経内科学教室
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表2　各群の比較

	視・聴覚ともに問題なし (n = 1,448)	聴覚のみ軽度低下 (n = 130)	視覚のみ軽度低下 (n = 97)	視・聴覚ともに軽度低下 (n = 92)	p
年齢（歳）	74±7	79±7	78±8	80±8	< 0.0001
性（男/女）	634/814	63/67	33/64	39/53	n.s.
Basic ADL（21点満点）	20.5±2.0	20.0±2.4	18.1±4.3	18.1±3.7	< 0.0001
Advanced ADL（12点満点）	10.6±2.2	9.6±2.3	7.4±3.5	6.9±3.5	< 0.0001
MMS（30点満点）	27.0±2.9	27.1±2.5	24.2±5.4	24.6±3.5	< 0.0001
GDS（15点満点）	6.6±3.7	7.0±4.0	8.8±3.8	9.4±3.5	< 0.0001

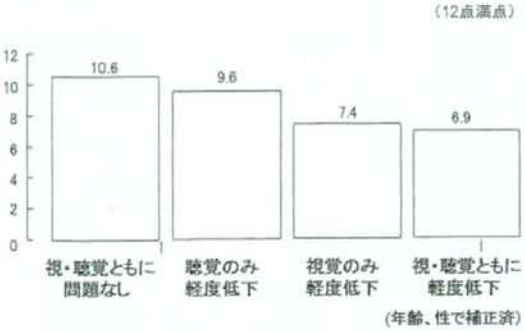

図1　高度ADL（Advanced ADL）

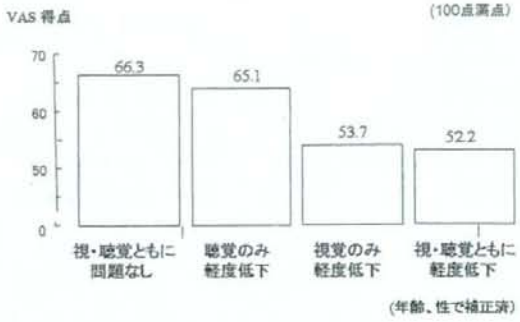

図3　生活満足度（VAS）

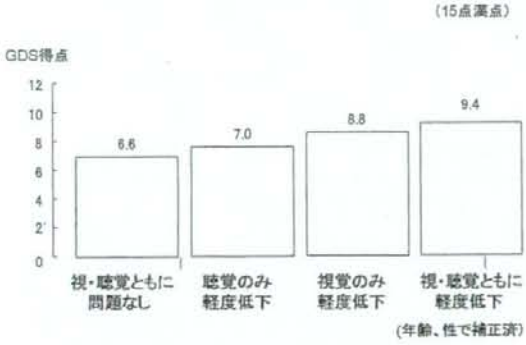

図2　GDS15（Geriatric Depression Scale 15）

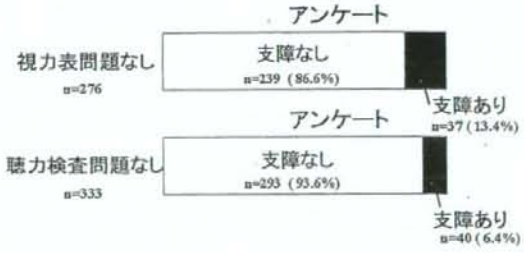

図4　健診時視・聴力検査と自記式アンケートとの関連

(83.5%)，軽度障害 235(12.5%)，中等度障害　43(2.3%)，重度障害 12（0.6%）とした．

(2) 視・聴覚支障なし 1,610 人・1,564 人と軽度低下 210・235 人（重複あり）の 4 群に分けて解析した．表 2 に示すように男女差は両群間になかったが，4 群間で視・聴覚に支障のない群では年齢が低く基本的 ADL や高度 ADL が維持され，うつ尺度は有意にうつ傾向を示し高かった．

(3) 年齢，性を補正しても高度 ADL および生活満足度（VAS）は視・聴覚支障なしでは有意に維持され，うつ尺度（GDS15）も有意に低かった．一方，ごく軽度の低下でも，視・聴覚障害が重なれば低下することが示された（図 1～3）．

(4) 視力表を用いた検査（20/40 以上を異常なし）や耳元でささやく聴覚検査（1 回で聞き取り可能を異常なし）健診時検査とアンケート検査との関連は比較的よく，軽度低下以上の視覚では 85% 以上，聴覚では 90% 以上が一致していた（図 4）．

考　察

高齢者の視・聴覚機能低下の原因の多くは加齢現象として見過ごされているおり，治療可能にもかかわらず，高齢であるからという理由で放置されることも少なくない[4)5)]．今回の検討では，極めて軽度と思われる視・聴覚機能の低下であっても，高次ADL（AADL）をはじめうつ尺度やQOLに与える影響は大きく，地域在住高齢者の生活機能維持に重要であることが明らかになった．平成18年度の介護保険改正により，介護予防に重点が置かれるようになるが，要介護ハイリスクグループ（特定高齢者）の選定に用いられる25項目のチェックリストの中に，視・聴覚機能に関する項目はみられない．主観的であっても，視・聴覚機能の低下は，地域在住高齢者の活動低下と関連し，かつ，以前の報告のように機能的予後との縦断的検討[6)]においても，情報関連機能（視・聴覚機能）の低下を知ることは高齢者の生活機能維持の観点から重要であると考えられる．

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Geriatric syndrome: Slightly reduced visual and hearing impairments reduce activities daily living (ADL) and quality of life (QOL) in the community-dwelling elderly

Masanori Nishinaga[1)], Sunggi Chi[2)], Yuri Kazusa[1)], Jun Takata[1)] and Yoshinori Doi[1)]

Abstract

Although it is well-known that moderate and severe visual and/or hearing impairments in elderly persons reduce their activities of daily living (ADL) and their quality of life (QOL), most elderly people, their caregivers and even nurses/doctors do not care about those disturbances considering them as normal aging. We studied 1,874 commnity-dwelling elderly (813 men, 1,061 women, mean age; 76±9 yrs.) and demonstrated that apparently healthy older persons with slightly reduced function clarified by self-reported questionnaires do not only have lower scores of ADL and VAS (QOL), but also higher score of GDS 15, that is, they have a more depressive state, compared to those without visual and/or hearing impairments. Therefore, because visual and/or hearing functional impairments in the elderly, even if slight, affect their ADL impairments, QOL and mood, we should assess whether the older persons have visual/hearing disturbance(s) in functional screening and should give them some kinds of assistance to succeed in achieving on enjoyable elderly life.

Key words: *Visual disturbance, Hearing loss, Dependence, Quality of life (QOL), Activities of daily living (ADL)*
(Nippon Ronen Igakkai Zasshi 2007; 44: 302–304)

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第48回日本老年医学会学術集会記録
〈ミニレビュー〉

高齢者急性心筋梗塞の治療

高田　淳　西永　正典　土居　義典

要　約　近年，心筋梗塞急性期治療の進歩はめざましく，特に1990年代から最近に至るまで，数多くのRandomized Control Trial（RCT）が行われ，従来の保存的治療に比較して，各種再灌流療法の良好な成績が次々と示された．しかしながら，これらのRCTにおける対象の多くは50歳代後半から60歳代前半が主体であり，近年治療戦略上問題となる70—80歳代の後期高齢者が除外されていたり，対象のごく一部を占めるのみに止まっていた．これに関しては，高齢者では各種再灌流療法施行する上で，重症病変が多く，また治療上問題となる腎機能障害や出血性合併症がより高率であるという背景もあったと考えられる．一方で，RCT以外の大規模登録調査の結果からは，心筋梗塞患者は年々高齢化しており，治療現場における現状とRCTの対象症例との間に解離が認められる．これらを踏まえて，心筋梗塞急性期治療の変遷について，専門施設でのRCTおよび大規模登録調査と，地域の高齢医療圏における登録調査（高知AMI研究会）を比較検討する．

Key words：後期高齢者，急性心筋梗塞，再灌流療法，血栓溶解療法，冠動脈形成術

（日老医誌　2006；43：693–696）

はじめに

高齢者の急性心筋梗塞は，典型的な胸部症状に乏しく受診までに長時間を要し，病初期に診断がつかない症例も少なくない．また冠動脈病変自体も重症多枝病変・石灰化病変が多く，経過中の心不全や機械的合併症も若年者と比べて高率になり，女性の重症例が多くなるのもその特徴である．これらに加えて，腎機能障害や末梢血管病変などの心臓外合併症も多く，従来から特に後期高齢者では院内死亡率が20%を超え，若年者にくらべて著しく予後不良とする報告も多かった．また治療戦略上も中・壮年者と異なり，病前のADLや認知障害の存在，場合によっては単に高齢であることによって積極的治療選択が制限される要因となってきた（図1）．

図1　高齢者急性心筋梗塞治療の問題点

急性期治療における高齢者の位置づけ

心筋梗塞に関して，1980年代から各種血栓溶解剤による急性期再灌流療法の大規模トライアルが相次いで実施された．従来の保存的治療と比較して，血栓溶解療法がより有効であることを示した報告が多い一方で，対象の平均年齢は60歳前後とかなり若く，特に75歳以上の後期高齢者が除外されていたり，含まれていても全体の10%内外と少ない傾向にあった．また，高齢者の多くを占める女性についても全体の20%前後にとどまり，このようなstudy designの傾向は1990年代にはいっても同様であった[1)–4)]．その後，急性期PCI（Percutaneous Coronary Intervention）の普及に伴って，血栓溶解療

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表1　急性心筋梗塞：大規模トライアルと対象年齢

トライアル	ISIS-3	LATE	GUSTO-1	EMERAS	NRMI 3/4
報告年	1992	1993	1993	1993	1998 ― 2002
症例数	41,299	5,711	41,021	4,534	153,486
再灌流療法	SK rt-PA	rt-PA	SK	SK	nPA
年齢	69歳以下 74%	平均63歳	平均62歳	64歳以下 64%	平均68歳
高齢者	70歳以上 26%	76歳以上 10%	75歳以上 12%	75歳以上 10%	65歳以上58% 75歳以上36%
女性	27%	27%	25%	23%	40%

ISIS：International Study of Infarct Survival
LATE：Late Assessment of Thrombolytic Efficacy study
GUSTO：Global Utilization of Streptokinase and t-PA for Occluded Coronary Arteries trial
EMERAS：Estudio Multicentrico Estreptoquinasa Repulicas de America del Sur
NRMI：National Registry of Myocardial Infarction

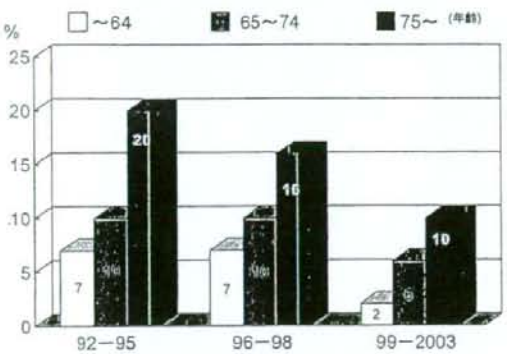

図2　再灌流療法施行例における院内死亡率の変遷
高知AMI研究会登録調査（KAMI registry）より

法とPCIの比較検討が行われるようになったが，対象年齢はやはり60歳代前半であった．これらの背景には，重症多枝病変に加えて合併症も多い後期高齢者を対象に加えることによる治療成績全体の悪化や，治療法選択とは独立した多数の危険因子の存在による解析の困難さが考慮されたとも考えられる．しかしながら，結果的には実際の現場における患者構成とは若干異なった対象に関する治療成績となってしまった感がある．事実，年間20万例以上が登録される米国の大規模登録調査，National Registry of Myocardial Infarction（NRMI）[5]での登録対象例を見ても，大規模トライアルに比べて，より高齢であり，高齢者および女性の頻度もより高率である（表1）．

これらの事実から分かるように，近年の血栓溶解療法ならびに各種PCIによる再灌流療法の進歩と目をみはる治療成績の向上の影で，実際は最も治療成績が悪く，改善が期待されてきた，後期高齢者や高齢女性例での検討が置き去りにされてきたとも言える．

わが国の虚血性心疾患の現状

厚生労働省の人口動態統計によれば，人口の高齢化と心血管疾患リスクの増加に伴って，虚血性心疾患による死亡数は1970年から1995年の25年間でほぼ倍に増加した．その後過去10年間はほぼ横ばいで目だった増加はないが，前述の急性期再灌流療法がわが国でも広く普及したことを考えると，死亡数の上では顕著な効果は現れていないとも言える．これらは疫学的データであり，虚血性心疾患の診断や実際の内容が，臨床データとは必ずしも一致しないという点を考慮する必要はあるが，一方で虚血性心疾患による総死亡数のうち75歳以上の占める割合が全体の60%以上を占めており，今後人口構成が確実に高齢化していくことを考えると，死亡数減少のための治療のターゲットは，中・壮年者に比べて数倍の死亡率を有する後期高齢者であると言える．

これらの社会的背景のもとに，わが国でも近年心筋梗塞急性期治療に関して，施設毎あるいは地域毎の取りくみが進みつつある．再灌流療法の普及およびmodalityの改善に加えて担当医師の技術的な進歩もあって，全体の治療成績は向上しつつある．しかしながら，患者搬送や設備，スタッフなどの点で比較的恵まれた条件の都市部の施設や，専門の多施設研究の結果と，高齢化のより進んだ地域における調査結果には若干の違いが認められる．特に院内死亡率については，専門施設からの報告では2～3%と低率であるが[5]，地域における調査では比較的最近の検討でも7～10%を示し，特に75あるいは80歳以上の検討では20%前後といまだに高率である[7][8]．

これらの背景には，都市部と地方の年齢構成の違いも大きいが，地方における搬送システムやアクセスの状況による搬送時間の問題なども関連する．加えて，循環器医以外の他科専門医や一般内科医との，急性期治療に関する知識の共有，あるいは広く一般への啓発活動も今後の重要な課題である．

地域における心筋梗塞治療の現状

我々は1992年から，急性心筋梗塞治療成績の向上のため，地域におけるネットワーク作りを目指して，高知AMI研究会登録調査を開始し，これまで急性期治療の変遷と，それによる急性期死亡率の改善について報告してきた．現在集計の終わっている1992年から2003年のデータを見ると，全体の年齢は68歳（男性66歳，女性73歳），65歳以上の高齢者が60%以上，75歳以上の高齢者が約30%を占める．また調査期間を通じて徐々に高齢化しており，特に女性は2000～03年の平均年齢は74歳を越えて後期高齢者が半数を占めるようになっている．一方で開始当時50%前後であった，再灌流療法施行率が現在では80%以上となり，再灌流療法の内容も92年当時大部分が血栓溶解療法であったものが，現在はほとんどPCIとなり，特に70～80%にはステントが用いられている．これらの治療法およびmodalityの変化は特に高齢者において顕著であり，有意に死亡率の改善が認められている（図2）．同様の傾向は重症心不全や高齢女性における治療成績にも現れている．若年者と比べると出血性合併症や心破裂などに注意する必要はあるが，PCIを主体とする再灌流療法を，より適切な対象に適切なタイミングで施行することにより，今後更に高齢者治療における有用性が期待できるものと考えられる．

ま と め

時代は，再灌流療法導入初期の血栓溶解療法から，PTCAを経て，各種の改良型deviceならびに再狭窄率の大幅な改善を目指したdrug eluting stent（DES）導入の時代となり，国内の登録調査においてもその治療成績は良好である．しかしながら，比較的長期にわたって強力な抗血小板療法を必要とする点や，晩期にも起こりうるとされる血栓形成の問題などを含め，長期的な不安が必ずしも払拭されたわけではない．今後これらの新しいmodalityについては，後期高齢者や超高齢者に対する治療戦略を考える上からも，注意深く長期的に検討していく必要があると考えられる．

最後に高知AMI研究会登録調査にご協力いただいた，基幹施設（近森会近森病院，高知医療センター，高知赤十字病院，高知県立幡多けんみん病院，高知県立安芸病院）ならびに全参加施設に深謝致します．

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Reperfusion therapy for acute myocardial infarction in elderly patients

Jun Takata, Masanori Nishinaga and Yoshinori Doi

Abstract

Although there has been great progress in reperfusion therapy, the role of coronary reperfusion for elderly patients with acute myocardial infarction has not been fully investigated. In general, mean age of the subjects in major trials was about 60 years old and approximately only 10 to 15% of patients were over age 75. On the other hand, large-scale registries such as the US national registry of myocardial infarction (NRMI) showed a higher prevalence of elderly (especially women) in the clinical setting. This discrepancy may be due to the fact that elderly patients with myocardial infarction have some difficulties in the treatment such as severe multi-vessel coronary lesions, non-cardiac complications and relatively high prevalence of adverse reactions to reperfusion therapy.

Here we focus on the situation of elderly patients (especially those 75 years or older) with myocardial infarction in the "real world" clinical setting, showing the clinical changes and outcome of our registry in rural Japan: the Kochi AMI (KAMI) registry.

Key words: *Old-old, Acute myocardial infarction, Reperfusion therapy, Thrombolysis, Percutaneous coronary intervension*
(Jpn J Geriat 2006; 43: 693–696)

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Adiponectin Antagonizes Stimulatory Effect of Tumor Necrosis Factor-α on Vascular Smooth Muscle Cell Calcification: Regulation of Growth Arrest-Specific Gene 6-Mediated Survival Pathway by Adenosine 5′-Monophosphate-Activated Protein Kinase

AQ: A

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Adiponectin exhibits diverse protective effects against atherogenesis and antagonizes many effects of TNFα. Here, we investigated the effect of adiponectin and TNFα on vascular calcification, a critical event in the development and progression of vascular disease, in human aortic smooth muscle cells (HASMC). TNFα augmented inorganic phosphate (Pi)-induced calcification, whereas adiponectin significantly suppressed it and abolished the stimulatory effect of TNFα in a concentration-dependent manner. Similarly, adiponectin ameliorated the "accelerating" effect of TNFα on Pi-induced apoptosis, the essential process of HASMC calcification. Furthermore, these effects of TNFα and adiponectin were associated with AMP-activated protein kinase (AMPK)-dependent growth arrest-specific gene 6 (Gas6) expression and Akt signaling. The AMPK activator, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), induced phosphorylation of AMPK and significantly inhibited Pi-induced calcification in HASMC. Conversely, pharmacological inhibition of AMPK by compound C blocked both AMPK activation and the inhibitory effect of adiponectin on calcification, providing evidence that AMPK plays a regulatory role in vascular calcification. Reporter assay revealed that adiponectin restored Gas6 promoter activity decreased by TNFα, and the effect of adiponectin was abrogated by compound C. These results demonstrate that adiponectin antagonizes the stimulatory effect of TNFα on vascular calcification by restoration of the AMPK-dependent Gas6-mediated survival pathway. (*Endocrinology* 149: 0000–0000, 2008)

VASCULAR CALCIFICATION IS often encountered in advanced atherosclerotic lesions and is a common consequence of aging (1, 2). Calcification of the coronary arteries has been shown to be positively correlated with atherosclerotic plaque burden, increased risk of myocardial infarction, and plaque instability (3–5). We recently demonstrated that apoptosis plays an important role in inorganic phosphate (Pi)-induced vascular smooth muscle cell (VSMC) calcification (6). This type of calcification is dependent on down-regulation of the growth arrest-specific gene 6 (Gas6)-mediated survival pathway.

Adiponectin is an adipocyte-derived cytokine that exhibits protective properties in the heart and blood vessels (7–10). It accumulates in injured arteries from plasma and suppresses the endothelial inflammatory response (11) and VSMC proliferation (12). Furthermore, low plasma adiponectin levels are associated with progression of coronary artery calcification in type 1 diabetic and nondiabetic subjects, independent of other cardiovascular risk factors (13). Experimental studies have shown that adiponectin reduces TNFα production in response to various stresses, whereas TNFα attenuates adiponectin production, resulting in a reduction of plasma adiponectin levels (14–16). In addition to the inverse relationship between their expression, increasing evidence supports suppressive effects on each other's function (11, 17, 18). Given the importance of the reciprocal effects of TNFα and adiponectin, it is not clear whether both play a regulatory role in VSMC calcification.

Most of the beneficial actions of adiponectin are accounted for by the activation of AMP-activated protein kinase (AMPK) (19, 20). AMPK is a serine/threonine protein kinase that plays a key role in metabolic homeostasis in all eukaryotic cell types (21). Cardioprotective effects of adiponectin, including antiapoptotic actions, are also likely to be dependent on AMPK (19, 22, 23). However, the role of AMPK in the effect of adiponectin on VSMC calcification has not been addressed.

In the present study, we investigated whether adiponectin and TNFα modulate Pi-induced VSMC calcification by regulating apoptosis. We found that TNFα had a stimulatory effect, whereas adiponectin had an inhibitory effect on Pi-induced apoptosis and calcification in human aortic smooth muscle cells (HASMC). Furthermore, these actions were mediated by regulation of Gas6 at the transcription level via AMPK activation.

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Abbreviations: AICAR, 5-Aminoimidazole-4-carboxamide ribonucleoside; AMPK, AMP-activated protein kinase; Gas6, growth arrest-specific gene 6; HASMC, human aortic smooth muscle cells; Pi, inorganic phosphate; PP2C, protein phosphatase 2C; siRNA, small interfering RNA; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; VSMC, vascular smooth muscle cells.

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Materials and Methods

Cell culture

HASMC were purchased from Clonetics Corp. (San Diego, CA). They were cultured in DMEM supplemented with 20% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin at 37 C in a humidified atmosphere with 5% CO_2. HASMC were used up to passage 8 for the experiments.

Induction and quantification of calcification

For Pi-induced calcification, Pi (a mixed solution of Na_2HPO_4 and NaH_2PO_4 whose pH was adjusted to 7.4) was added to serum-supplemented DMEM to a final concentration of 2.6 mM (calcification medium). Ca deposition was evaluated by the *o*-cresolphthalein complexone method (C-Test; WAKO, Osaka, Japan) and von Kossa's staining, as previously described (6, 24).

Determination of apoptosis

To examine the effect of TNFα (Sigma-Aldrich, St. Louis, MO) and adiponectin (R&D Systems, Minneapolis, MN) on Pi-induced apoptosis, they were added simultaneously when the medium was switched to the calcification medium. Apoptosis was detected by DNA fragmentation with a cell-death detection ELISA^{plus} kit (Roche, Manheim, Germany) and terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay with ApopTag Plus obtained from Chemicon International, Ltd. (Hampshire, UK), according to the manufacturer's instructions.

AQ: C

Generation of promoter reporter construct and luciferase activity assay

The 1925-bp Gas6 promoter (−1827/+99) corresponding to the Gas6 promoter sequences was generated by PCR from human genomic DNA with the appropriate sets of primers (6). These inserts were cloned into a pGL3 basic vector (Promega, Charbonnières, France) by standard molecular biological techniques. The constructs were verified by sequencing. HASMC were transiently transfected in 12-well plates with 0.8 μg plasmid DNA and lipofectamine 2000 (Invitrogen Corp., Paisley, UK) according to the procedure recommended by the manufacturer. Cells were treated with TNFα, adiponectin, and compound C at 24 h after transfection, followed by incubation for an additional 44 h. Firefly luciferase activity was determined using a luciferase assay system (Promega) and normalized by total cell protein.

Preparation of small interfering RNA (siRNA) targeting Gas6 and transfection

To evaluate the role of Gas6 in the inhibitory effect of adiponectin on calcification, we knocked down Gas6 using siRNA. Two kinds of siRNA were designed to target human Gas6 and nonspecific control siRNA was synthesized using standard templates (6). siRNA (100 nM) was transfected using transfection reagent (Upstate, Charlottesville, VA) when HASMC had reached 80–90% confluence and then was transfected every 2 d with TNFα and adiponectin up to 6 d. The efficiency of Gas6 siRNA was confirmed with immunoblotting (6).

RNA extraction and Northern blot analysis

Total RNA was extracted from HASMC using an RNeasy minikit (QIAGEN, Courtaboeuf, France). For Northern blot analysis, harvested RNA (5 μg) was fractionated on 1.4% formaldehyde-agarose gel and transferred to a nylon filter. The filter was hybridized at 68 C for 2 h with ^{32}P-labeled Gas6 cDNA (6) and an 18S probe in QuickHyb solution (Stratagene, La Jolla, CA) and autoradiographed.

Immunoblotting

The effect of TNFα and adiponectin on the expression of Gas6, phospho-Akt, and Akt was examined, as described previously (24). Analysis of AMPK activation was performed using an antibody specific for the phosphorylated Thr172 of AMPK (Cell Signaling Technology Inc., Beverly, MA).

Statistical analysis

All results are presented as mean ± SE. Statistical comparisons were made by ANOVA, followed by Bonferroni test. A value of $P < 0.05$ was considered statistically significant.

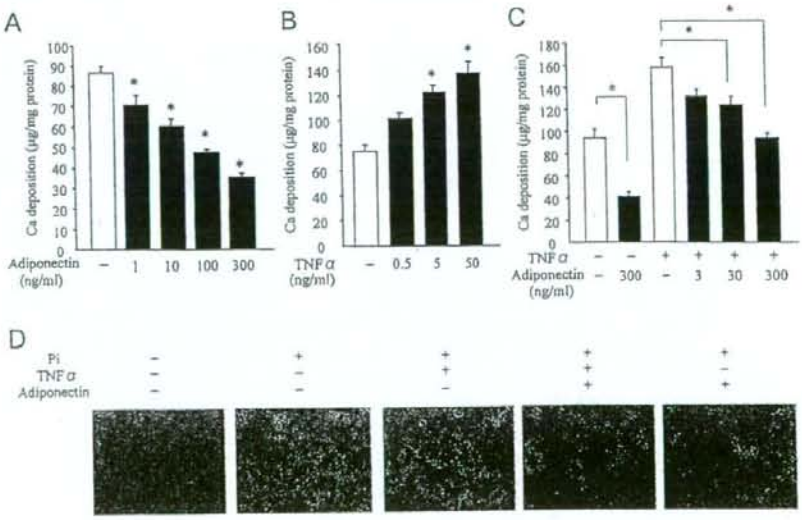

FIG. 1. Effect of adiponectin and TNFα on Pi-induced calcification. A and B, HASMC were cultured with the indicated concentrations of adiponectin (A) or TNFα (B) in calcification medium. They were added simultaneously when the medium was changed every 2 d. C, The effect of TNFα (20 ng/ml) and adiponectin with the indicated concentrations on Ca deposition was determined at 6 d. D, The effect of TNFα (20 ng/ml) and adiponectin (300 ng/ml) on Ca deposition was evaluated with von Kossa's staining at the light microscopic level. All values are presented as mean ± SE (n = 6). *, $P < 0.05$ by Bonferroni test. Each experiment was performed at least in triplicate for each condition.

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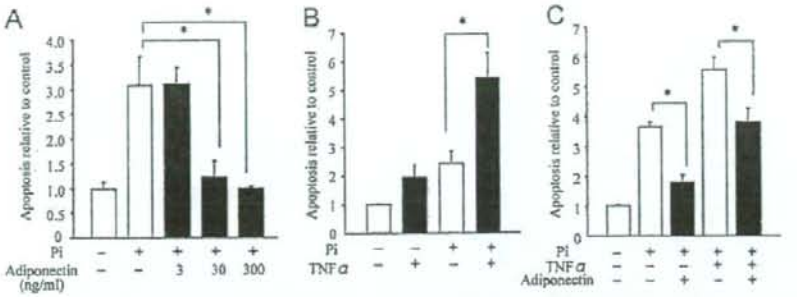

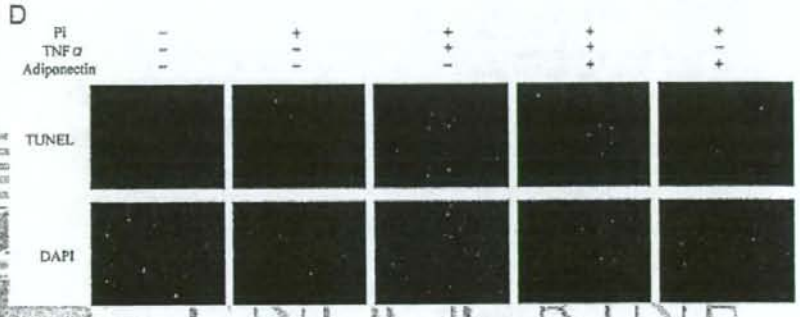

FIG. 2. Effect of adiponectin and TNFα on Pi-induced apoptosis. HASMC were cultured with the indicated concentrations of adiponectin for 6 d. Calcification medium was exchanged every 2 d. A, A quantitative index of apoptosis, determined by ELISA, is presented as the value relative to that without Pi treatment. B, HASMC were incubated with or without TNFα (20 ng/ml) in the absence or presence of 2.6 mM Pi for 6 d. C and D, On d 6, the effect of adiponectin (300 ng/ml) and TNFα (20 ng/ml) on apoptosis in calcification medium was determined by ELISA (C) and evaluated with TUNEL staining (D, *green*). Nuclei were counterstained with DAPI (*blue*). All values are presented as mean ± SE (n = 3). *, $P < 0.05$ by Bonferroni test. Each experiment was performed in triplicate for each condition.

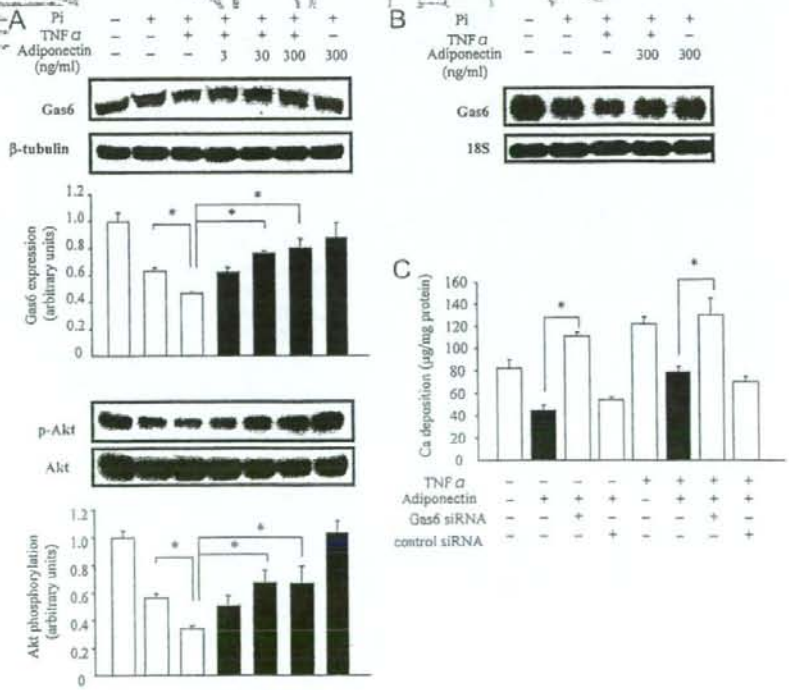

FIG. 3. Gas6 is the target of the effect of adiponectin and TNFα on Pi-induced calcification. HASMC were cultured with the indicated concentrations of adiponectin and TNFα (20 ng/ml). On d 6, cell lysates were collected and immunoblotted with antibodies that recognize Gas6, phospho-Akt (p-Akt), Akt, or β-tubulin. A, The untreated condition is the serum-supplemented status without Pi. B, Total RNA (5 μg) was harvested for Northern blot analysis after HASMC were incubated with adiponectin (300 ng/ml) and TNFα (20 ng/ml) for 6 d. When HASMC had reached 80–90% confluence, siRNA (100 nM) was transfected and then was transfected every 2 d with adiponectin (300 ng/ml) and TNFα (20 ng/ml) up to 6 d. C, Ca deposition was measured and normalized by cell protein content. All values are presented as mean ± SE (n = 3). *, $P < 0.05$ by Bonferroni test. Each experiment was performed in triplicate for each condition.

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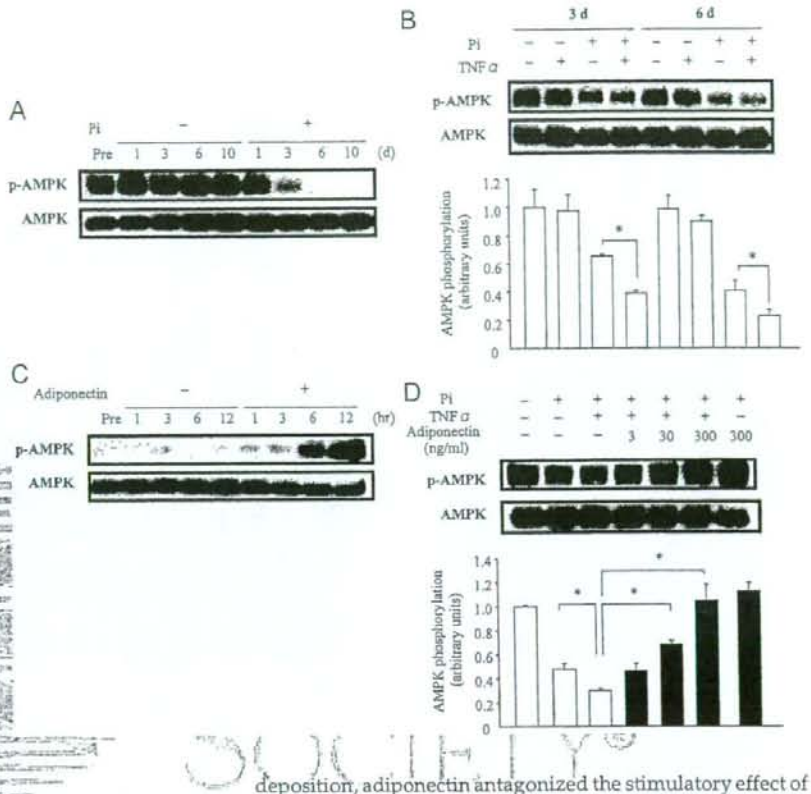

FIG. 4. Effect of adiponectin and TNFα on AMPK activity during Pi-induced calcification. HASMC were cultured in the absence or presence of Pi (2.6 mM) for up to 10 d. After the indicated incubation period, cell lysates were harvested and immunoblotted with antibodies to phospho-AMPK (p-AMPK) and AMPK. A, The untreated condition is the serum-supplemented status without Pi. B, Immunoblotting analysis showing the effect of TNFα (20 ng/ml) on p-AMPK and AMPK expression in the absence or presence of serum containing Pi (2.6 mM). C, Serum-starved HASMC were incubated with or without adiponectin (300 ng/ml) for 12 h. HASMC were cultured with the indicated concentrations of adiponectin and TNFα (20 ng/ml). D, On d 6, cell lysates were harvested and immunoblotted with antibodies to p-AMPK and AMPK. All values are presented as mean ± SE ($n = 3$). *, $P < 0.05$ by Bonferroni test. Each experiment was performed in triplicate for each condition.

Results

Adiponectin and TNFα regulate Pi-induced calcification in HASMC

To investigate the effect of adiponectin and TNFα on Pi-induced calcification, HASMC were incubated with adiponectin and TNFα in the presence of 2.6 mM Pi. On d 6, Ca deposition was suppressed by adiponectin in a concentration-dependent manner (40 ± 2% of control at 300 ng/ml, Fig. 1A), whereas TNFα significantly augmented Ca deposition (182 ± 13% of control at 50 ng/ml, Fig. 1B). Furthermore, adiponectin clearly inhibited Ca deposition stimulated by TNFα in a concentration-dependent manner (Fig. 1C). This was also found by von Kossa's staining (Fig. 1D). These results suggest that adiponectin has an inhibitory effect on both Pi-induced and TNFα-stimulated calcification in HASMC.

F1

Adiponectin antagonizes stimulatory effect of TNFα on Pi-induced apoptosis by restoration of Gas6-mediated survival pathway

Because apoptosis has been shown to be an important pathway regulating Pi-induced calcification (6, 24), we examined the effect of adiponectin and TNFα on apoptosis in HASMC. Adiponectin, at concentrations exerting inhibitory effects on calcification, significantly reduced apoptosis, as quantified by cytoplasmic histone-associated DNA fragments (Fig. 2A). On the other hand, apoptosis was enhanced by TNFα in the presence of Pi (Fig. 2B). As shown in Ca deposition, adiponectin antagonized the stimulatory effect of TNFα on apoptosis. This inhibition was also observed by TUNEL assay (Fig. 2, C and D).

F2

We previously demonstrated that Pi-induced apoptosis was mediated by down-regulation of the Gas6-mediated survival pathway (6, 24). Therefore, we examined the effects of adiponectin and TNFα on this pathway. Both Gas6 mRNA and protein expression were down-regulated by TNFα in the presence of Pi, whereas adiponectin clearly restored their expression (Fig. 3, A and B). Next, because the Gas6-mediated survival pathway is Akt-dependent, the effect of adiponectin and TNFα on Akt phosphorylation was examined. As shown in the Gas6 expression, the similar effect of adiponectin and TNFα was observed in Akt phosphorylation that is high at basal level in the untreated condition containing serum (Fig. 3A). We confirmed that total Akt was not changed by adiponectin and TNFα treatment (Fig. 3A). On the other hand, adiponectin and TNFα did not affect Gas6 expression and Akt phosphorylation in the condition without Pi treatment (data not shown).

F3

Furthermore, to evaluate the role of Gas6 in the inhibitory effect of adiponectin on calcification, we examined whether the knockdown of Gas6 abrogated the effects of adiponectin using siRNA. On d 6, transfection of Gas6 siRNA markedly decreased its expression (data not shown), as reported previously (6). The inhibitory effect of adiponectin on Pi- and TNFα-induced calcification was reversed by Gas6 siRNA, supporting the critical role of Gas6 in the effect of adiponectin on calcification (Fig. 3C).

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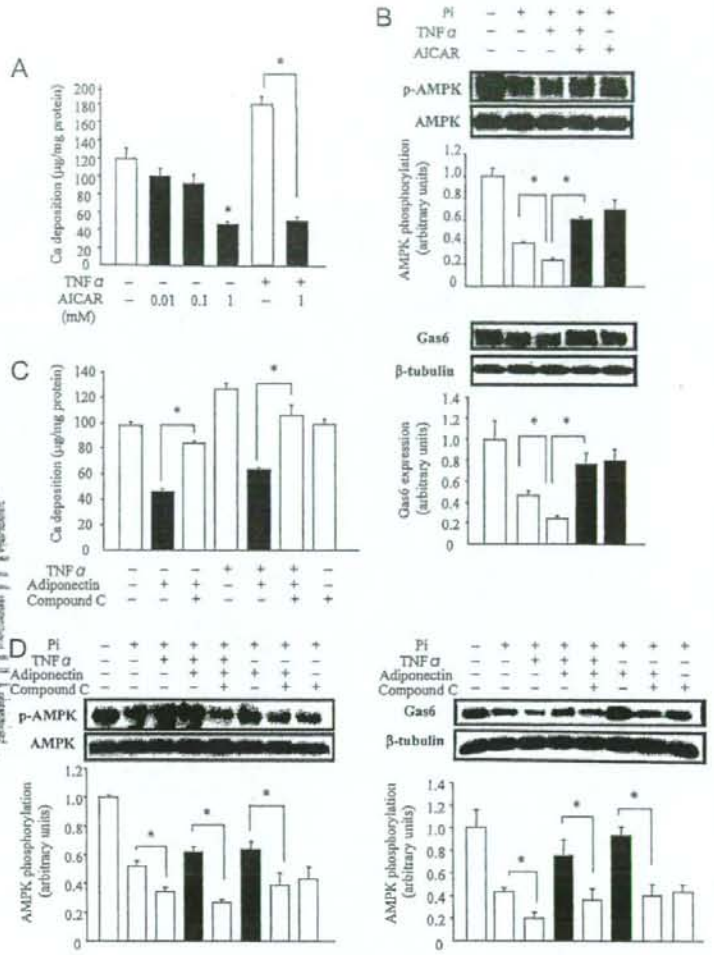

FIG. 5. AMPK plays an important role in Pi-induced calcification. HASMC were treated with or without AICAR (1 mM), a pharmacological activator of AMPK and TNFα (20 ng/ml) in calcification medium for 6 d. A and B, Ca deposition (n = 6) (A) was measured, and immunoblotting with antibodies to p-AMPK, AMPK, Gas6, and β-tubulin (B) was performed (n = 3). HASMC were cultured with or without compound C (1 μM), a chemical inhibitor of AMPK, adiponectin (300 ng/ml), and TNFα (20 ng/ml) in calcification medium for 6 d. C and D, Ca deposition (C) was evaluated (n = 6) and immunoblotting with antibodies to p-AMPK, AMPK, Gas6, and β-tubulin (D) was performed (n = 3). All values are presented as mean ± SE. * $P < 0.05$ by Bonferroni test. Each experiment was performed in triplicate for each condition.

AMPK plays a critical role in VSMC calcification and is regulated by adiponectin and TNFα

It has been reported that AMPK is a central signaling molecule in adiponectin's action (19, 20). We investigated whether AMPK is involved in the effect of adiponectin on Pi-induced calcification. First, we examined the activity of AMPK during calcification. Immunoblot analysis showed that phosphorylated AMPK was markedly down-regulated in the presence of Pi for 10 d, whereas the expression of total AMPK was not changed (Fig. 4A). TNFα further inhibited its phosphorylation in the presence of Pi, without changing total AMPK (Fig. 4B). In the case of adiponectin, AMPK phosphorylation was remarkably stimulated in a time-dependent manner (Fig. 4C). As shown in Fig. 4D, adiponectin further restored AMPK phosphorylation that was inhibited by Pi and TNFα in a calcification-promoting condition.

To clarify the causal relationship between AMPK and calcification, we tried to activate AMPK by treatment with 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) (25). In HASMC, AICAR significantly inhibited Ca deposition in a concentration-dependent manner (Fig. 5A). In addition, TNFα-stimulated Ca deposition was also blunted by AICAR. Interestingly, AICAR restored Gas6 expression down-regulated by Pi and TNFα (Fig. 5B). Next, to investigate whether the effect of adiponectin is dependent on AMPK, we tried to block AMPK using compound C, a chemical inhibitor of AMPK. As shown in Fig. 5C, compound C clearly abrogated the inhibitory effect of adiponectin both on Pi- and TNFα-induced calcification. The increase in Gas6 expression as well as AMPK phosphorylation by adiponectin was also abolished by compound C (Fig. 5D). These results suggest that AMPK regulates Gas6 expression, followed by regulation of Ca deposition in HASMC.