表❸ 検査学的アスピリン抵抗性と心血管系疾患の再発（臨床学的アスピリン抵抗性）との関連，メタアナリシス（Snoep JD *et al* 2007[10]より引用）

研究（対象者数）	検査学的アスピリン抵抗性を示した患者数（%）	検査学的アスピリン抵抗性を示した患者数（%）	検査学的アスピリン抵抗性を示さない患者数*（%）	オッズ比（95%CI）	P値
		心血管疾患イベントを発症した人数			
Grotemeyer *et al*, 1993 (180)	60 (33)	24/60 (40)	5/114 (4)	14.5 (5.2～40.9)	<0.001
Buchanan *et al*, 2000 (289)	158 (55)	15/158 (10)	9/131 (7)	1.4 (0.6～3.4)	0.42
Andersen *et al*, 2002 (71)	25 (35)	9/25 (36)	11/46 (24)	1.8 (0.6～5.2)	0.28
Eikelboom *et al*, 2002 (448 cases, 488 controls)	NR	NR	NR	1.8 (1.2～2.9)	0.01
Grundmann *et al*, 2003 (35 cases, 18 controls)	12 (23)	12/35 (34)	0/18 (0)	6.8 (1.8～26.2)	0.004
Gum *et al*, 2003 (326)	17 (5)	4/17 (24)	30/309 (10)	2.9 (0.9～9.3)	0.09
Cotter *et al*, 2004 (73)	21 (29)	6/21 (29)	3/52 (6)	6.5 (1.5～29.3)	0.01
Cheng *et al*, 2005 (422)	113 (27)	NR	NR	2.9 (1.5～5.7)	0.002
Pamukcu *et al*, 2006 (105)	20 (19)	9/20 (45)	10/85 (12)	6.1 (2.0～18.5)	<0.001
Stejskal *et al*, 2006 (103)	57 (55)	50/57 (88)	21/46 (46)	8.5 (3.2～22.7)	<0.001
Mueller *et al*, 1997 (100)	65 (65)	8/65 (12)	0/35 (0)	10.5 (0.6～187.5)	0.048
Ziegler *et al*, 2002 (52)	5 (10)	0/5 (0)	13/47 (28)	0.2 (0.0～4.5)	0.31
Yilmaz *et al*, 2005 (14 cases, 14 controls)	8 (29)	7/14 (50)	1/14 (7)	13.0 (1.3～128.1)	0.03
Poston *et al*, 2006 (225)	22 (10)	4/22 (18)	12/203 (6)	3.5 (1.0～12.1)	0.06
Chen *et al*, 2004 (151)	29 (19)	15/29 (52)	30/122 (25)	3.3 (1.4～7.6)	0.004
Lev *et al*, 2006 (150)	19 (13)	7/18 (39)	23/126 (18)	2.9 (1.0～8.1)	0.045

＊分子：心血管系疾患イベント発症者数，分母：対象患者数

で求めた検査学的アスピリン抵抗性の頻度は24%（95%信頼区間：20～28%）であり，測定方法，対象疾患，アスピリンの用量で補正した頻度は27%（95%信頼区間：22～33%）であった．また，補正後では，100 mg以下のアスピリン投与でのアスピリン抵抗性の頻度は36%，300 mg以上の投与では19%であった．測定法でもアスピリン抵抗性の頻度に大きな違いがみられており，アラキドン酸惹起血小板凝集能ではその頻度は6%であったものの，PFA-100やRPFAを用いた場合は，それぞれ28%および19%であったと報告されている．

これらの検査学的アスピリン抵抗性が，実際どのような臨床的意義をもつかについても検討がなされている．アスピリンを服薬しているにもかかわらず血栓塞栓症を発症した患者群を臨床的アスピリン抵抗性と定義し，上述した検査学的アスピリン抵抗性が，臨床的アスピリン抵抗性のリスク因子であるとの報告[9][10]がなされている．

表❸に，2007年に発表された検査学的アスピリン抵抗性と心血管系疾患の再発（臨床的アスピリン抵抗性）との関連を検討したメタアナリシスの結果を示す[10]．この解析では16の試験のデータを用いている．これらの結果をまとめると，そのオッズ比は3.8（95%信頼区間：2.3～6.1）であり，アスピリン服薬患者において，血小板の機能が効果的に抑制されていない患者では，効果的に抑制されている患者にくらべ，心血管系疾患の再発率が高いことが示唆される．2008年にも同様のメタアナリシスの結果が報告[11]されている．心筋梗塞，急性冠症候群，脳梗塞，経皮的冠動脈形成術（percutaneous coronary intervention：PCI），冠動脈バイパス術，血管疾患に対して二次予防としてアスピリンを投与された患者に対するアスピリン抵抗性を検討した20試験に対するメタアナリシスであり，アスピリン投与量は75 mg/日から325 mg/日であった．用いられた血小板機能検査でアスピリ

表❹ アスピリン抵抗性のメカニズム
(De Gaetano G *et al*：Aspirin resistance：a revival of platelet aggregation tests? *J Thromb Haemost* 1：2048-2050, 2003より引用)

1．アスピリンのバイオアベイラビリティ
- ●服薬不全，
- ●アスピリン投与量の不足，
- ●salicylate の蓄積によるアスピリンの COX-1 結合部位への結合の妨害
- ●非ステロイド性抗炎症薬の同時服用によるアスピリンの効果の妨害
- ●プロトンポンプ阻害薬によるアスピリンのバイオアベイラビリティの減少

2．血小板機能
- ●血小板の代謝によるアスピリンにさらされていない新しい血小板の血中への出現
- ●新しく形成された血小板中の COX-2 の発現量の違い
- ●ADP とコラーゲンへの血小板感受性の増強

3．遺伝子多型
- ●血小板コラーゲン受容体の遺伝子多型
- ●COX-1，COX-2，TXA_2合成酵素，アラキドン酸代謝酵素の遺伝子多型
- ●血小板フィブリノーゲン受容体 GP II b/III a 遺伝子多型
- 1．低用量アスピリンによる FXIII 活性化の阻害の差異につながる FXIII Val34Leu 遺伝子多型

4．他の血球細胞や細胞由来産物と血小板の相互作用
- ●赤血球による血小板活性化の不十分な阻害
- ●アスピリン処理された血小板と血管細胞の間におこるアラキドン酸代謝の細胞間の移動
- ●単球-マクロファージ由来 TxA_2
- ●血小板 TxA_2の制御として COX-1/COX-2 で生成する PGI_2 もしくは血管の t-PA の放出

5．他の因子
- ●過度の運動や心理的ストレスによるノルエピネフリン量の増加
- ●喫煙
- ●酸化ストレスとアラキドン酸非酵素的な過酸化によって生成する活性をもつ 8-iso-$PGF_{2\alpha}$の生成
- ●アスピリンとアセチルコリンによって生じる一酸化窒素（NO）の抗血小板作用と血管拡張作用の相互作用

ン抵抗性と分類された患者が全体の 28%で，そのなかの 39%で心血管イベントが発生した．一方，アスピリンに対して感受性を示した患者では，心血管イベントの発生率は 16%であったと報告され，アスピリン抵抗性群における心血管イベント発生のオッズ比は，3.85（95%信頼区間：3.08～4.80）であった．

アスピリンは，プロスタグランジンの生合成を阻害することにより薬理学的作用を発揮する．プロスタグランジンの生合成は，ホスホリパーゼ A が膜リン脂質からアラキドン酸を生成することではじまる．このアラキドン酸はプロスタグランジン（PG）H 合成酵素により PGG_2 に変換され，さらに同酵素の過酸化酵素作用により PGH_2となる．この PGH 合成酵素はシクロオキシゲナーゼ（COX）ともよばれ，COX-1 と COX-2 が知られる．アスピリンは，COX-1 の Ser529 をアセチル化し活性中心のコンホメーション変化を起こし，基質であるアラキドン酸への結合が阻害され，その結果 PGH_2産生を抑制する．アスピリンの COX-1 の阻害効果は COX-2 の阻害より約 170 倍強い．このように，アスピリンはトロンボキサン依存性の血小板活性化を抑制する．

アスピリン抵抗性の原因として，血小板活性化経路は，トロンボキサン依存経路だけでなく，カテコールアミン，トロンビン，adenosine diphosphate（ADP）といった刺激による血小板活性化経路など，さまざまにその活性化が制御されているため，トロンボキサン依存性の血小板活性化を抑制しても，血小板活性化経路の一部を抑制しているに過ぎないことが考えられる．それ以外にも，現時点で，さまざまなアスピリン抵抗性のメカニズムが指

摘されている（表❸）.

それらのなかで，健常人や心血管系疾患患者を対象にして，検査学的アスピリン抵抗性と遺伝子多型，とくにCOX-1遺伝子多型との関連についても報告されている[12)~14)]．また，ADP受容体である$P2Y_{12}$の遺伝子多型とアスピリン服用後の残存アラキドン酸惹起血小板凝集能との関連について指摘されている[15)]．

わが国においても，アスピリン抵抗性に関する優れた研究が発表されている[16)]．これによると，アスピリン服薬患者のコラーゲン惹起血小板凝集能を四分位（quartile）に分けると，凝集能が最も残存している群は，他の群より有意にイベント発症が高かった（ハザード比=8）と報告されている．われわれも，脳梗塞/一過性脳虚血発作ならびに急性冠症候群に対する二次予防としてアスピリン投与を受けている患者群を対象に，わが国におけるアスピリン抵抗性の頻度，その予後ならびに遺伝子背景を明らかにするために，多施設共同前向き観察研究「アスピリン抵抗性の実態ならびにその遺伝子背景に関する研究（The Study on Profile and Genetic factors of Aspirin Resistance：ProGEAR study）を実施し，患者登録を完了している．今後，登録後2年間の臨床イベントを追跡し，わが国においてアスピリン抵抗性が臨床的意義をもつのかどうか，もつとしたら，どのような測定系（遺伝子背景を含めて）が臨床的アスピリン抵抗性を最適に反映できるのかどうか，検討をおこなう予定である．

アスピリン抵抗性に対する対応策として，検査学的アスピリン抵抗性を示した患者に対して，他の抗血小板薬に変更することにより予後が改善する可能性がある[17)]．実際このようなコンセプトにもとづいた介入試験[18)]が実施されており，検査学的アスピリン抵抗性を示す患者に対して，他の抗血小板薬に変更することで患者予後が改善することがこのような介入試験で証明されれば，抗血小板療法の個別化が現実味を帯びてくると思われる．

3 クロピドグレル抵抗性

チエノピリジン（Thienopyridine）化合物であるクロピドグレルは，ADP受容体$P2Y_{12}$を特異的に阻害することで，血小板凝集を抑制する．薬物そのものには抗血小板作用はなく，肝臓で代謝され活性化体となってはじめて抗血小板作用を発揮するプロドラッグである．

クロピドグレルについても，その血小板機能抑制効果にかなりの個人差があることが指摘されている[19)20)]．その測定には，古典的な血小板凝集計を用いたADP惹起血小板凝集，ずり応力下での血小板凝集能を測定するPFA-100，フィブリノーゲンが固相化された使い捨てタイプのカートリッジを用いるRPFA，さらに特異的にクロピドグレルによる$P2Y_{12}$受容体抑制効果を測定可能なvasodilator-stimulated phosphoprotein（VASP）のリン酸化を測定する方法などが用いられている．それら検討の結果が多数報告されており，測定方法によって異なるものの，検査学的クロピドグレル抵抗性，すなわちクロピドグレルを投与されているが，血小板機能が効果的に抑制されていない患者の頻度は5%から44%の幅であった[19)20)]．

これら検査学的クロピドグレル抵抗性と心血管系疾患の再発（臨床的クロピドグレル抵抗性）との関連を検討した研究についても近年，その報告が増加している[19)20)]．待機的PCIをおこなった379症例について，クロピドグレル導入後，70%を超えるADP惹起血小板凝集が残存している患者群では，70%以下に抑制されている患者群と比較して，PCI実施3ヵ月後までの心血管イベントが有意（オッズ比：4.9，95%信頼区間：1.66~14.96）に多く，心血管イベントに関係している因子に対して調整をおこなった後も，クロピドグレルに対する低反応性は，独立した危険因子であったと報告[21)]されている．急性ST上昇型心筋梗塞でプライマリPCIを予定している連続60症例について検討（全員アスピリン投与継続され，クロピドグレルは3ヵ月間服薬された）した報告[22)]がなされている．ADP凝集の結果で患者群を四分位（quartile）に分けた（1群：103%±8%，2群：69%±3%，3群：58%±7%，4群：33%±12%）．6ヵ月のフォローアップ期間中に7名が心血管イベントを発症し，そのうち6名は1群（クロピドグレル抵抗性群）に属し，残り1名は2群に属した．3群ならびに4群には発症者はいなかった（$p=0.007$）．非ST上昇型急性冠症候群でPCIをおこなった292症例において，クロピドグレル導入後において70%を超えるADP惹起血小板凝集が残存していることが，1ヵ月後までの心血管イベントに対し，唯一独立したリスク因子であったと報告[23)]されている．さらに，クロピドグレル抵抗性に対し，治療介入することで患者予後に改善がみられるかどうかに関する多施設共

同ランダム化比較試験が報告[24]されている．PCIを実施する406症例のなかで，クロピドグレル600 mgのloading doseで投与後，VASP指数を測定し，50%を超える（すなわちクロピドグレルによる$P2Y_{12}$受容体抑制効果が弱い）患者162症例を無作為に割り付け，VASP-guided群（78症例）は，VASP指数が50%以下に低下するまで，最大3回24時間ごとにクロピドグレル600 mgが追加投与され，その後PCIが実施された．コントロール群（84症例）は，クロピドグレルの追加投与なしにPCIが実施された．その結果，PCI後1ヵ月までのフォローアップ期間に，心血管イベントは有意にVASP-guided群で，コントロール群と比較して少なかった（0% vs 10%，p=0.007）．これらの報告は，症例数が少ない検討であり，今後更なるデータの蓄積が望まれる．

現時点で指摘されているクロピドグレル抵抗性のメカニズムを表❺に示す．これらの原因のなかで，クロピドグレル抵抗性に対する遺伝子多型の関与について検討した結果も報告されている．

健常人にクロピドグレルを服用させ，ADP惹起血小板凝集やVASPのリン酸化といった指標を用いてクロピドグレルの効果を評価し，これに関する個人差を説明する遺伝子多型が，候補遺伝子を対象に探索された．ADP凝集が十分に抑制されない遺伝的背景として，ADP受容体である*P2Y$_{12}$*，*P2Y$_1$*，血小板膜受容体*GP Ia*，プロドラッグであるクロピドグレルの活性化体への変換に関与するCytochrome P450である*CYP3A4*，*CYP3A5*，*CYP2C19*の多型が報告された．これらはいずれも小規模な研究であったので，他の集団を用いて確認する必要がある．そこで，クロピドグレルとアスピリンを併用した1,419名の心筋梗塞患者を対象にADP凝集能とアラキドン酸凝集能を測定し，*CYP3A4*，*CYP2C19*，*P2Y$_{12}$*の多型を調べた研究がイタリアから報告された[25]．この三つの遺伝子のなかで*CYP2C19*の多型のみがADP凝集に強く関連を示した．この多型は，スプライス異常を生じる681 G>Aであり，*CYP2C19*2*として知られている．本変異のAアレル保有者はADP凝集能が高く，その凝集能はアレル数に依存していた．また，この効果は弱いながらアラキドン酸凝集能にも関連を示した．以前の報告で関連がみられた*CYP3A4*と*P2Y$_{12}$*の多型は，本研究ではADP凝集能と関連を示さなかった．フランスの報告では，*CYP3A4*，*CYP3A5*，*CYP2C19*の多型を検討し，

表❺ クロピドグレル抵抗性のメカニズム
（De Miguel A *et al* 2008[20]より引用）

1．クロピドグレルのバイオアベイラビリティ
- ●服薬不全，
- ●クロピドグレル投与量の不足，
- ●吸収不全
- ●Cytochrome P450 3Asに対する薬剤相互作用

2．遺伝子多型
- ●$P2Y_{12}$遺伝子多型
- ●Cytochrome P450 3Asの遺伝子多型
- ●GP Ⅰaの遺伝子多型
- 2．GP Ⅱb/Ⅲaの遺伝子多型

3．ADP放出の増加
4．$P2Y_{12}$受容体の増加
5．治療前の血小板活性化の病態
- ●急性冠症候群
- ●糖尿病/インスリン抵抗性
- ●高いbody mass index

6．$P2Y_{12}$に依存しない血小板刺激伝達系の亢進
7．P2Yに依存しない血小板刺激伝達系の亢進
- ●トロンビン
- ●トロンボキサンA_2
- ●コラーゲン
- ●エピネフリン

8．血小板turn-overの亢進

この研究でも*CYP2C19*の多型だけが関連を示す結果となった[26]．この研究では，クロピドグレルとアスピリンの併用療法をおこなっている急性冠症候群患者603名を対象にADP凝集，VASPのリン酸化，血小板表面へのPセレクチンの発現を調べた．*CYP2C19*2*はこれらいずれの測定値に対しても強い関連を示し，*CYP2C19*2*変異保有者ではADP惹起血小板凝集能が残存する結果となった．これらの報告を総合すると，クロピドグレル服用者の抗血小板作用のモニターとしてADP惹起血小板凝集を用いてクロピドグレル抵抗性を評価すると，*CYP2C19*多型がクロピドグレルの効果に関連を示す．クロピドグレルはプロドラッグであり，おもにCYP2C19を通して活性化体へ変換され，活性化体が$P2Y_{12}$のCys97を修飾し，$P2Y_{12}$が膜脂質ラフトから離れることによりADP凝集が抑制される[27]．このように，*CYP2C19*がクロピドグレルの活性化体への変換反応に大きくかかわっている可能性が考えられた．

日本人を対象としたクロピドグレルの効果と遺伝子多型に関する研究はこれまでのところみつからない．*CYP2C19*の多型頻度に関しては，日本人を対象に詳しく

表❻ 日本人の *CYP2C19* 遺伝型のアレル頻度（国立食品薬品衛生研究所でタイピングした 253 人から得られた頻度）

クロピドグレルへの反応性			よい		悪い
	CYP2C19	*CYP2C19* *1*2	*1/*1	*1/*2	*2/*2
クロピドグレルへの反応性	*CYP2C19* *1*3	genotype frequency	0.5376	0.3912	0.0712
よい	*1/*1	0.7596	0.41	0.30	0.05
	*1/*3	0.2239	0.12	0.09	0.02
悪い	*3/*3	0.0165	0.01	0.01	0.00

*CYP2C19*2、*3は酵素ができないアレル		CYP2C19	*1/*1	*1/*2	*2/*2
	白人での頻度		0.71	0.27	0.03

日本人はクロピドグレルを活性化体へ変換する CYP2C19 量が少ないヒトが多い．

調べられており，多くの論文が発表されている．なかでも，国立医薬品食品衛生研究所がミレニアムゲノムプロジェクトの一環としておこなった多型頻度に関する研究は，日本人 253 名を対象とし，ハプロタイプの構築までおこなっており，日本人の *CYP2C19* の変異研究の集大成である[28]．これによると *CYP2C19**2 のアレル頻度は 0.267，**3* のアレル頻度は 0.128 である．**2* は先ほども紹介したようにスプライス異常変異であり，**3* は停止コドンとなるナンセンス変異である．ともに，蛋白質に重大な影響を与える．両変異の頻度をもとに計算すると，日本人の約 40%しか *CYP2C19* の野生型をもたないことが理解されよう（表❻）．日本人の約 1 割は，*CYP2C19* をまったく発現しない**2* のホモ体もしくは**3* のホモ体である．白人では *CYP2C19**2 のアレル頻度は 0.16 であり**3* は存在しない．したがって，日本人は *CYP2C19* を欠損する遺伝型を高頻度に保有すると理解されている．

おわりに

ここまで，抗血栓薬の抵抗性について，テーラーメイド（個別化）医療実施の可能性の観点から述べてきたが，これら薬剤抵抗性に関しては，いまだ臨床的に受け入れられているとはいいがたい[20)29)]．その大きな理由として，*in vivo* における血小板機能を鋭敏に反映する *in vitro* もしくは *ex vivo* 血小板機能測定系はいまだ確立されておらず，普遍的に認められた診断上のカットオフ値の設定もできないため，これら薬剤抵抗性の診断基準が確立できていないことが指摘されている．また，抗血栓薬に限らず，どんな薬剤でもすべての患者に有効であるわけはない．したがって，薬剤抵抗性と定義せずに "treatment failure"（治療不成功）とすべきで，特別な疾患として分類する必要はないとも指摘されている．さらに，薬剤服薬のコンプライアンスを確認する必要があること，また，血栓塞栓症は，血小板機能のみに依存するのではなく，さまざまな要因に依存すること，抗血小板薬は血小板凝集にかかわる刺激伝達系のたった一つの経路を抑えるに過ぎないことなどの理由で，抗血小板薬に過大な期待をもつこと自体が誤りであることも指摘されている．したがって，現時点では血小板機能を測定して，その結果をもって抗血小板療法に介入することを臨床現場でおこなうべきではないことを，強調しておきたい．今後，われわれが実施している研究も含めて，更なる検討が進められ，今後，個別化医療により，さらに有効で安全な抗血栓療法が確立されることを期待したい．

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みやた・しげき

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1961年，奈良県生まれ．
奈良県立医科大学小児科入局，同助手．1992年から4年間The Scripps Research Institute（CA，USA）に留学．2000年から現職．研究テーマは，流動状況下（ずり応力下）血小板血栓形成メカニズム，アスピリンレジスタンス，人工心肺使用手術周術期の血小板機能低下のメカニズム，ヘパリン起因性血小板減少症（HIT）など．

Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials

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Summary

Background Patients who have acute myocardial infarction remain at major risk of cardiovascular events. We aimed to assess the effects of either human atrial natriuretic peptide or nicorandil on infarct size and cardiovascular outcome.

Methods We enrolled 1216 patients who had acute myocardial infarction and were undergoing reperfusion treatment in two prospective, single-blind trials at 65 hospitals in Japan. We randomly assigned 277 patients to receive intravenous atrial natriuretic peptide (0·025 μg/kg per min for 3 days) and 292 the same dose of placebo. 276 patients were assigned to receive intravenous nicorandil (0·067 mg/kg as a bolus, followed by 1·67 μg/kg per min as a 24-h continuous infusion), and 269 the same dose of placebo. Median follow-up was 2·7 (IQR 1·5–3·6) years for patients in the atrial natriuretic peptide trial and 2·5 (1·5–3·7) years for those in the nicorandil trial. Primary endpoints were infarct size (estimated from creatine kinase) and left ventricular ejection fraction (gauged by angiography of the left ventricle).

Findings 43 patients withdrew consent after randomisation, and 59 did not have acute myocardial infarction. We did not assess infarct size in 50 patients for whom we had fewer than six samples of blood. We did not have angiographs of left ventricles in 383 patients. Total creatine kinase was 66459·9 IU/mL per h in patients given atrial natriuretic peptide, compared with 77878·9 IU/mL per h in controls, with a ratio of 0·85 between these groups (95% CI 0·75–0·97, p=0·016), which indicated a reduction of 14·7% in infarct size (95% CI 3·0–24·9%). The left ventricular ejection fraction at 6–12 months increased in the atrial natriuretic peptide group (ratio 1·05, 95% CI 1·01–1·10, p=0·024). Total activity of creatine kinase did not differ between patients given nicorandil (70520·5 IU/mL per h) and controls (70852·7 IU/mL per h) (ratio 0·995, 95% CI 0·878–1·138, p=0·94). Intravenous nicorandil did not affect the size of the left ventricular ejection fraction, although oral administration of nicorandil during follow-up increased the left ventricular ejection fraction between the chronic and acute phases. 29 patients in the atrial natriuretic peptide group had severe hypotension, compared with one in the corresponding placebo group.

Interpretation Patients with acute myocardial infarction who were given atrial natriuretic peptide had lower infarct size, fewer reperfusion injuries, and better outcomes than controls. We believe that atrial natriuretic peptide could be a safe and effective adjunctive treatment in patients with acute myocardial infarction who receive percutaneous coronary intervention.

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Introduction

Despite availability of effective medical treatments, chronic heart failure remains a major cause of morbidity and mortality worldwide.[1–3] Ischaemic heart disease, in turn, is one of the main causes of chronic heart failure.[4] The most important treatment objectives are prevention of acute myocardial infarction, and, in individuals who have an acute myocardial infarction, reduction in infarct size and ischaemia or reperfusion injury.[5] Only a few medications have been shown to decrease ischaemia or reperfusion injury.[6–8]

Reperfusion of ischaemic myocardium reduces infarct size and improves left ventricular function, both of which contribute to better clinical outcomes in patients with acute myocardial infarction.[9–11] However, reperfusion can also cause tissue damage.[12] Several drugs have been trialled for the prevention or amelioration of such injuries, but results have not been consistently satisfactory.[13–15] Recently, human atrial natriuretic peptide and nicorandil have both been shown to be effective for reduction of myocardial damage after acute myocardial infarction in basic and clinical studies.[16–23] Atrial natriuretic peptide is a candidate for adjunctive treatment after acute myocardial infarction, because it has been shown to suppress the renin–angiotensin–aldosterone system and endothelin-1, both of which which modulate infarct size and cardiac remodelling.[19] Nicorandil is a combined adenosine triphosphate (ATP)-sensitive potassium channel opener and nitrate preparation that has also shown promise as an adjunctive treatment for acute myocardial infarction. In the clinical setting, however,

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the beneficial effects of atrial natriuretic peptide and nicorandil have only been tested in single-centre studies with small sample sizes.[20–25] The Japan working group studies on acute myocardial infarction for the reduction of necrotic damage by human atrial natriuretic peptide or nicorandil (J-WIND-ANP and J-WIND-KATP, respectively) aimed to assess the value of these drugs as adjuncts to percutaneous coronary intervention for patients with acute myocardial infarction.

Methods

Patients

We have described the protocols for the two trials previously.[26,27] In brief, we recruited patients to two independent, investigator-initiated, investigator-led, multicentre, prospective, randomised, single-blind, controlled trials at 65 hospitals. 27 hospitals participated in the atrial natriuretic peptide trial, and 38 separate hospitals in the nicorandil trial (table 1); the two studies were completely independent. We initially planned to include fewer hospitals, but we increased the number to promote enrolment of sufficient patients.

Eligibility criteria were age between 20 and 79 years; chest pain for more than 30 min; at least 0·1 mV of ST segment elevation in two adjacent ECG leads; admission to hospital within 12 h of the onset of symptoms; and one instance of acute myocardial infarction. Exclusion criteria were a history of myocardial infarction; left main trunk stenosis; severe liver or kidney dysfunction or both; suspected aortic dissection; previous coronary artery bypass grafting; and a history of drug allergy.

All patients gave written informed consent immediately after admission to hospital, and were asked to sign the same consent form again after 2 weeks when they had more time to decide. This system was applied on the recommendation of the institutional review boards. Only one patient, who was in the nicorandil group, withdrew their consent at their second opportunity. We enrolled patients from Oct 24, 2001, to Dec 13, 2005. The study protocol was approved by the institutional review boards and ethics committees of all participating hospitals, and was in accordance with the Declaration of Helsinki.

Procedures

An independent statistician generated our randomisation lists with a computer, by the permuted-block method. Within each centre, the block length was eight. Treatment allocations were concealed in opaque sealed envelopes until patients were enrolled. Physicians were not aware of the random assignments of patients until the follow-up stage; patients and those who analysed the data were unaware of the treatment assignment for the duration of the study. Both trials were designed as single-blind studies.

277 patients who were enrolled in the atrial natriuretic peptide trial were randomly assigned to receive an intravenous infusion of this drug after reperfusion treatment, at 0·025 μg/kg per min for 3 days, and 292 a placebo of 5% glucose solution by the same method. 276 patients in the other trial were randomly assigned to intravenous nicorandil, infused at 1·67 μg/kg per min for 24 h after bolus injection of nicorandil at a dose of 0·067 mg/kg, and 269 were assigned to 0·9% saline solution, by the same method. Previous studies have shown substantial cardiovascular protection with atrial natriuretic peptide and nicorandil at these doses.[20,22] Of the 276 patients assigned to receive nicorandil, 61 were given nicorandil orally, at the discretion of individual investigators, during the follow-up period.

We planned to stop the administration of treatment drugs in case of severe hypotension, which was defined as systolic blood pressure of less than 90 mm Hg, because of the vasodilator effect of these drugs. The study protocol did not restrict or specify any other diagnostic or therapeutic methods in the acute phase (2–8 weeks after acute myocardial infarction) or chronic phase (6–12 months).

We obtained data on baseline characteristics, emergent catheterisation, and medication at discharge after 1 month; data on follow-up catheterisation and medication after 6 months; and data on medication after 24 months. We also followed up all patients for cardiovascular events (ie, cardiac death, readmission to hospital due to heart failure, new onset of acute coronary syndrome, or revascularisation of new lesions) until the end of August, 2006. We took blood samples to measure concentrations of creatine kinase at a central laboratory, before the procedure and at 1, 3, 6, 9, 12, 18, 24, 36, 48, and 72 h after the onset of reperfusion.[14] We analysed total creatine kinase for all patients with at least six blood samples. We obtained right anterior oblique views with angiography of the left ventricle once in the acute phase (2–8 weeks), and once in the chronic phase (6–12 months).

Our primary endpoints were infarct size (which was estimated as the area under the concentration versus time curve for creatine kinase)[14] and ventricular ejection fraction (which was assessed by angiography of the left ventricle at 6–12 months after hospital admission).[15] The prespecified secondary endpoints were survival rate; cardiovascular events (such as cardiac death, readmission to hospital for heart failure, new onset of acute coronary syndrome, or revascularisation of new lesions); incidence of cardiac death or readmission to hospital for

	J-WIND-ANP study	J-WIND-KATP study
1–4 patients	7 hospitals	9 hospitals
5–9 patients	3 hospitals	13 hospitals
10–19 patients	7 hospitals	6 hospitals
More than 20 patients	10 hospitals	10 hospitals

Table 1: Distribution of patients between participating hospitals

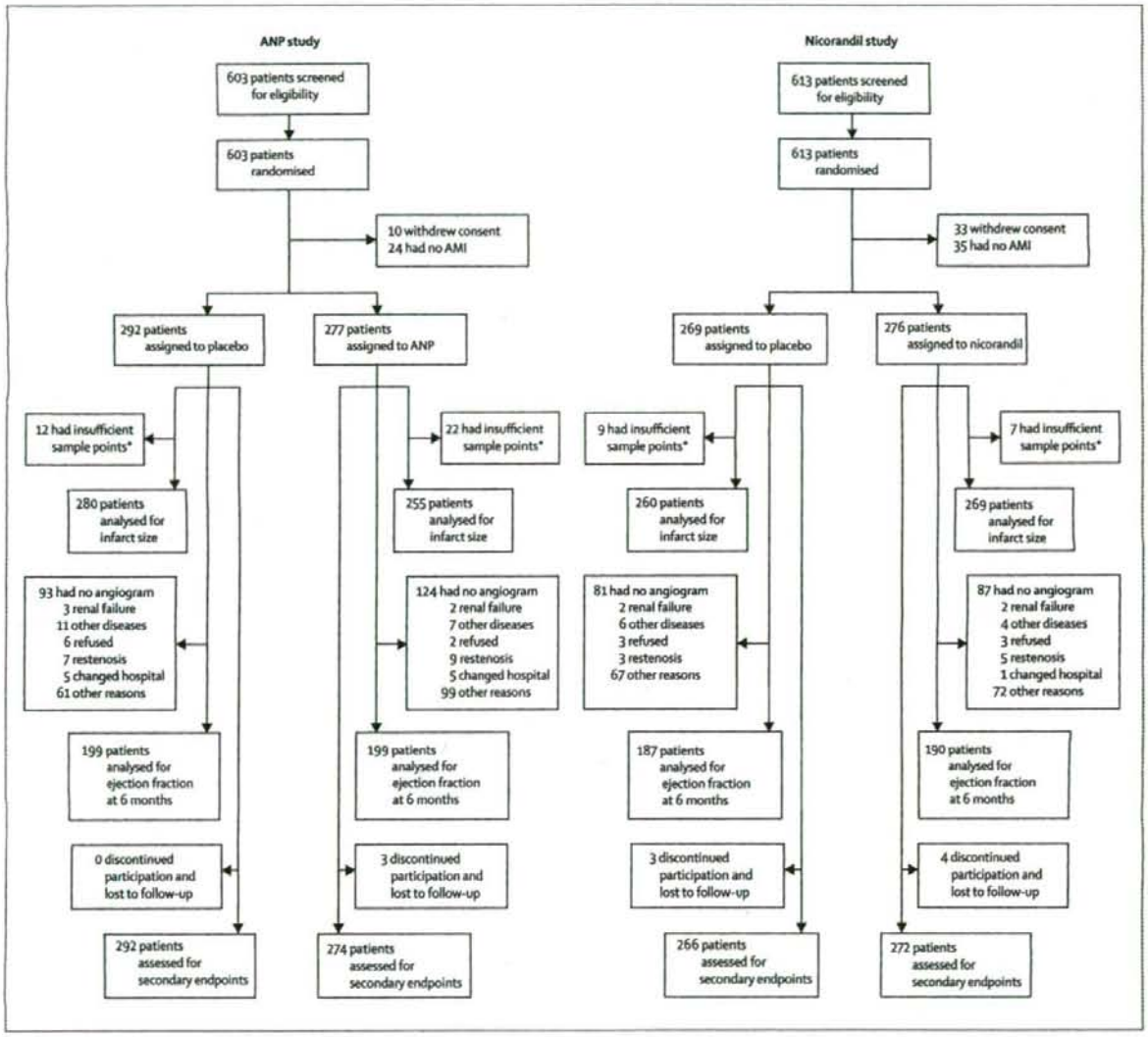

Figure 1: **Trial profiles**
ANP=atrial natriuretic peptide. AMI=acute myocardial infarction. *Fewer than six blood samples.

heart failure; or reperfusion injury before discharge from coronary care unit (such as malignant ventricular arrhythmia during reperfusion, recurrence of ST segment elevation, or worsening of chest pain). We also assessed infarct size, estimated by peak creatine kinase and troponin T;[28,29] left ventricular ejection fraction at acute phase; and end-diastolic or end-systolic volume index (assessed by angiography of the left ventricle). We looked at the effects of each drug on the primary endpoints in prespecified subgroups (sex, age, body-mass index, pre-angina, elapsed time between acute myocardial infarction and intervention, diabetes mellitus, hyperlipidaemia, smoking, and family history of acute myocardial infarction). We also did post-hoc analyses on the effect of chronic administration of nicorandil on the ejection fraction.

All data were collected by Koteisho-kyokai (Tokyo), an organisation established by the Japanese government in 2001–2003 and by NTT Data (Tokyo) in 2004–2006. Left ventricular ejection fraction and end-diastolic volume were measured by the area–length method, from angiography of the left ventricle. Two independent

interpreters, who were unaware of the treatment assigned to patients, measured left ventricular ejection fractions from the angiographs. We calculated the average value, unless the two investigators disagreed, in which case we referred to a third opinion.

Clinical findings and medications during the follow-up period were reported to a data and safety committee after registration. This committee, which consisted of three physicians and one statistician who did not participate in the trial, monitored all adverse events. Research nurses or doctors visited all participating hospitals to check that patients were registered, drugs were given, and data collected according to the protocol. Committee members did not provide any results to the steering committee, because discontinuation of the study was not recommended.

Statistical analysis

We calculated that a sample size of 300 patients would be needed in each group to detect a 20% reduction in the most important primary endpoint (total creatine kinase) with a statistical power of 80% at significance level of 0·05 (with a two-sided t test), accounting for dropout of some patients. We set equal sample sizes in both groups, because we expected to see almost the same reduction in infarct size with either treatment. Since creatine kinase and total creatine kinase are both log-normally distributed,[30] total creatine kinase was log-transformed before analysis. The left ventricular ejection fraction was also log-transformed before the analysis since the distribution was skewed.

Statistical analysis was done according to a prespecified analytical plan. Efficacy analysis was based on intention to treat. The primary efficacy analyses for total creatine kinase and left ventricular ejection fraction were done simply by t test. The estimated mean and differences on the log scale were transformed back to the original scale and were expressed as geometric means and ratios of geometric mean. If the calculated 95% CI for the ratio of the geometric mean did not cross the point of no effect (ie, 1) the difference between groups was regarded as significant. Furthermore, analysis of covariance for the two endpoints was used to estimate adjusted mean comparison, with effect of covariates and the interactions. We imputed missing data for patients by the predicted mean imputation method, with nonlinear regression. We applied multiple imputation techniques (with group means, Markov Chain Monte Carlo, Bayesian bootstrap, and last-observation-carried-forward methods) to assess the robustness and sensitivity of our conclusions.

Proportions were examined by Fisher's exact test. We examined time-to-event by the Kaplan-Meier method to estimate the survival for each group and then the differences in survival between groups by the log-rank test. The Cox proportional hazards model was used to assess baseline risk factors and an adjusted hazard ratio. The proportional hazards assumption was investigated graphically, with a test based on Schoenfeld residuals.[31,32]

All tests were two-sided, and a p value of less than 0·05 was regarded as significant. All analyses were done with SAS software (version 8.2). The trials are registered with Clinicaltrials.gov, numbers NCT00212056 and NCT00212030.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data at the end of the study, and had final responsibility for the decision to submit for publication.

Results

Figure 1 shows the trial profile. Table 2 shows baseline characteristics. Median follow-up was 2·7 (IQR 1·5–3·6) years in the atrial natriuretic peptide trial and 2·5 (1·5–3·7) years in the nicorandil trial. Table 3 shows

	Atrial natriuretic peptide study			Nicorandil study		
	ANP (n=277)	Control (n=292)	p	Nicorandil (n=276)	Control (n=269)	p
Age (years)	63·0 (10·4)	61·8 (10·7)	0·1652	61·1 (11·4)	63·7 (10·2)	0·0035
Sex (male)	211 (76·2%)	243 (83·2%)	0·0374	246 (89·1%)	220 (81·8%)	0·0153
Body-mass index	24·3 (3·5)	24·0 (2·9)	0·3733	24·2 (3·0)	23·4 (2·8)	0·0007
Killip classification (I, II, III, IV)	88·6%, 9·5%, 1·1%, 0·8%	90·3%, 7·5%, 1·4%, 0·7%	0·5274	91·1%, 8·2%, 0·4%, 0·4%	92·0%, 4·2%, 2·7%, 1·1%	0·7843
Pre-angina	105 (44·5%)	118 (46·1%)	0·7862	111 (44·6%)	111 (43·9%)	0·9284
Risk factors						
Hypertension	137 (56·1%)	162 (62·1%)	0·2046	127 (48·5%)	137 (53·9%)	0·2190
Diabetes mellitus	81 (33·8%)	86 (33·9%)	1·0000	104 (39·5%)	82 (32·9%)	0·1413
Hyperlipidaemia	127 (54·3%)	131 (50·6%)	0·4181	121 (46·7%)	114 (46·2%)	0·9291
Smoking	158 (63·7%)	175 (67·3%)	0·4022	178 (68·7%)	170 (66·1%)	0·5732

Data are number (%) or mean (SD), unless otherwise specified. ANP=atrial natriuretic peptide.

Table 2: Baseline characteristics on admission

	Atrial natriuretic peptide study		Nicorandil study	
	ANP (n=277)	Control (n=292)	Nicorandil (n=276)	Control (n=269)
Elapsed time (h)*	4·00 (3·00–6·00)	4·00 (2·50–6·00)	3·50 (2·50–5·00)	3·50 (2·50–5·00)
Infusion time (h)	1·00 (0·50–1·00)	1·00 (0·50–1·00)	0·70 (0·50–1·00)	0·75 (0·50–1·00)
IRA (LAD, LCx, RCA)	55·3%, 6·4%, 38·3%	52·3, 10·6, 37·1%	53·9, 7·4, 38·7%	44·5, 9·9, 45·6%
Stents	176 (63·5%)	193 (66·1%)	187 (67·8%)	183 (68·0%)
Rescue	64 (23·1%)	92 (31·5%)	94 (34·1%)	92 (34·2%)
Intra-aortic balloon pump	17 (6·1%)	14 (4·8%)	14 (5·1%)	15 (5·6%)
Final stenosis (<75%)	246 (93·5%)	266 (94·7%)	257 (96·6%)	255 (97·0%)
Final thrombolysis in myocardial infarction (0, 1, 2, 3)	3·9%, 1·9%, 5·0%, 89·1%	5·2%, 0·7%, 4·1%, 90·0%	3·7%, 0·7%, 5·2%, 90·3%	3·4%, 1·1%, 6·9%, 88·5%
Medications at 1 month				
ACE inhibitor	155 (57·8%)	173 (60·7%)	164 (61·0%)	163 (62·0%)
ARB	77 (28·7%)	99 (34·7%)	72 (26·8%)	69 (26·2%)
Spinorolactone	28 (10·4%)	33 (11·6%)	17 (6·3%)	22 (8·4%)
β blocker	112 (41·8%)	128 (44·9%)	110 (40·9%)	121 (46·0%)
Aspirin	225 (84·0%)	252 (88·4%)	251 (93·3%)	250 (95·1%)
Nitrates	81 (30·2%)	86 (30·2%)	50 (18·6%)	63 (24·0%)
Statins	129 (48·1%)	156 (54·7%)	126 (46·8%)	115 (43·7%)
Nicorandil	62 (23·1%)	52 (18·2%)	79 (29·4%)	34 (12·9%)
Medications at 6 months				
ACE inhibitor	103 (48·1%)	117 (44·8%)	120 (50·6%)	131 (53·9%)
ARB	69 (32·2%)	110 (42·1%)	68 (28·7%)	75 (30·9%)
Spinorolactone	26 (12·1%)	26 (10·0%)	11 (4·6%)	15 (6·2%)
β blocker	93 (43·5%)	118 (45·2%)	104 (43·9%)	113 (46·5%)
Aspirin	179 (83·6%)	233 (89·3%)	217 (91·6%)	229 (94·2%)
Nitrates	51 (23·8%)	63 (24·1%)	37 (15·6%)	49 (20·2%)
Statins	112 (52·3%)	150 (57·5%)	123 (51·9%)	118 (48·6%)
Nicorandil	46 (21·5%)	39 (14·9%)	55 (23·2%)	23 (9·5%)
Medications at 24 months				
ACE inhibitor	66 (47·5%)	63 (37·5%)	83 (52·5%)	75 (49·3%)
ARB	42 (30·2%)	72 (42·9%)	39 (24·7%)	43 (28·3%)
Spinorolactone	13 (9·4%)	21 (12·5%)	9 (5·7%)	4 (2·6%)
β blocker	57 (41·0%)	61 (36·3%)	77 (48·7%)	71 (46·7%)
Aspirin	113 (81·3%)	133 (79·2%)	143 (90·5%)	137 (90·1%)
Nitrates	29 (20·9%)	45 (26·8%)	23 (14·6%)	25 (16·4%)
Statins	66 (47·5%)	78 (46·4%)	81 (51·3%)	71 (46·7%)
Nicorandil	26 (18·7%)	26 (15·5%)	28 (17·7%)	11 (7·2%)

Data are median (IQR), number (%) or mean (SD), unless otherwise specified. ANP=atrial natriuretic peptide. IRA=infarct-related artery. LAD=left anterior descending coronary artery. LCx=left circumflex artery. RCA=right coronary artery. ARB=antiotensin receptor blocker. ACE=angiotensin-converting enzyme. *Period between acute myocardial infarction and start of intervention.

Table 3: Treatments and prescribed drugs

treatments and drugs throughout the study. Drugs used in the chronic stage did not differ between groups in either study, except that some patients in the nicorandil trial were given oral nicorandil during follow-up.

Table 4 and figure 2 show infarct size and left ventricular function at 2–8 weeks and 6–12 months in both studies. The ratio of total creatine kinase between the atrial natriuretic peptide and placebo groups was 0·85 (95% CI 0·75–0·97, p=0·0155); which indicates that atrial natriuretic peptide was associated with a reduction of 14·7% in infarct size. Subanalyses identified no factors that enhanced or reduced the influence of atrial natriuretic peptide on infarct size (figure 2). Nicorandil did not reduce infarct size compared with placebo, and no factors affected this finding. Treatment with atrial natriuretic peptide tended to increase the left ventricular ejection fraction (ratio 1·043, 95% CI 1·000–1·089, p=0·0525) at 2–8 weeks after the onset of acute myocardial infarction, and at 6–12 months (ratio 1·051, 95% CI 1·006–1·099, p=0·0236). By contrast, table 4 and figure 2 show that left ventricular ejection fraction did not differ in patients given nicorandil and controls at either 2–8 weeks or 6–12 months.

	J-WIND-ANP study			J-WIND-KATP study		
	Atrial natriuretic peptide	Control	p	Nicorandil	Control	p
Infarct size						
n	255	280		269	260	
Creatine kinase (area under curve) (IU/L h)	66 459·9 (60 258·2–73 300·0)	77 878·9 (71 590·2–84 720·1)	0·016	70 520·5 (64 309·8–77 331·0)	70 852·7 (65 066·7–77 153·2)	0·941
Peak creatine kinase (IU/L)	2487·5 (2217·6–2790·3)	2784·2 (2526·7–3067·9)	0·141	2557·1 (2306·1–2835·4)	2428·7 (2199·8–2681·5)	0·479
Troponin-T concentration (12–18 h) (ng/mL)	5·36 (4·76–6·03)	6·13 (5·55–6·79)	0·084	6·18 (5·51–6·93)	5·60 (4·97–6·32)	0·244
Troponin T (96 h) (ng/mL)	2·57 (2·25–2·94)	2·94 (2·64–3·27)	0·125	2·63 (2·36–2·94)	2·89 (2·61–3·19)	0·225
Left ventricle (2–8 weeks)						
n	187	207		168	170	
Median elapsed time (days)*	18·5 (IQR 15·0–27·0)	19·0 (IQR 16·0–25·0)		17·0 (IQR 14·0–23·0)	17·0 (IQR 14·0–24·0)	
Ejection fraction	43·0% (41·8–44·3)	41·3% (40·0–42·6)	0·053	42·0% (40·7–43·3)	41·6% (40·4–42·9)	0·680
End diastolic volume index (mL/m²)	98·8 (94·4–103·4)	102·3 (98·1–106·6)	0·272	111·2 (106·4–116·3)	105·9 (100·9–111·3)	0·147
End systolic volume index (mL/m²)	54·2 (51·2–57·4)	58·3 (55·5–61·4)	0·058	62·8 (59·2–66·6)	60·4 (57·0–64·1)	0·360
Left ventricle (6–12 months)						
n	155	199		190	187	
Median elapsed time (days)*	196·5 (IQR 180·5–230·5)	200·5 (IQR 183·0–226·0)		195·0 (IQR 180·0–231·0)	195·5 (IQR 183·0–232·0)	
Ejection fraction	44·7% (43·4–46·0)	42·5% (41·2–43·9)	0·024	42·5% (41·2–43·8)	43·2% (42·0–44·4)	0·460
End diastolic volume index (mL/m²)	100·6 (95·2–106·2)	100·9 (96·8–105·1)	0·930	109·8 (105·4–114·4)	105·7 (100·8–110·8)	0·230
End systolic volume index (mL/m²)	54·2 (50·6–58·0)	56·0 (53·1–58·9)	0·452	61·7 (58·4–65·2)	58·5 (55·1–62·1)	0·198

Data are mean (95% CI) or median (IQR). *Time between acute myocardial infarction and start of intervention.

Table 4: **Primary endpoints and other outcomes obtained by angiography of left ventricles**

Figure 3 shows reperfusion injuries, survival rates, and cardiovascular events. Reperfusion injuries were less common in the atrial natriuretic peptide group than in the placebo group (ratio 0·743, 95% CI 0·58–0·952, p=0·019). Although there were no differences between groups in either survival rates or the incidence of cardiovascular events, both cardiac death and readmission to hospital for heart failure were lower in patients given atrial natriuretic peptide than in controls (HR 0·267, 95% CI 0·089–0·799, p=0·0112). By contrast, cardiac death and readmission to hospital for heart failure were not significantly lower in patients given nicorandil than in controls (HR 0·799, 95% CI 0·307–1·973, p=0·5972). When nicorandil was given orally throughout the study after reperfusion treatment, the change of left ventricular ejection fraction increased substantially between the acute and chronic phase. The ejection fraction was 3·66% in the 61 patients who were given nicorandil orally, and 1·47% in the 241 patients who were not (difference 2·20, 95% CI 0·17–4·22, p=0·0338).

In the atrial natriuretic peptide trial, 29 patients given that drug had severe hypotension during the acute phase, compared with one control. In the other trial, three patients in the nicorandil group had severe hypotension, compared with no controls. No other severe adverse events were reported during the course of either study.

Discussion

We showed that adjunctive, acute-phase treatment with atrial natriuretic peptide after reperfusion therapy in patients with acute myocardial infarction reduced infarct size by 14·7%, increased the left ventricular ejection fraction during the chronic phase, and decreased the incidence of cardiac death and readmission to hospital because of heart failure. Intravenous treatment with nicorandil did not affect the primary endpoints, although patients who were given nicorandil orally had better cardiac function outcomes.

Interest in the cardioprotective effects of adenosine has increased, because of its variety of cardioprotective mechanisms. Unfortunately, in trials of adenosine, it only marginally improved infarct size and showed no clinical benefits.[7,33] We hypothesised that treatment with atrial natriuretic peptide and nicorandil in the acute phase might prove more effective than chronic-phase treatment for limitation of infarct size. The first window of ischaemic preconditioning is mediated by opening of the KATP channel,[34] which is the mechanism of action of nicorandil; and the second window is mediated by nitric oxide and activation of G kinase, which is the mechanism of action of atrial natriuretic peptide.

Before this clinical trial, we had tested whether atrial natriuretic peptide could limit infarct size in a canine model in which the left anterior coronary artery was ligated for 90 min, followed by 6 h of reperfusion. Treatment with atrial natriuretic peptide reduced infarct size by about 40% after reperfusion (unpublished data). Our results are consistent with the finding of Hayashi and coworkers[35] that infusion of atrial natriuretic peptide immediately after reperfusion in patients with their first anterior acute myocardial infarction increased left ventricular ejection fraction.

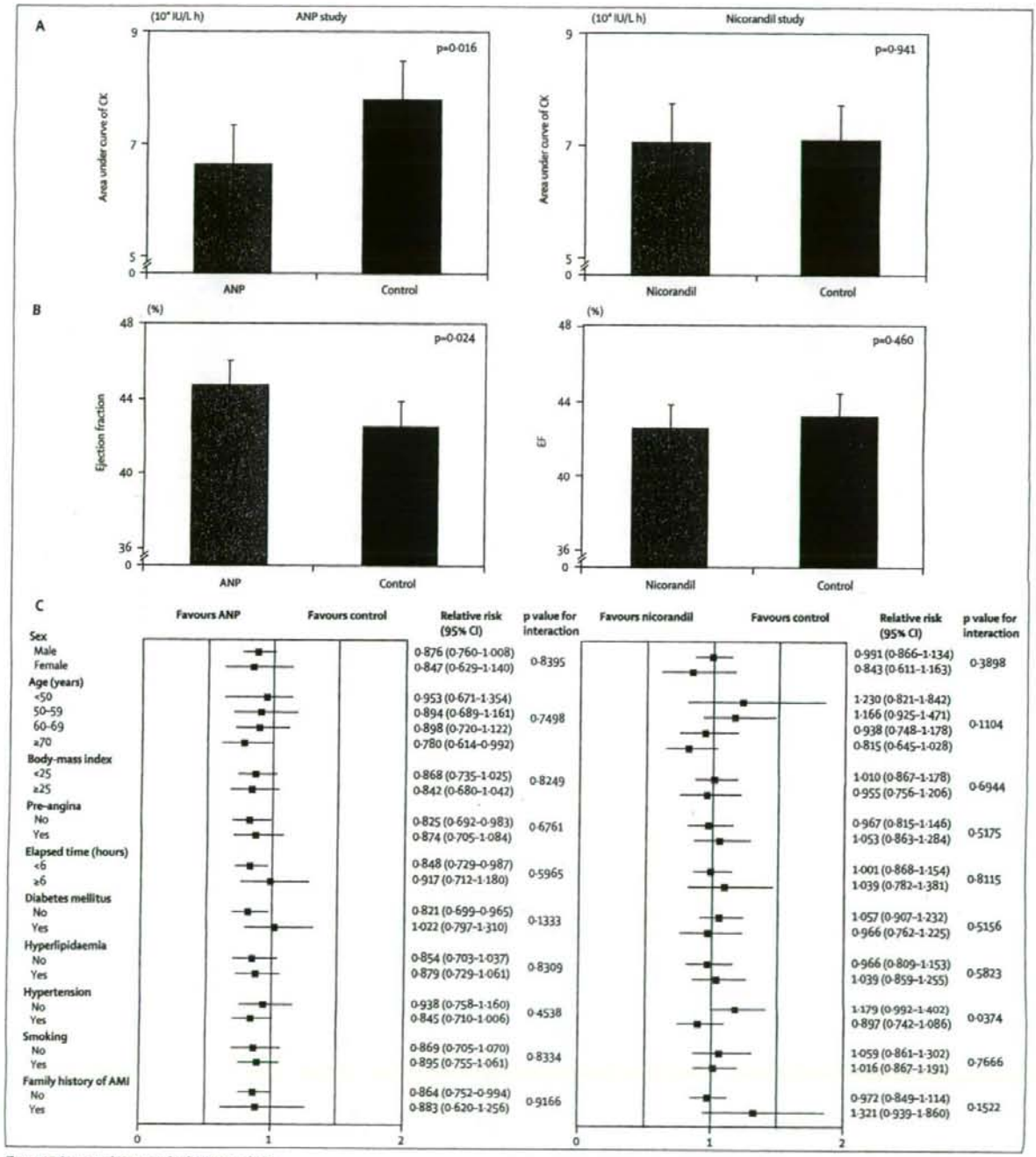

Figure 2: **Primary endpoints and subgroup analyses**

CK=creatine kinase. AMI=acute myocardial infarction. ANP=atrial natriuretic peptide. Panel A shows area under curve of creatine kinase concentration versus time. Panel B represents left ventricular ejection fraction measured at 6–12 months.

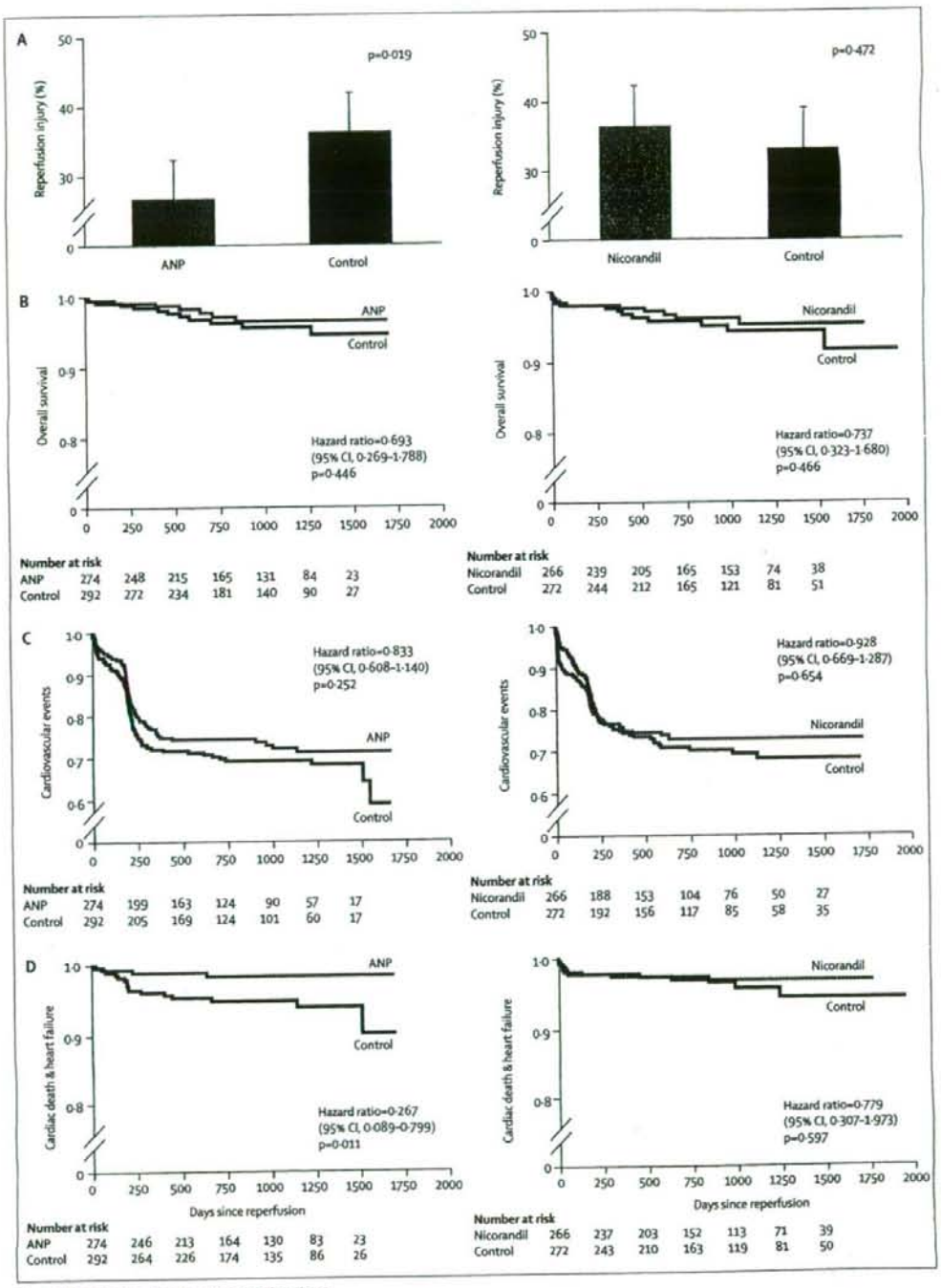

Figure 3: Secondary endpoints and other subanalyses
ANP=atrial natriuretic peptide.

The reduction of infarct size and the improvement of left ventricular ejection fraction might decrease mechanical stress on the non-infarcted myocardium, which might decrease hypertrophy and dilatation of the non-infarcted myocardium. Since cardiac hypertrophy and dilatation cause diastolic and systolic heart failure, a reduction of infarct size and an increase of left ventricular ejection fraction could mediate beneficial clinical outcomes. However, we need to do another large-scale clinical trial to target clinical outcomes such as cardiovascular death, because our primary aim here was to test the reduction of infarct size. Moreover, Hayashi and colleagues[20] showed that plasma concentrations of angiotensin II, aldosterone, and endothelin-1 were lower in patients given atrial natriuretic peptide than in controls. Sudden exposure to high concentrations of angiotensin II, aldosterone, and endothelin-1 for several days caused vascular or ventricular remodelling, and attenuation of these harmful effects by infusion of atrial natriuretic peptide could reduce the incidence of cardiac death and readmission to hospital for chronic heart failure.[20]

One reason that nicorandil treatment did not limit infarct size in our study could be the size of the dose. Ishii and colleagues[25] have reported that one intravenous administration of a dose of nicorandil that was three times higher than that which we used decreased the infarct size and reduced the rate of cardiovascular death or readmission to hospital for chronic heart failure in 368 patients with acute myocardial infarction.

Patients in the nicorandil study who were given nicorandil orally in the chronic phase had greater increases in left ventricular ejection fraction, irrespective of whether nicorandil was given intravenously or orally. Since microvascular obstruction ten days after myocardial infarction was associated with left ventricular remodelling and poor prognosis, coronary perfusion might be improved by opening KATP channels in coronary blood vessels during the healing stage. The IONA study[15] showed that nicorandil could reduce the incidence of unstable angina in patients with stable angina.

Our finding that treatment with atrial natriuretic peptide in the acute phase reduced the incidence of readmission to hospital for chronic heart failure could help to reduce the physical, medical, and economic burdens on people around the world. Moreover, since intravenous nicorandil in the acute phase, followed by oral administration in the chronic phase, increased the left ventricular ejection fraction, chronic treatment with nicorandil could improve ventricular function for patients with myocardial infarction in the chronic phase.

Several limitations of our study should be discussed. First, physicians knew the random assignment of patients, and treatment for acute myocardial infarction in the chronic phase was not restricted accordingly; this could have affected the difference in nicorandil treatment at the chronic phase. Second, although we planned to do angiography of the left ventricle when patients were admitted to hospital, some hospitals could not take angiographs, because of the additional medical cost. Therefore, baseline angiographs were absent for some patients. Third, the patterns of missing angiography data on left ventriculography differed between the two studies (which were done at different hospitals) and also between the atrial natriuretic peptide group and corresponding placebo group. We cannot explain this difference, but since we did not intervene in this procedure, we believe that it must be due to chance.

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Conflict of interest statement

We declare that we have no conflict of interest.

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Original Article

Impact of Adenosine Receptor Signaling and Metabolism on Pathophysiology in Patients with Chronic Heart Failure

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Adenosine is well known to be a cardioprotective substance in ischemic heart disease. However, the modulation of adenosine receptors and the production and degradation of endogenous adenosine in chronic heart failure (CHF) are not fully understood. We analyzed the gene expression patterns of adenosine-related genes in human failing and nonfailing myocardium using DNA microarray analysis and quantitative real time–polymerase chain reaction (RT-PCR). DNA microarray analysis revealed that the gene expression of adenosine A2a, A2b, and A3 receptors (A2aR, A2bR, and A3R) as well as that of adenosine deaminase (ADA) decreased in failing myocardium. The down-regulation of these genes was verified by quantitative RT-PCR. We also measured the activities of these adenosine metabolism–related enzymes in failing myocardium and cardiac adenosine levels in patients with CHF. In CHF patients, we observed the decreased enzyme activity of ADA and the elevation of cardiac adenosine levels in CHF patients. To enhance the signaling of adenosine receptors, we increased plasma adenosine levels using dipyridamole, which decreased the severity of CHF. The gene expression of A2aR, A2bR, A3R, and ADA was decreased in the failing hearts, and this decrease may impair adenosine-related signal transduction. The activities of adenosine-related enzymes were altered, thus increasing the myocardial adenosine levels; this increase may compensate for the impairment of adenosine-related signal transduction in patients with CHF. The impairment of adenosine-related signal transmission contributes to the pathophysiology of CHF. (*Hypertens Res* 2007; 30: 781–787)

***Key Words*: DNA microarray, adenosine, single nucleotide polymorphism, heart failure, adenosine deaminase, adenosine A2a receptor**

Introduction

Chronic heart failure (CHF) represents the common characteristics secondary to various cardiac diseases, such as systemic hypertension, dilated cardiomyopathy, hypertrophic cardiomyopathy, ischemic heart disease, valvular heart disease, and myocarditis (*1*). Interestingly, catecholamine, angiotensin, aldosterone, and cytokines are known to be involved in the pathophysiology of CHF (*2–5*), as evidenced

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Table1. Patient Characteristics

Case	Age (years old)	Sex	Diagnosis	Operation	LAD (mm)	LVDd (mm)	EF (%)	MR	ANP (ng/mL)	BNP (ng/mL)
01	53	M	ICM	Batista	31	88	24	IV	25	90
02	45	M	DCM	Batista	63	81	39	IV	85	217
03	72	M	DCM	Batista	52	71	14	III	86	201
04	58	F	ICM	Dor	44	76	24	I	NA	NA
05	57	M	HCM	Dor	54	52	44	III	20	80
06	69	M	DCM	Batista	49	86	15	IV	100	465
07	40	M	AR	Dor	44	76	38	I	39	200
08	75	M	ICM	Dor	28	48	35	II	37	150
09	32	M	DCM	Batista	54	81	26	IV	170	403
10	51	F	Myocarditis	Dor	26	68	35	IV	70	196
11	54	M	ICM	Dor	47	64	27	I	84	302
12	58	M	Myocarditis	Dor	48	77	18	III	800	2,710

LAD, left atrial diameter; LVDd, diastolic left ventricular diameter; EF, ejection fraction; MR, severity of mitral regurgitation; ANP, the concentration of plasma atrial natriuretic peptide (ng/mL); BNP, the concentration of plasma brain natriuretic peptide; M, male; F, female; ICM, ischemic cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; AR, aortic valve regurgitation; NA, not available.

by the fact that β-adrenoceptor antagonists, angiotensin-converting enzyme (ACE) inhibitors, and aldosterone receptor antagonists are widely accepted as drugs for CHF (*6*, *7*). Adenosine has biological effects on various tissues (*8–10*). Since several lines of evidence (*9*, *10*) support the idea that adenosine is cardioprotective against deleterious sequels in CHF as well as ischemic heart disease, it is intriguing and important to analyze the adenosine receptor– or adenosine metabolism–related genes using DNA microarray analysis. Adenosine is known to be an endogenous nucleoside acting as a cardioprotective substance that modulates numerous physiological processes, including the regulation of coronary blood flow (*9*, *10*). Adenosine is produced or degraded by several enzymes, including 5′-nucleotidase, adenosine deaminase (ADA), and adenosine kinase (AK). Adenosine elicits its physiological actions by binding to four specific receptors: A1, A2a, A2b, and A3. A1 and A3 receptors are coupled through Gi protein to adenylate cyclase inhibition, while A2a and A2b receptors are coupled to adenylate cyclase activation through Gs protein. However, the adenosine metabolism and its receptor-mediated signaling in patients with CHF remain unclear.

In the present study, we first examined gene expression in failing and nonfailing myocardium by focusing on adenosine-related genes using DNA microarray analysis followed by quantitative real time–polymerase chain reaction (RT-PCR). Then, to examine whether or not the consequences of the altered gene expression are related to the pathophysiology of human CHF, we also measured cardiac adenosine levels and the activities of adenosine-related enzymes. Finally, we tested whether or not increased adenosine levels using dipyridamole, an adenosine uptake inhibitor, improves the pathophysiology of patients with CHF.

Table 2. The Comparison of Gene Expressions between the Nonfailing and Failing Hearts

Gene name	Fold change
Adenosine receptors	
A1 receptor	1.51±0.32
A2a receptor	0.29±0.04
A2b receptor	0.75±0.07
A3 receptor	0.61±0.04
Adenosine-related enzymes	
Adenosine deaminase	0.52±0.03
Adenosine kinase	1.14±0.16

Methods

RNA Samples from Human Heart Tissues

Tissue samples of human failing heart were obtained from 12 patients (average age 55 years [range 32–75 years]; 10 males and 2 females) who had undergone partial left ventriculectomy (the Batista or Dor procedure) for end-stage heart failure at Hayama Heart Center. All heart tissues were stored in RNA Later (Ambion, Austin, USA). Because of the difficulty of acquiring nonfailing heart tissues in Japan, we obtained total RNAs of nonfailing myocardium of Mongolian people from BioChain Institute Inc. (Hayward, USA). The collection and use of tissue were approved by independent ethics committees of the National Cardiovascular Center at Osaka University and of the Hayama Heart Center.