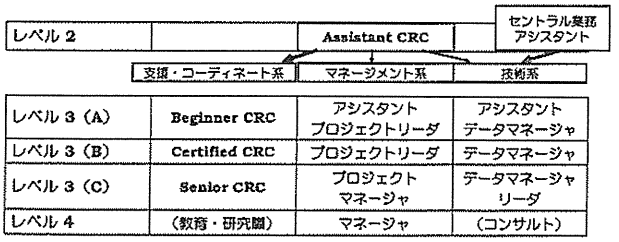

レベル 3 (A)	Beginner CRC	アシスタント プロジェクトリーダ	アシスタント データマネージャ
レベル 3 (B)	Certified CRC	プロジェクトリーダ	データマネージャ
レベル 3 (C)	Senior CRC	プロジェクト マネージャ	データマネージャ リーダ
レベル 4	(教育・研究職)	マネージャ	(コンサルト)

Fig. 2 CRC のキャリアパスの一例

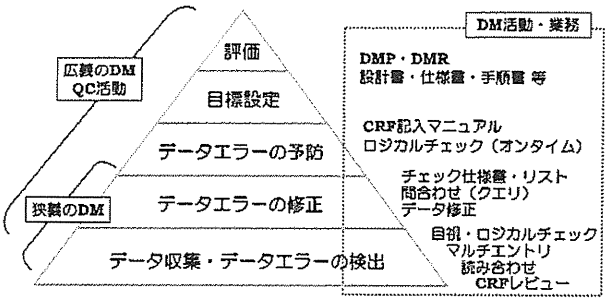

Fig. 3 データマネージメントレベルと活動

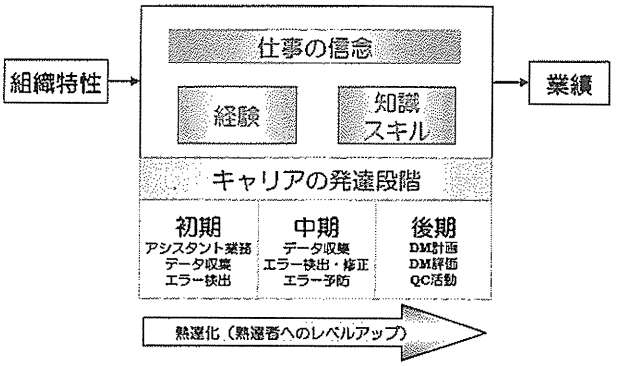

Fig. 4 データマネージャにおけるキャリアアップ

も含めた，セントラルとローカルの融合した広義の CDM (Fig. 3) が品質管理活動として重要である[3,4]．CRC のキャリアパスの一部として DM を考えるのであれば，CDM 全体を考慮し，Fig. 4 のようなキャリアの発達段階を設定する必要がある[5]．

DM としての熟達化を実現するには，知識とスキルと経験が必要である．知識を得るには，やはり CDM の基礎から習得できるセミナーが最適であり，CDM の基礎は依頼者側であっても医療機関側であっても共通している．また，CDM が実践科学である以上，フィールドでの経験は極めて重要であり，その領域の熟達者[5]になるにはフィールドを離れないことが求められるだろう．

4．まとめ

CRC は多種多様な業務に対応する専門職であるため，advanced 研修を考える際，その職種の特殊性を考慮するとキャリアパスは 1 つではない．多様性を認めるフレキシビリティが求められる．CRC 個人も，キャリアアップを実現するには，各領域の知識や技術を身につけるだけでなく，個人の能力を活かすキャリアパスにおいて経験を積むことが良いだろう．

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A survey of attitudes toward clinical research among physicians at Kyoto University Hospital

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Abstract

Background: In Japan, only clinical research related to investigational new drug trials must be notified to regulatory bodies, and this lack of a uniform standard for clinical research has caused a number of difficulties. The objective of this study was to assess the willingness of physicians to participate in clinical research and to identify effective methods to promote and enhance clinical research.

Methods: We conducted a cross-sectional survey by administrating questionnaires to physicians in 31 departments in Kyoto University Hospital from October through November 2007.

Results: A total of 51.5% (310 of 602) of physicians completed the questionnaire. More than two-thirds of them reported currently participating in clinical research, and nearly all believed that clinical research is necessary for physicians. Less than 20% of respondents had specific training regarding clinical research, and most reported a need to acquire concepts and skills regarding clinical research, especially those related to statistics. "Paperwork was complicated and onerous" was the most frequently cited obstacle in conducting clinical research, followed by "few eligible patients" and "lack of time". Previous participation in and prospective participation in clinical research, previous writing a research protocol were positively associated with current participation in clinical research.

Conclusions: Physicians in university hospitals need more training regarding clinical research, particularly in biostatistics. They also require administrative assistance. Our findings indicate that the quality of clinical research could be improved if training in clinical research methodology and biostatistics were provided, and if greater assistance in the preparation of study documents requested by the institutional Independent Ethics Committee were available.

Background

Good Clinical Practice (GCP) should be used for designing, conducting, recording, and reporting trials that involve the participation of human subjects[1]. This guideline should be followed when generating clinical trial data that are intended to be submitted to regulatory authorities. In the United States, many research sites conduct clinical trials in compliance with GCP standards [2], and the European Clinical Trial Directive made GCP mandatory for all clinical drug trials [3].

In Japan, clinical trials of new drugs can be classified into two categories: investigational new drug (IND) application trials, and studies that do not seek marketing approval (non-notified trials). The former are strictly regulated by the Pharmaceutical Affairs Law[4] and by the Ministry of Health, Labour and Welfare (MHLW) Ordinance on GCP[5], which was adopted in Japan in 1997 and is based on the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) E6 Guidelines[1]. In striking contrast to other countries, Japanese researchers can conduct clinical trials without notifying or applying to the authorities, unless they require new drug approval (NDA). In fact, there is little legal regulation of non-notified trials in Japan. The only guidance provided by the MHLW is Ethical Guidelines for Clinical Studies [6], which was published in 2003 and has no legal implications. The main difficulty in conducting non-notified trials is that the policies of Japanese ethics committees vary by medical institution or hospital. Thus, a trial that is disallowed by one institution might be approved by another, perhaps without sufficient discussion of its ethical or scientific implications.

Before 2003, applications for IND trials were only submitted by the company responsible for manufacturing and marketing the drug. After revision of the Pharmaceutical Affairs Law, an investigator can now initiate and notify the relevant authorities of an IND trial, which involves strict observance of GCP. As of 2007, the number of investigator-initiated IND trials has been very small, and notified by certain university hospitals, including Kyoto University Hospital (KUH), and the Japan Medical Association. However, many non-notified trials, undertaken in observance of the Ethical Guidelines, have been conducted by KUH and other hospitals.

KUH is one of the seven largest university hospitals in Japan, and a total of 348 faculty, 176 senior residents, and 125 junior residents were employed there as of October 2007. Since 2007, it has been one of seven distinguished research sites chosen by the Ministry of Education, Culture, Sports, Science and Technology to participate in the Coordinating Support and Training Program for Translational Research, which seeks to promote quality in clinical research.

The objective of the present study was to investigate both the willingness of physicians to participate in clinical research and their attitudes toward such research. In addition, we aimed to identify methods of support and training that might assist physicians in conducting research. We aimed that our findings may foster future academic clinical research both at KUH and other university hospitals in Japan.

Methods

Respondents and survey administration

From October through November 2007 we conducted a cross-sectional survey of 31 departments in KUH. We initially contacted the directors of each of the 34 departments in KUH to explain the study and to ask for their participation in the study. Thirty-one departments consented to participate. The person in charge of each department distributed the study description and questionnaire by hand or by mail to the physicians belonging to the department, and later collected them. Residents, faculty, and doctoral students (physicians) with medical degree were invited to participate. It was not necessary to obtain ethical approval for this survey, as this survey was out of jurisdiction of Ethical Guidelines for Epidemiological Research[7], which shall be applied to studies on etiology of human disease and diagnostic or therapeutic procedures.

Questionnaire

An initial questionnaire was prepared to gain a better understanding of the current state of clinical research and to guide development of activities at the Translational Research Center, Kyoto University Graduate School of Medicine. To prepare the questionnaire, we modified and added questions to a questionnaire from a similar study conducted in Tokushima University Hospital [8].

The questionnaire inquired about demographic data--including age range and employment status--and attitudes regarding clinical research, clinical research training, and submission of articles on clinical research. Since ICH E6 guidelines for GCP, an international standard for research ethics, is based on and consistent with the principles of the Declaration of Helsinki, we queried the respondents' knowledge of the World Medical Association Declaration of Helsinki [9].

Using multiple-choice questions, respondents were asked about (1) the benefits of conducting clinical research and desired lecture topics on clinical research; (2) the difficulties of conducting research, among physicians who had participated in such research; and (3) the content of reviewers' comments, among physicians who had submitted a clinical research article. The questionnaire was anonymous, and included a separate form to state freely their name or additional opinions for those physicians who wished to collaborate on any further research project.

Statistical Analysis

Descriptive analysis was used to examine respondents' perception of the benefits and difficulties of clinical research. Answers to multiple-choice questions were summed and listed in order of frequency. The chi-square test was used to compare the age range and the proportion

of physicians employed by internal medicine departments (ie, internal medicine, pediatrics, psychiatry and radiology) among respondents with those among both nonrespondents and national physicians.

Bivariate analyses were performed to identify factors that might be associated with current participation in clinical research. We used chi-square tests for categorical variables and *t* test for continuous variables. The continuous variables in this dataset were age range (decade) and knowledge of Helsinki. Correlation analyses were performed to test for multicollinearity between 5 sets of factors we hypothesized might be highly correlated (age range and status, past participation in clinical research and past submission for publication of a manuscript on clinical research, past participation in clinical research and past writing of a research protocol, past participation in clinical research and prospective participation in clinical research, and past submission for publication of a manuscript on clinical research and past writing of a research protocol). Decisions to include factors in the multiple logistic regression analysis were based on the strength of correlated factors ($r < 0.75$) or a P value < .05 on bivariate analyses. We performed multiple logistic regression analysis to identify factors that were correlated with participation in clinical research.

A P value of less than 0.05 was considered to be statistically significant. Analysis was performed using STAT View (SAS Institute Inc, Cary, NC).

Results

Characteristics of respondents

Among 602 physicians from the 31 departments who received the questionnaire, a total of 51.5% (310 of 602) completed the questionnaire. A total of 175 faculty and 58 residents responded; 173 faculty and 243 residents did not respond ($P < 0.001$). As to age range, 47.8% of nonrespondents were aged 20 to 29, 16.8% of nonrespondents were aged 30 to 39, and 24.2% of nonrespondents were 40 to 49. Table 1 provided age range of respondents. There were statistically significant difference between respondents and nonrespondents on age range ($P < 0.001$). The survey respondents were not representative of all physicians at KUH: Faculty was more likely to complete survey than were residents, possibly because many junior residents did not receive the questionnaire. As junior residents rotate through various specialties, some of the person in charge of each department hesitated to distribute the questionnaire to junior residents. A total of 96 faculty and residents employed in internal medicine departments responded to the questionnaire, and 137 faculty and residents in surgical or other departments responded to the questionnaire. There were 164 nonrespondents in internal medicine departments and 252 nonrespondents in surgical or other departments (P =

Table 1: Characteristics of the 304 respondents

Characteristic	Percent*		
	resident or doctoral student (n = 129)	faculty (n = 175)	total (n = 304)
Age range			
<= 29	15.5	0.6	6.9
30-39	82.2	31.4	53.0
40-49	2.3	53.1	31.6
>= 50	0.0	14.9	8.6
Internal medicine departments	50.4	36.0	42.1
Current participation in clinical research	48.8	82.3	68.1
Past participation in clinical research	53.5	89.1	74.0
Prospective participation in clinical research	61.2	89.7	77.6
Previous training course in clinical research	11.6	18.9	15.8
Do you consider it is necessary for physicians to conduct clinical research?			
yes	96.1	97.1	96.7
Have you ever written research protocol?			
yes	14.0	51.4	35.5
Have you submitted for publication of a manuscript on clinical research?			
yes	24.8	50.9	39.8
Do you know "World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects"?			
I know very well	10.9	28.0	20.7
I know to some degree	81.4	68.0	73.7
I don't know	5.4	2.9	3.9

* Percent values were expressed as ratio in respondents of each age range. Percentage may not total 100% due to missing or blank data.

0.657 vs respondents). In comparison, there were 77358 physicians in internal medicine departments and 90969 physicians in surgical or other departments in hospitals in Japan in December 2006 [10] ($P = 0.146$ vs respondents). Respondents did not differ from nonrespondents and national physicians in the proportion of physicians who belonged to internal medicine departments.

Table 1 lists the respondents' characteristics by status: resident or doctoral student vs faculty. Six respondents with other status or with blank data for status were deleted. Among respondents, 68% of physicians reported current participation in clinical research; 74% reported past participation in clinical research. More faculty than resident or doctoral student reported past participation in, current participation in and prospective participation in clinical research. Most physicians (97%) believed that it is necessary for physicians to conduct clinical research. More than half of faculty had written a research protocol and reported submitting for publication of a manuscript on clinical research, whereas 14% of counterpart had written a research protocol and 25% of counterpart reported submitting for publication of a manuscript on clinical research. However, only 16% had taken a training course in clinical research offered by either the Japan Clinical Oncology Group (9), Kyoto University Graduate School of Medicine (9), other domestic universities and scientific societies (9), or foreign institutions (2). Most physicians (94%) were aware of the World Medical Association Declaration of Helsinki; 4% were not.

Attitudes

Respondents were queried regarding the benefits of conducting clinical research. Obtaining a better understanding of disease was the most frequently cited benefit, and was mentioned by 255 physicians (47.3%). Enhanced standing in society or the hospital was the second most frequently cited benefit, and was mentioned by 150 physicians (27.8%), followed by obtaining research grants or awards. Eleven respondents (2.0%) felt that there was no benefit (Table 2).

Table 2: Attitude towards clinical research

Question	Percent*(%)
What benefits do you think are brought to physicians of conducting clinical research?	
Physicians can obtain a better understanding of disease	47.3
Physicians will enhance standing in society or in hospital	27.8
Physicians will obtain research grants or awards	12.8
Physicians will obtain credits to be board certified doctor	4.1
There is no benefit to physicians	2.0
Which lecture topics related to clinical research are interesting or useful?	
Statistical analysis	25.3
How to write a protocol	20.7
Paperwork and procedures†	13.2
Cost management for clinical research	12.7
Informed consent form to patients	10.5
Compensation	9.2
Medical ethics	8.0
What were the criticisms of reviewers when you submitted for publication a manuscript on clinical research ?	
Statistical analysis	36.9
Selection of patients	21.0
Aim or meaning of research	19.1
Definition of the technical terms	10.2
Ethical problems	5.7
What difficulties did you meet of conducting clinical research?	
The paperwork was complicated and onerous	26.2
Eligible patients were very few	18.9
Lack time	17.6
Too many examinations were scheduled	11.5
There was no benefit to patients	8.6
I could not continue clinical research because of transfer of physicians	6.3
Patients missed appointments	5.4
Patients didn't consent to take placebo	3.6
Doctor-patient relationships were damaged by offering clinical research	0.8

* Percent values were expressed as ratio in total answers. Percentage may not total 100% due to missing or blank data.
†Paperwork and procedures mean production and management of study documents regarding submission to institutional review board and completion of case report form.

Most physicians (93.2%) wanted to attend lectures or seminars on one or more topics related to clinical research. The most frequently cited desired lecture topics were statistical analysis, how to write a protocol, paperwork and procedures (production and management of study documents regarding submission to institutional review board and completion of case report form), and cost management in clinical research (Table 2).

Respondents who had submitted research papers for publication were asked to indicate the criticisms of reviewers. Statistical analysis was the most frequent reviewer criticism, followed by selection of patients, aim or meaning of research, and definition of technical terms (Table 2).

Regarding the difficulties of conducting clinical research, respondents indicated that the "paperwork was complicated and onerous", that there were "few eligible patients", and that the respondents "lack time" (Table 2).

Factors associated with current participation in clinical research

Age range had moderate correlation with status (r = 0.635), as did past participation in clinical research with prospective participation in clinical research (r = 0.505). Past participation in clinical research had some correlation with past submission for publication of a manuscript on clinical research (r = 0.413), as did past submission for publication of a manuscript on clinical research with past writing of a research protocol (r = 0.311) and past participation in clinical research with past writing of a research protocol (r = 0.282).

In bivariate analyses, current participation had statistically significant correlation with status, age range, past participation in clinical research, prospective participation in clinical research, past submission for publication a manuscript on clinical research, training course in clinical research, past writing a research protocol and knowledge of the World medical Association Declaration of Helsinki. A multivariable logistic regression model was developed including all these correlated factors as variables. Current participation was positively associated with past participation in, prospective participation in clinical research and past writing of a research protocol (Table 3). Age range of 30-39 was negatively associated with current participation in clinical research: Respondents aged 30 to 39 were less than quarter (odds ratio, 0.24; 95% confidence interval,

Table 3: Effect of status, age range, and attitudes to current participation in clinical research

Characteristic	Odds ratio (95% CI)	*P* value*
Status		
resident or doctoral student	reference	
faculty	1.416(0.568-3.531)	0.4554
Age range, y		
<=29	reference	
30-39	0.240(0.064-0.907)	0.0353
40-49	0.354(0.069-1.822)	0.2142
>=50	0.218(0.028-1.684)	0.1442
Past participation in clinical research		
yes	5.680(2.40-13.441)	< 0.0001
no	reference	
Prospective participation in clinical research		
yes	5.756(2.508-13.212)	< 0.0001
no	reference	
Previous training course in clinical research		
yes	2.081(0.678-6.389)	0.2002
no	reference	
Previous writing of a research protocol		
yes	2.631(1.130-6.125)	0.0249
no	reference	
Previous submission for publication of a manuscript on clinical research		
yes	1.798(0.815-3.967)	0.1464
no	reference	
Do you know "WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI Ethical Principles for Medical Research Involving Human Subjects"?		
I know very well	4.219(0.561-31.728)	0.1619
I know to some degree	2.457(0.413-14.623)	0.3233
I don't know	reference	

* P < 0.05 is considered statistically significant.
The R2 value was 0.378. CI, confidence intervals

0.064-0.907) as likely to participate in clinical research currently as respondents aged 20 to 29. There was no association between current participation and either status or previous training course in clinical research.

Discussion

In this questionnaire survey of physicians at KUH, most respondents were currently participating in clinical research and felt that clinical research was necessary. As compared to physicians participating in clinical research, smaller proportions of physicians had formal training in clinical research. The majority reported a need to acquire concepts and skills regarding clinical research, especially those related to statistics. Both previous participation in and prospective participation in clinical research were positively associated with current participation in clinical research, suggesting that physicians who were accustomed to clinical research were participating in and would participate in clinical research.

Our findings indicate that the contention that "doctors (in Japan) simply don't want to take part in clinical trials"[11] is a misunderstanding. Indeed, our results indicate that if an adequate trial infrastructure is present, Japanese physicians are eager to conduct clinical research.

KUH is an important research center in Japan, and this likely explains why the rates of participation in and acknowledgement of the importance of clinical research were high among respondents. Studies have reported a wide range in the percentage of physicians participating in clinical research, from 13% to 90% [12-14]. In a questionnaire survey at Tokushima University Hospital[8], 61% of faculty had contributed to IND application trials and 58% of those wanted to participate in IND application trials, whereas in our survey at KUH, 89% of faculty reported past participation in clinical research. The difference in participation rates could be the result of different criteria of clinical research in the questionnaire. As mentioned above, many non-notified trials are carried out at KUH and other hospitals. Perhaps the rate of participation was high because, with the exception of notified trials, physicians in Japan are able to initiate clinical research with only minimal ethical oversight.

In the present study, the difficulties that physicians faced in conducting clinical research are similar to those noted in previous studies [14-16]. Paperwork was cited as a major hurdle, even though the limited number of regulatory obstacles in Japan would be expected to lessen paperwork demands. Perhaps because physicians have a low opinion of the necessity for preparing and managing study documents, they perceive extra paperwork as onerous. Therefore, we suggest that a clinical support center should be available to provide initial advice and support regarding the production and design of documents, thereby establishing good practice. Lack of time was also reported as a major hurdle. Most physicians in university hospitals in Japan are involved in both patient care and research on molecular and cellular biology including experiments with animals. Because researchers could study molecular and cellular biology on a smaller budget than clinical research, which is the evaluation of new treatment involving human subjects, they studied it since it was introduced to Japan. As a result, there are few highly skilled clinical researchers in Japan and opportunities to learn the principles and methodology of clinical research are limited for young Japanese physicians.

Physicians who are familiar with clinical research are able to conduct clinical research more easily than those who are not, as they know the guidelines and laws necessary for conducting clinical research and can use their pre-existing network of experienced research collaborators [17]. In addition, physicians who have completed clinical trials can obtain funding more easily than those who have not; however, they gain no special treatment or financial incentives [11]. As the majority of physicians indicated that obtaining a better understanding of disease was the greatest benefit of conducting clinical research, the pleasure of discovery would appear to have more than repaid them for their efforts.

In our model with respect to current participation in clinical research, the previous training in clinical research was not found to be a significant factor. As various training providers were reported in this questionnaire, the programs and the length of these training courses should be variable. Universities or university hospitals should develop a standardized training program on clinical research that physicians can learn essential knowledge before they initiate such research.

The current study did have some limitations. The most significant of these is that the clinical research referred to in this survey comprised a variety of research types, ranging from epidemiological and observational studies to clinical trials, including IND application trials. Nevertheless, the research support section that serves the university hospital assists with a variety of clinical research designs, and a commonality of needs among physicians was demonstrated in our survey. Another limitation was that the response rate was much higher among faculty than among resident, which may influence the final logistic regression analysis. In addition, this survey took place at a single institution, so the possibility for generalization is limited. However, the difficulties indicated by respondents were quite consistent with those of prior reports. Moreover, an ongoing international collaboration project is attempting to compare the status and attitudes of physicians, and to

seek strategies to promote clinical research. The results of this study have contributed much to the refinement and modification of the questionnaire used for the international attitude study. We aim to identify unique and universal problems regarding academic clinical research, and to submit them to academic societies and governing bodies in order to improve the situation. In addition, after completion of our questionnaire survey, Ethical Guidelines for Clinical Studies were just revised and enacted in April 2009. Under the revised guidelines, investigators are now required to register their trials at a public trial registry, to obtain insurance for trial subjects, and to have adequate training in clinical research. Concern for the welfare of trial subjects may have increased, but this may create another barrier to perform clinical research by requesting more paperwork and more funding for insurance for trial subjects.

Conclusions

Physicians in university hospitals need more administrative assistance and greater knowledge of the principles and techniques of clinical research, especially the concepts of biostatistics. Our results highlight the need for training in clinical research and biostatistics and the necessity for administrative assistance in the production of study documents requested by the institutional Independent Ethics Committee.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ES conceived the study and participated in the design, management, data analysis, and preparation of the manuscript. TM participated in the study design and in the preparation of the manuscript. MY participated in the study design and participant recruitment. All authors read and approved the final manuscript.

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Plasma des-acyl ghrelin, but not plasma HMW adiponectin, is a useful cardiometabolic marker for predicting atherosclerosis in elderly hypertensive patients

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ABSTRACT

Objective: The coming obesity epidemic in elderly persons necessitates the establishment of new and easy-to-use cardiometabolic markers to identify individuals most likely to develop atherosclerosis among hypertensives.
Methods: We measured plasma HMW adiponectin and des-acyl ghrelin levels, and carotid-artery intima-media thickness (cIMT) in 263 elderly hypertensives (mean 72.6 years; 37%men). Other cardiometabolic markers, including metabolites, inflammation, and hemostasis, were also measured.
Results and conclusion: Both HMW adiponectin and des-acyl ghrelin levels were inversely correlated with obesity. The HMW adiponectin level was favorably associated with glucose and lipid metabolites, PAI-1 (all $P<0.05$), and hs-CRP ($P=0.07$) after adjustment for age, sex, and BMI; however, it had no correlations with cIMT. In contrast, although there were no correlations between des-acyl ghrelin and cardiometabolic markers, except for a positive association with the nitrite/nitrate (NO_x) level ($P=0.002$), des-acyl ghrelin had a significant inverse correlation with cIMT ($P=0.003$). A multivariable regression analysis showed that des-acyl ghrelin, but not HMW adiponectin, was significantly associated with cIMT after adjusting for age, obesity, sex, smoking, 24-h BP, and other cardiometabolic factors ($\beta=-0.178$, $P=0.001$). Moreover, the increased risk of cIMT among those with abdominal obesity compared with non-obesity (0.833 ± 0.185 mm vs. 0.782 ± 0.163 mm, $P=0.019$) was explained by the elevated 24-h BP and reduced des-acyl ghrelin level, but not by other cardiometabolic parameters. These associations were unchanged after adding NO_x to the model. In conclusion, the des-acyl ghrelin level is a useful cardiometabolic marker for predicting atherosclerosis in elderly hypertensives, and the pathologic pathway linking these factors is independent of its NO bioactivity.

1. Introduction

Due to the growing obesity epidemic and the growing elderly population, it is essential that we improve our understanding of the impact of obesity on the cardiovascular system in elderly persons, and establish new and easy-to-use cardiometabolic markers to identify individuals most likely to develop atherosclerosis among hypertensives [1]. A recent study has revealed that endocrine functions (i.e., adipocytokines) are involved in the pathophysiological mechanisms underlying the risk factors associated with obesity and atherosclerosis [2], but the roles of adipocytokines in these mechanisms are only partially understood.

Adiponectin, an adipocyte-derived hormone, has favorable effects on insulin-sensitizing, anti-inflammatory, and anti-atherogenic properties [3], and thus high adiponectin levels have been associated with a reduction of cardiovascular disease (CVD) [3,4]. However, recent prospective studies have shown conflicting results, particularly in elderly persons [5], suggesting that adiponectin may have different clinical implications in the elderly. High adiponectin in the elderly is a consequence of weight loss

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and sarcopenia with aging [5–7], both of which are predictors of mortality [8]. In addition, adiponectin could exert its own effects by increasing energy expenditures and thereby leading to wasting [7]. Recently, increasing attention has been paid to the multimeric isoforms of adiponectin. In the circulation, adiponectin exists in at least three multimeric isoforms: a low-, medium- and high-molecular-weight (HMW) form. These different oligomeric forms of adiponectin might activate different signaling pathways and exert distinct functions on its target tissues. Because HMW adiponectin may be the major active form of the metabolic and vascular protective effects of adiponectin [3,6,9], it would be worthwhile to examine its clinical implications in elderly persons.

In contrast, ghrelin, an endogenous ligand for the growth hormone secretagogue receptor (GHSR) and acts as an anabolic hormone in elderly persons [10,11], has been shown to exert not only energy homeostasis, but also cardiometabolic regulations in both healthy and obese subjects [12,13]. The reduction of ghrelin, which is associated with obesity, has also been shown to be proportional to aging and catabolic rates, and thus it may be more representative of a true biological phenomenon in elderly persons. Des-acyl ghrelin, a more abundant form of ghrelin in humans and allows easier measurement than acylated ghrelin, has some unique cardiometabolic properties, such as blood pressure (BP) lowering [12], nitric oxide (NO) production [13], and prevention of cardiac and endothelial cell apoptosis [14]. However, there have been no studies examining these effects in humans.

In the present study, we measured plasma HMW adiponectin and des-acyl ghrelin levels, and examined their relationship to obesity-related cardiometabolic factors, including glucose and lipid metabolism, inflammation (high-sensitivity C-reactive protein: hsCRP), hemostasis (plasminogen activator inhibitor-1: PAI-1), and atherosclerosis (carotid artery intima-media thickness: cIMT) in elderly hypertensives.

2. Subjects and methods

276 consecutive ambulatory patients with essential hypertension who were >40 years old and who had been referred to our outpatient clinic (Kitaura National Health Insurance Hospital, Miyazaki, Japan) were recruited for the Miyazaki Elderly-Fat (EL-FAT) project (see Supplementary materials). The project was approved by the institutional review board at Jichi Medical University, and written informed consent was obtained from all the participants. All patients completed a health questionnaire and provided their complete medical history (smoking and drinking status, physical activity, use of medications, and past medical history), and underwent measurement of body mass index (BMI) and waist circumference (WC), office and 24-h ambulatory BP monitoring (ABPM), blood sampling, and carotid ultrasonography. Hypertension was defined as use of anti-hypertensive drugs or an office BP level of ≥140/90 mmHg. Type 2 diabetes was defined as use of anti-hyperglycemic drugs or a fasting glucose of ≥126 mg/dl. The exclusion criteria were as follows: a history of coronary arterial disease (CAD), cerebrovascular disease, or heart failure in the past 3 months; presence of inflammatory diseases (acute infection, autoimmune diseases); presence of malignant diseases; presence of gastrectomy; and absence of or incomplete sampling data.

2.1. Laboratory testing

To measure the des-acyl ghrelin level, a fasting venous sample was carefully collected via a 21-gauge needle into a syringe containing EDTA-2Na (1.25 mg/ml) and aprotinin (Ohkura Pharmaceutical, Inc., Kyoto, Japan; 500 kallikrein inactivator U/ml) at 08:00–08:30 h. Plasma was obtained by centrifuging the whole blood at 1500 × *g* for 15 min at 4 °C, and was immediately frozen and stored at −40 °C until analysis. We used a commercially available ELISA Kit (Mitsubishi Kagaku Iatron Co., Tokyo, Japan) [15]. HMW adiponectin concentrations were measured using a two-step sandwich ELISA system (FujiRevio Inc., Tokyo, Japan), and the levels of nitrite/nitrate (NO_x) (NO_2^- and NO_3^-) in serum were measured using a high-performance liquid chromatography ultraviolet (HPLC-UV) system. Methods of other parameters are described in detail in the Supplementary materials. The intraassay and interassay coefficients of laboratory tests were all <7%.

2.2. ABPM and carotid ultrasonography

For full details, see the Supplementary materials. Briefly, as described in a previous report [16], cIMT was calculated by averaging the values from three different sites on each common carotid artery: the point of greatest thickness, and points 1 cm upstream and 1 cm downstream from the point of greatest thickness. The average of the right and left cIMT was defined as the mean cIMT.

2.3. Statistical analysis

All statistical analyses were performed with SPSS version 16.0J software (SPSS, Chicago, IL). Data are expressed as the means ± S.D. or median (25th to 75th percentile). The associations between the individual parameters were calculated using Spearman's correlation method. To identify factors associated with the development of cIMT, we used a step-wise multivariable linear regression analysis in which a *P*-value of 0.05 or less in a simple regression analysis was used as the criterion for entry into the model. Variables with skewed distribution were logarithmically transformed before analysis. We also used the κ^2 test for categorical analysis and unpaired *t*-test to compare differences between subjects with or without abdominal obesity, and the difference in cIMT was assessed using ANOVA with post hoc Bonferroni corrections. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Characteristics of the study population

Two patients who refused to participate, seven patients with incomplete ABPM, and four patients with unsatisfactory blood sampling were excluded. The characteristics of the remaining 263 subjects are shown in Table 1.

3.2. Association of HMW adiponectin with cardiometabolic markers

The plasma HMW adiponectin level was inversely correlated with BMI ($r = -0.215$, $P < 0.001$), WC ($r = -0.253$, $P < 0.001$), ever smoker ($r = -0.203$, $P < 0.001$), GFR ($r = -0.305$, $P < 0.001$), and diabetes ($r = -0.255$, $P < 0.001$), and positively correlated with age ($r = 0.353$, $P < 0.001$) and female gender ($r = 0.185$, $P < 0.001$); however, the significant correlations between HMW adiponectin and BMI and WC disappeared with aging (≥75 years; $n = 118$). The age-, sex-, and BMI-adjusted partial correlations between HMW adiponectin and cardiometabolic markers are shown in Table 2. Additional adjustments for medications, including anti-hypertensive and lipid-lowering drugs, did not alter the associations (data not shown).

Table 1
Characteristics of the study population.

	Total subjects (n = 263)
Age (years)	72.6 ± 8.4
Men, n (%)	96 (37)
BMI (kg/m^2)	24.4 ± 3.5
Waist, cm	86.0 ± 9.5
Ever smoker, n (%)	89 (34)
Anti-hypertensive medications, n (%)	214 (81)
Type 2 diabetes, n (%)	55 (21)
Stain, n (%)	62 (24)
CAD, n (%)	16 (6)
Cerebrovascular diseases, n (%)	25 (10)
GFR	69.6 ± 24.2
ABP measurement	
24-h SBP (mmHg)	134.9 ± 15.4
24-h DBP (mmHg)	77.7 ± 7.8
24-h HR (bpm)	67.2 ± 7.6
Laboratory testing	
Fasting glucose (mg/dl)	99.0 (91.0–112.0)
Insulin (μIU/ml)	5.7 (3.8–8.2)
High-density lipoprotein (mg/dl)	52.0 (44.0–61.0)
Triglycerides (mg/dl)	97.0 (71.0–131.0)
Low-density lipoprotein (mg/dl)	109.0 (91.0–132.0)
hs-CRP (mg/l)	0.58 (0.29–1.09)
PAI-1 (ng/ml)	34.0 (29.0–46.0)
IGF-1 (ng/ml)	103.0 (82.0–130.0)
NO_x (μmol/l)	32.0 (23.0–50.0)
HMW adiponectin (μg/ml)	7.0 (4.2–10.3)
Des-acyl ghrelin (fmol/ml)	108.7 (99.1–159.3)

Data are expressed as means ± S.D. or median (25th to 75th percentile). IGF-1: insulin-like growth factor-1.

3.3. Association of des-acyl ghrelin with cardiometabolic markers

The plasma des-acyl ghrelin level was inversely correlated with BMI (r = −0.155, P = 0.012) and WC (r = −0.162, P = 0.008), but showed no correlation with age, sex, smoking and drinking status, physical activity, or use of any drugs (all P = NS). Intriguingly, stronger inverse correlations between des-acyl ghrelin and BMI (r = −0.256, P = 0.005) and between des-acyl ghrelin and WC (r = −0.323, P < 0.001) were evident with aging (≥75 years). There were no correlations between plasma des-acyl ghrelin and any of the cardiometabolic markers, with the exception of the NO_x level, which showed a positive correlation with the des-acyl ghrelin level (Table 2).

3.4. Determinants of cIMT in elderly hypertensives

Table 3 shows the relation between the cIMT value and patients characteristics, and cardiometabolic markers. In a step-wise multivariate regression analysis including these significant covariates, age, smoking status, 24-h SBP, and the des-acyl ghrelin level remained independently correlated with the cIMT value (Table 4).

Table 2
Age-, sex-, and BMI-adjusted partial correlation among biomarkers.

	HMW adiponectin	Des-acyl ghrelin
Insulin	−0.364 (P < 0.001)	−0.064 (P = 0.301)
High-density lipoprotein	0.191 (P = 0.002)	0.036 (P = 0.560)
Triglycerides	−0.196 (P = 0.002)	0.014 (P = 0.821)
hs-CRP	−0.113 (P = 0.070)	0.076 (P = 0.220)
PAI-1	−0.148 (P = 0.017)	0.041 (P = 0.507)
IGF-1	0.034 (P = 0.585)	0.030 (P = 0.632)
NO_x	−0.009 (P = 0.886)	0.190 (P = 0.002)
HMW adiponectin	–	0.051 (P = 0.415)
Des-acyl ghrelin	0.051 (P = 0.415)	–

Statistical significance was defined as P < 0.05.

Table 3
Univariate analyses with cIMT in elderly hypertensives.

Variable	Spearman's correlation coefficient	P value
Age	0.407	<0.001
Sex (0 = men, 1 = women)	−0.125	0.044
Ever smoker (0 = No, 1 = Yes)	0.150	0.015
BMI	0.051	0.406
Waist	0.109	0.078
24-h SBP	0.259	<0.001
24-h DBP	−0.059	0.340
Insulin	−0.021	0.729
High-density lipoprotein	−0.117	0.059
Low-density lipoprotein	0.060	0.336
GFR	−0.262	<0.001
hs-CRP	−0.046	0.453
PAI-1	−0.122	0.048
IGF-1	−0.110	0.075
NO_x	0.051	0.419
HMW adiponectin	0.122	0.049
Des-acyl ghrelin	−0.180	0.003

Statistical significance was defined as P < 0.05.

Table 4
Multivariate analyses for determination of cIMT in elderly hypertensives.

Variable	Multivariate regression analysis*		
	$\beta^\dagger$	β (95% CI)	P value
Age	0.387	0.004 (0.003–0.005)	<0.001
Sex (0 = men, 1 = women)	0.021	–	0.785
Ever smoker (0 = No, 1 = Yes)	0.184	0.035 (0.015–0.056)	0.001
24-h SBP	0.212	0.001 (0.001–0.002)	<0.001
GFR	0.033	–	0.676
PAI-1	−0.101	–	0.068
HMW adiponectin	−0.010	–	0.863
Des-acyl ghrelin	−0.178	−0.061 (−0.097–−0.025)	0.001

$\beta^\dagger$: standardized coefficient; CI: confidence interval. Statistical significance was defined as P < 0.05.

* Variables with a P-value of 0.05 or less in a simple regression analysis with cIMT were used. Model summary: R^2 = 0.270, P < 0.001.

When NO_x was added to the model, the results were unchanged (data not shown). The significance of these explanatory variables was confirmed by dividing the population into two groups, those with cIMT above or below the median level (see Supplementary materials, Table S1). In addition, receiver–operator curves (ROC) were built to assess the power of biomarkers to predict a high cIMT level. In this way, des-acyl ghrelin was shown to be the best predictor (see Supplementary materials, Figure S1).

3.5. Effects of abdominal obesity and des-acyl ghrelin on atherosclerosis

We divided the subjects into two groups according to the presence of abdominal obesity (defined as WC ≥85 cm in men and ≥90 cm in women) [17]. cIMT was more elevated among those with abdominal obesity than in those without it (Table 5), which difference persisted even after adjustment for sex, smoking, insulin, triglycerides, hs-CRP, PAI-1, and HMW adiponectin (P = 0.022). However, this association was no longer significant after adding 24-h SBP (P = 0.151) or des-acyl ghrelin (P = 0.061) to the model. When added NO_x added to the model, the result was unchanged (data not shown).

4. Discussion

Our data indicate that although reductions of both plasma des-acyl ghrelin and plasma HMW adiponectin are associated with obesity, only the former is a useful cardiometabolic marker for pre-

Table 5
Anthropometric, hemodynamic, and cardiometabolic parameters in elderly hypertensives with or without abdominal obesity.

	Total subjects (n = 263)		P value
	Non-obesity (n = 136)	Abdominal obesity (n = 127)	
Age (years)	73.3 ± 8.2	71.9 ± 8.7	0.153
Men, n (%)	35 (26)	61 (48)	<0.001
BMI (kg/m^2)	22.3 ± 2.3	26.6 ± 3.1	<0.001
Waist (cm)	79.3 ± 6.1	93.1 ± 7.0	<0.001
A BP measurement			
24-h SBP (mmHg)	131.8 ± 12.7	138.3 ± 17.2	0.001
24-h DBP (mmHg)	76.5 ± 7.1	78.9 ± 8.3	0.010
Laboratory testing			
Insulin (μIU/ml)	4.8 (3.2–6.6)	6.9 (5.1–10.6)	<0.001
Triglycerides (mg/dl)	88.0 (68.0–119.8)	107.0 (79.0–154.0)	<0.001
hs-CRP (mg/l)	0.44 (0.21–0.93)	0.66 (0.36–1.28)	0.004
PAI-1 (ng/ml)	32.0 (27.0–40.0)	38.0 (30.0–54.0)	0.001
NO_x (μmol/l)	33.0 (23.0–50.0)	31.0 (23.0–51.0)	0.974
HMW adiponectin (μg/ml)	7.8 (5.0–11.3)	5.2 (3.5–8.9)	<0.001
Des-acyl ghrelin (fmol/ml)	116.8 (81.4–193.9)	98.3 (70.7–140.5)	0.003
cIMT (mm)	0.782 ± 0.163	0.833 ± 0.185	0.019

Data are expressed as means ± S.D. or median (25th to 75th percentile). Statistical significance was defined as $P < 0.05$.

dicting atherosclerosis in elderly hypertensives. Although there was a significant association between the des-acyl ghrelin and NO_x levels, the association of des-acyl ghrelin with atherosclerosis appears to be independent of the NO_x level. Because of previous experimental studies in which the cardiovascular protective effect of des-acyl ghrelin was suggested to be robust [12–14], the hypothesis that des-acyl ghrelin protects against the development of atherosclerosis is attractive. Our data warrant further investigation of the pathologic mechanisms responsible for this phenomenon and to clarify the prognostic value of this peptide with respect to cardiovascular events in the future.

4.1. Effects of HMW adiponectin on atherosclerosis in elderly hypertensives

Although the cardiovascular risks of obesity in elderly persons are still debatable [1,7], our data showed that elderly persons with abdominal obesity had a significantly higher level of cIMT. The mechanisms of this relation were not explained by the HMW adiponectin level or its related cardiometabolic parameters (glucose and lipid metabolites, inflammation, and hemostasis), despite the fact that the absolute magnitude of their difference between those with and without abdominal obesity was larger than for the des-acyl ghrelin level. Furthermore, the HMW adiponectin level could not predict the higher level of cIMT. These observations raised at least two possibilities. First, the detrimental effects of these parameters on atherosclerosis are generally weaker in elderly than middle-aged persons [1,5,8], which may be partially explained by the survival-effects [18]. Second, because HMW adiponectin could be influenced by many physiological and pathophysiological factors, interpretation of the HMW adiponectin level in elderly persons should be undertaken with caution. In fact, our study showed that the significant inverse association between HMW adiponectin and obesity [3–5] becomes attenuated with aging (≥75 years). This may be partly explained by the fact that the HMW adiponectin level is proportionally increased with age and impaired renal function, and thus an individual difference in advanced aging might become inconspicuous. Furthermore, our data show a significant inverse correlation between weight change from 20 years of age to current weight and HMW adiponectin levels (data not shown), suggesting a possibility that HMW adiponectin levels among the elderly are modulated by systemic energy valance [5–7]. Because weight decline (called *wasting*) is associated with an adverse cardiovascular outcome in elderly persons [8], high levels of HMW adiponectin might also reflect harmful signals in the body in advanced age [5,6].

4.2. Des-acyl ghrelin and atherosclerosis in elderly hypertensives

In contrast to HMW adiponectin, the inverse correlation between des-acyl ghrelin and obesity was particularly strong in our subjects with advanced aged (≥75 years). The mechanisms responsible for the reduction of des-acyl ghrelin in obesity remain unknown, but at least two possible explanations could be considered. First, the decreased activity of acylation enzyme or increased activity of endogenous esterase may occur in obesity. Barazzoni et al. reported that abdominal fat accumulation leads to accelerated ghrelin acylation in conjunction with a decrease in the des-acyl ghrelin levels [19]. Our study did not measure the plasma level of acylated ghrelin, because the measurement of this parameter is technically complex (e.g., acidified plasma with a 1/10 volume of 1N HCl is needed), and thus this hypothesis remains untested. Second, the decreased des-acyl ghrelin level may be a consequence of unmeasured abnormal adipochemokines or physiological adaptation to the positive energy balance associated with obesity. Further research is needed in this area.

In the current study, the des-acyl ghrelin level showed a negative correlation with cIMT independently of age, WC, sex, smoking, 24-h BP and renal function. Because the des-acyl ghrelin level parallels the acylated ghrelin level [15], our data may simply confirm those of the previous report in which decreased total ghrelin levels were associated with the progression of cIMT in elderly patients with metabolic syndrome [20]. However, numerous studies suggest that des-acyl ghrelin itself shows a wide array of cardiovascular activities [10–14], and thus des-acyl ghrelin may exert independent effects on the cardiovascular system. The pathologic pathways linking des-acyl ghrelin and cIMT remain unclear, and are probably not related to factors such as hemodynamic, metabolic or inflammatory pathways. That des-acyl ghrelin was significantly correlated only with the NO_x level suggests the possibility that des-acyl ghrelin may increase NO bioactivity. This consideration is supported by the recent report of Tesauro et al., which demonstrated that administration of des-acyl ghrelin reversed endothelial dysfunction by increasing NO bioactivity [13]. Des-acyl ghrelin is as effective as ghrelin in activating intracellular signaling pathways (i.e., ERK-

1/2, Akt) [14] that could be involved in endothelial NO production. However, our data indicate that the association of des-acyl ghrelin with atherosclerosis is independent from the NO_x level, thus the mechanisms responsible for this phenomenon could not be clarified in the present investigation. Nevertheless, because previous experiments showed a cardiovascular protective effect of des-acyl ghrelin [12–14], it is feasible that des-acyl ghrelin protects against the development of atherosclerosis. The atherosclerotic risks of abdominal obesity in our elderly persons were largely explained by elevated 24-h BP and reduced des-acyl ghrelin levels, suggesting the possibility that an increase in the des-acyl ghrelin level, in addition to BP control, may counteract the obesity-related atherosclerosis in elderly persons.

Our study has several limitations. First, because of the cross-sectional nature of our data, we cannot infer any causality. Hopefully longitudinal follow-up data will be able to provide some insights. Second, because our study was conducted in elderly hypertensives, caution should be used in applying the results to different groups. Third, all cardiometabolic parameters were measured only once. Finally, medication use may be potentially confounding, although our results were not changed after adjustment of these factors as a covariate.

In conclusion, the current study has demonstrated that the plasma des-acyl ghrelin level is a suitable predictor of cardiovascular risk in elderly hypertensives. The prognostic value of this peptide with respect to cardiovascular events will be addressed in a follow-up study in the present study population. In addition, the cardiovascular protective effect of des-acyl ghrelin suggests that the peptide could play a modulating role in atherosclerosis, especially in obese subjects. Further intervention studies will be needed to clarify this possibility.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2008.10.013.

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Effects of Long-Term Intravenous Administration of Adrenomedullin (AM) Plus hANP Therapy in Acute Decompensated Heart Failure

— A Pilot Study —

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Background: It was reported previously that 30 min administration of adrenomedullin (AM) improves hemodynamics in chronic stable heart failure patients. The present study was designed to examine whether long-term AM+human atrial natriuretic peptide (hANP) administration can be used as a therapeutic drug in patients with acute decompensated heart failure (ADHF) in clinical setting.

Methods and Results: Seven acute heart failure patients (74±5 years) with dyspnea and pulmonary congestion were studied. AM ($0.02\mu g \cdot kg^{-1} \cdot min^{-1}$)+hANP ($0.05\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused for 12 h and then hANP ($0.05\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused for 12 h. Hemodynamic, renal, hormonal and oxidative stress responses were evaluated. AM+hANP significantly reduced mean arterial pressure, pulmonary arterial pressure and systemic and pulmonary vascular resistance without changing heart rate, and increased cardiac output for most time-points compared with those at baseline. In addition, AM+hANP reduced aldosterone, brain natriuretic peptide and free-radical metabolites compared with those at baseline (all $P<0.05$). AM+hANP increased urine volume and $U_{Na}V$ compared with baseline data.

Conclusions: In this small, pilot trial, AM+hANP therapy had beneficial hemodynamic and hormonal effects in ADHF. Intravenous infusion of AM with hANP could be used as a therapeutic drug in ADHF. These data are preliminary and require confirmation in a larger clinical study. (*Circ J* 2009; **73:** 892–898)

Key Words: Acute decompensated heart failure; Adrenomedullin; Atrial natriuretic peptide; Brain natriuretic peptide; Oxidative stress

Adrenomedullin (AM), a strong vasodilatory peptide, was originally isolated from human pheochromocytoma[1] Infusion of AM causes vasodilatation, diuresis and natriuresis in normal animals[2] AM also increases cardiac output and left ventricular contractility in vivo and exerts a direct inotropic effect in vitro[3] We and others have shown that plasma AM levels are increased in patients with congestive heart failure[4,5] Tissue levels of the AM peptide and mRNA have also been shown to be increased in the heart, kidney and lungs of rats with congestive heart failure[6] These findings suggest that AM may play a role in the regulation of volume and pressure homeostasis in congestive heart failure as a paracrine and/or autocrine factor, and as a circulating hormone. In addition, we reported previously beneficial hemodynamic and renal effects of AM infusion in animals with congestive heart failure[7] In humans, systemically administered AM has been shown to decrease mean arterial pressure (MAP) significantly in healthy subjects without any adverse effects[8] These findings raise the possibility that intravenous infusion of AM may also be beneficial in human subjects with heart failure. Indeed, we and other investigators demonstrated previously that short-term infusion of AM increased the cardiac index (CI) and decreased mean pulmonary arterial pressure (mPA) only in patients with chronic stable heart failure[9,10] In comparison with human atrial natriuretic peptide (hANP), AM is more potent in decreasing vascular resistance and enhancing cardiac output, and less potent in diuresis and natriuresis![11] The infusion of hANP is currently used as a treatment for acute decompensated heart failure (ADHF) in Japan; however, some of the patients with ADHF are resistant to hANP monotherapy. Taken together, these results suggest that AM+ANP therapy may be used as a therapeutic drug in ADHF. However, it is not known whether long-term AM+ANP infusion in ADHF is beneficial or not.

Therefore, our aim in the present study was thus to investigate if long-term AM+ANP infusion therapy was effective in terms of hemodynamics, renal function and hormone levels in patients with ADHF in a real clinical setting.

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Methods

The present study was approved by the ethics committee of the Dokkyo Medical University, and all patients gave written informed consent.

Study Subjects

Seven patients with ADHF who were admitted to our hospital with a prime complaint of dyspnea were studied. Chest X-rays in all patients showed cardiomegaly with pulmonary congestion. After written informed consent was obtained, baseline blood tests and echocardiography were performed. Patients with one of the following conditions were excluded: (1) chronic renal impairment (serum creatinine level 2.0 mg/dl); (2) systolic blood pressure <100 mmHg; or (3) the presence of aortic stenosis or mitral stenosis. The baseline clinical characteristics and hemodynamics in the present study are shown in **Table**.

Table. Patient Characteristics

Age (years)	74±5
M/F	5/2
BMI (kg/m^2)	25.7±5.2
NYHA (III/IV)	5/2
Cause of HF (IHD/valvular)	4/3
BNP (pg/ml)	1,350±1,187
Cre (mg/dl)	1.0±0.5
Echocardiographic findings	
LVDd	61±6
LVDs	46±8
EF	39±10
MR (II/III/IV)	2/1/4
AR (III)	1
Baseline hemodynamic data	
MAP	98±17
SVR	1,699±529
CI	2.54±0.80
HR	73±14
mPA	45±15
PAR	370±252
PCWP	27±9

BMI, body mass injdex; NYHA, New York Heart Association; HF, heart failure; MAP, mean arterial pressure; SVR, systemic vascular resistance; CI, cardiac index; HR, heart rate; mPA, mean pulmonary arterial pressure; PAR, pulmonary arterial resistance; PCWP, pulmonary capillary wedge pressure.

Preparation of Human AM

Human AM was obtained from Peptide Institute Inc, Osaka, Japan. The homogeneity of human AM was confirmed using reverse-phase, high-performance liquid chromatography and amino acid analysis. AM was dissolved in saline with 4% D-mannitol and sterilized through a 0.22-μm filter (Millipore Co, Billerica, MA, USA). Then, randomly selected vials were submitted for sterility and pyrogen testing, as reported previously.[10] The chemical nature and content of the human AM in the vials were verified using high-performance liquid chromatography and radioimmunoassay.

Study Protocol

All patients were hospitalized in our intensive care unit. A 7.5-F Swan-Ganz catheter (TOO21H-7.5F, Baxter Co, Deerfild, IL, USA) was positioned in the pulmonary artery through a jugular vein. One 22-gauge cannula was inserted into a radial artery for hemodynamic measurements and blood sampling. Another 22-gauge cannula was inserted into a forearm vein for the infusion of 0.9% hANP, with or without AM. A bladder catheter was inserted for urine sampling. During an equilibration period of 60 min, baseline hemodynamic, renal and blood samples for hormonal measurements were obtained. Then, AM (0.02 $\mu g \cdot kg^{-1} \cdot min^{-1}$) + hANP (0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$) were administered intravenously at a rate of 0.5 ml/min for 12 h, followed by 12 h of hANP (0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$) infusion (**Figure 1**). Hemodynamic parameters, including heart rate (HR), MAP, mPA, pulmonary capillary wedge pressure (PCWP) and cardiac output, were continuously monitored and measured at 60-min intervals during the protocol. Blood samples were taken before, 12 h after AM + hANP infusion and 12 h after hANP monotherapy. Urine samples were obtained every 60 min. Urine volume, urinary sodium excretion, urinary potassium excretion, urinary cAMP and cGMP excretion were measured and calculated using standard formulas.

Hormone and Oxidative Stress Marker Measurement

Plasma total AM, mature AM, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) levels were measured using immunoradiometric assays with a specific kit for each marker (Shionogi Co, Ltd, Osaka, Japan).[11] Plasma cyclic adenosine 3', 5'-monophosphate (cAMP), cyclic guanosine 3', 5'-monophosphate (cGMP), renin, aldosterone and norepinephrine (NE) were measured with commercially available kits.[12] Reactive oxygen metabolite (d-ROM) was

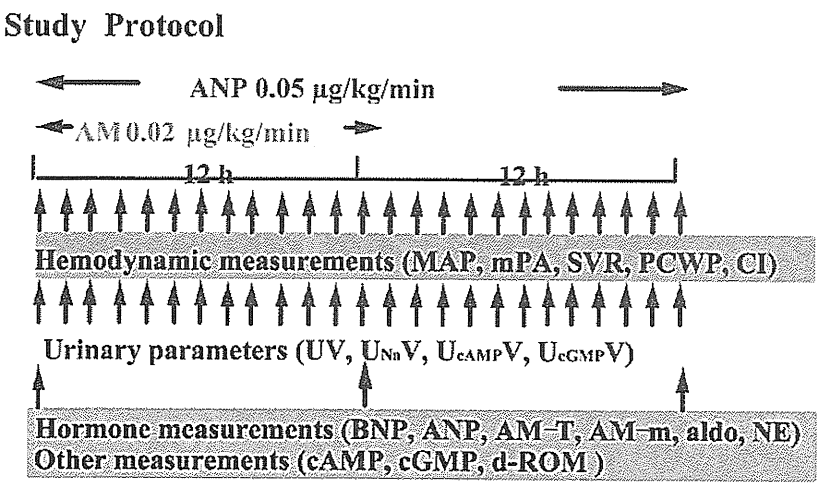

Figure 1. Study protocol. After a 60-min baseline period, AM (0.02 $\mu g \cdot kg^{-1} \cdot min^{-1}$) + hANP (0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was administered intravenously for 12 h, followed by hANP (0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$) monotherapy for 12 h. AM, adrenomedullin; AM-m, AM-mature; AM-T, AM-total; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; cAMP, cyclic adenosine 3', 5'-monophosphate; CI, cardiac index; cGMP, cyclic guanosine 3', 5'-monophosphate; d-ROM, reactive oxygen metabolite; hANP, human atrial natriuretic peptide; MAP, mean arterial pressure; mPA, mean pulmonary arterial pressure; NE, norepinephrine; PCWP, pulmonary capillary wedge pressure; SVR, systemic vascular resistance.

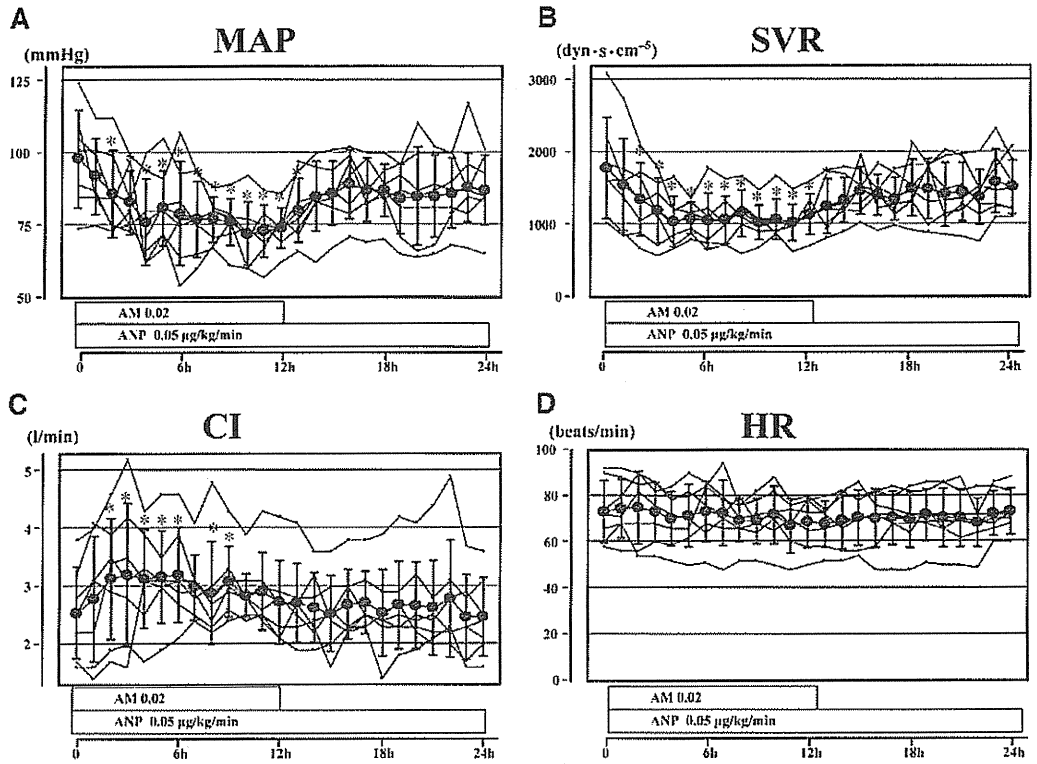

Figure 2. Systemic hemodynamic [(A) MAP, (B) SVR, (C) CI and (D) HR] changes during the infusion of AM+hANP and hANP therapy. Data are mean±SD. *P<0.05 vs value at time 0. HR, heart rate. Other abbreviations see in Figure 1.

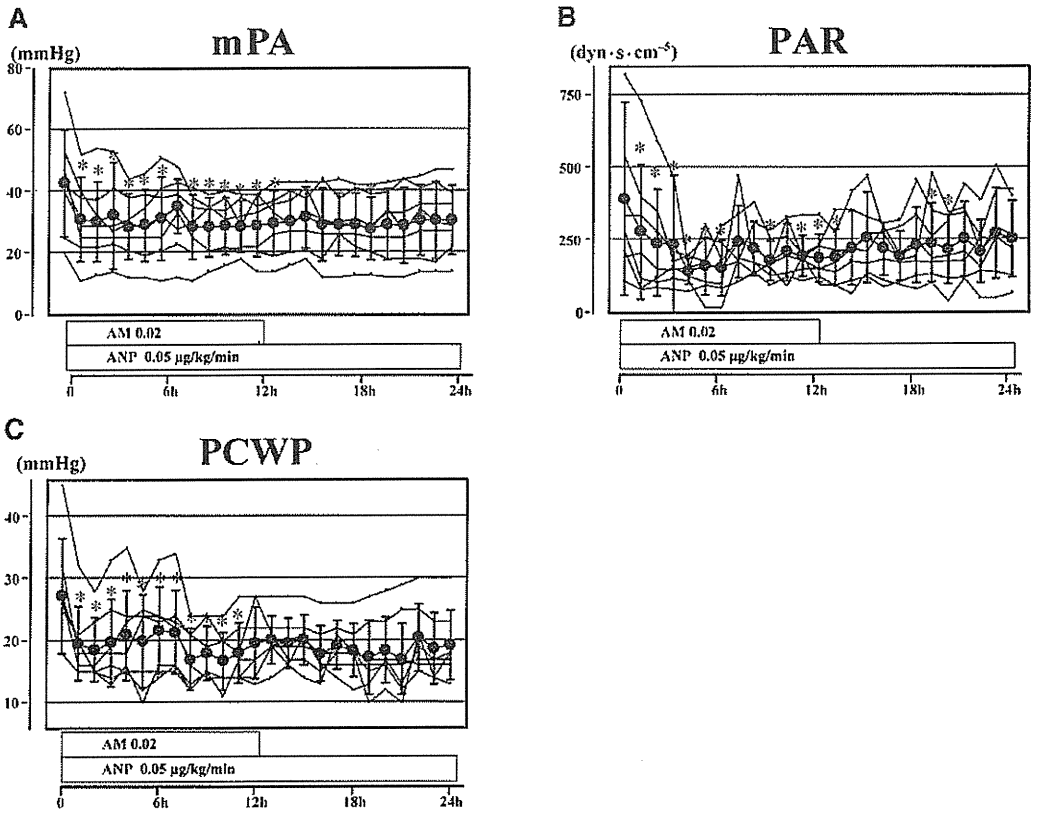

Figure 3. Pulmonary hemodynamic [(A) mPA, (B) PAR and (C) PCWP] changes during the infusion of AM+hANP and hANP therapy. Data are mean±SD. *P<0.05 vs value at time 0. PAR, pulmonary arterial resistance. Other abbreviations see in Figure 1.

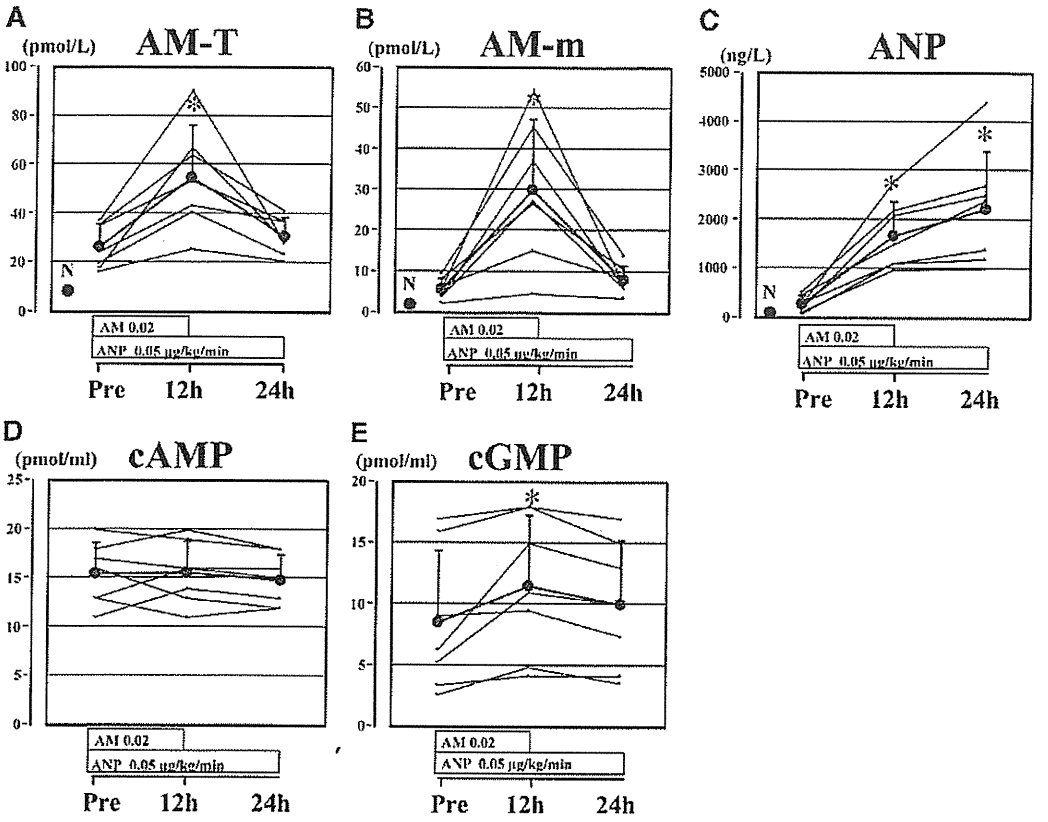

Figure 4. Hormonal [(A) AM-T, (B) AM-m, (C) ANP, (D) cAMP and (E) cGMP] changes during infusion of AM+hANP and hANP therapy. Data are mean±SD. *P<0.05 vs value at time 0. Abbreviations see in Figure 1.

measured using a commercially available kit (H&D, s.r.l., Parma, Italy).

Statistical Analysis

All data were expressed as mean±SD unless otherwise indicated. Comparisons of the parameters between the baseline data and each time point were made using a paired Student's t-test. Log transformation was completed for plasma BNP and ANP levels. P<0.05 was considered statistically significant.

Results

All subjects tolerated the present study protocol. No obvious side effects were observed in blood chemistry tests such as liver function, renal function, electrolytes or hemograms. The 2 of 7 patients had mild skin flushing in the body during the AM+hANP therapy. This disappeared soon after switching to ANP monotherapy.

Hemodynamic Responses to AM and hANP

The infusion of AM+hANP significantly decreased MAP, systemic vascular resistance (SVR), and increased CI (**Figures 2A–C**) at most of the time-point compared with the baseline levels, whereas there were no changes in HR (**Figure 2D**). Infusion of AM+hANP also significantly decreased mPA, pulmonary vascular resistance and PCWP at most time-points compared with the baseline values (**Figures 3A–C**).

Hormonal and Oxidative Stress Responses to AM and hANP

Baseline plasma total AM, mature AM and ANP were significantly elevated in patients with heart failure and were comparable with previous reports (**Figures 4A–C**). At the end of AM+hANP infusion, plasma total AM, mature AM and ANP increased about 2-fold, 6-fold and 6-fold, respectively. After switching to hANP monotherapy, plasma total AM and mature AM decreased to near baseline levels, whereas plasma ANP increased further (**Figures 4A–C**).

Infusion of AM+hANP significantly increased the plasma level of cGMP, which is a secondary messenger for ANP, BNP and nitric oxide (**Figure 4E**). Plasma levels of cAMP, one of the secondary messengers of AM, did not change significantly during the study period (**Figure 4D**).

The effects of infusion of AM+hANP on plasma BNP, aldosterone, NE, PRA, and d-ROM are shown in **Figure 5**. Infusion of AM+hANP significantly decreased plasma BNP and aldosterone levels (**Figures 5A,B**). In contrast, NE or PRA levels did not change (**Figures 5C,D**). Interestingly, the infusion of AM+hANP significantly decreased d-ROM levels, a marker of oxidative stress (**Figure 5E**).

Renal Urinary Responses to AM and hANP

Infusion of AM+hANP tended to increase urine volume, urinary sodium excretion and urinary cAMP excretion compared with the baseline level and these changes reached statistical significance at several points; however, there seemed to be no differences in these parameters between AM+hANP and hANP monotherapy. Whereas urinary cGMP excretion was significantly higher for almost entire period compared

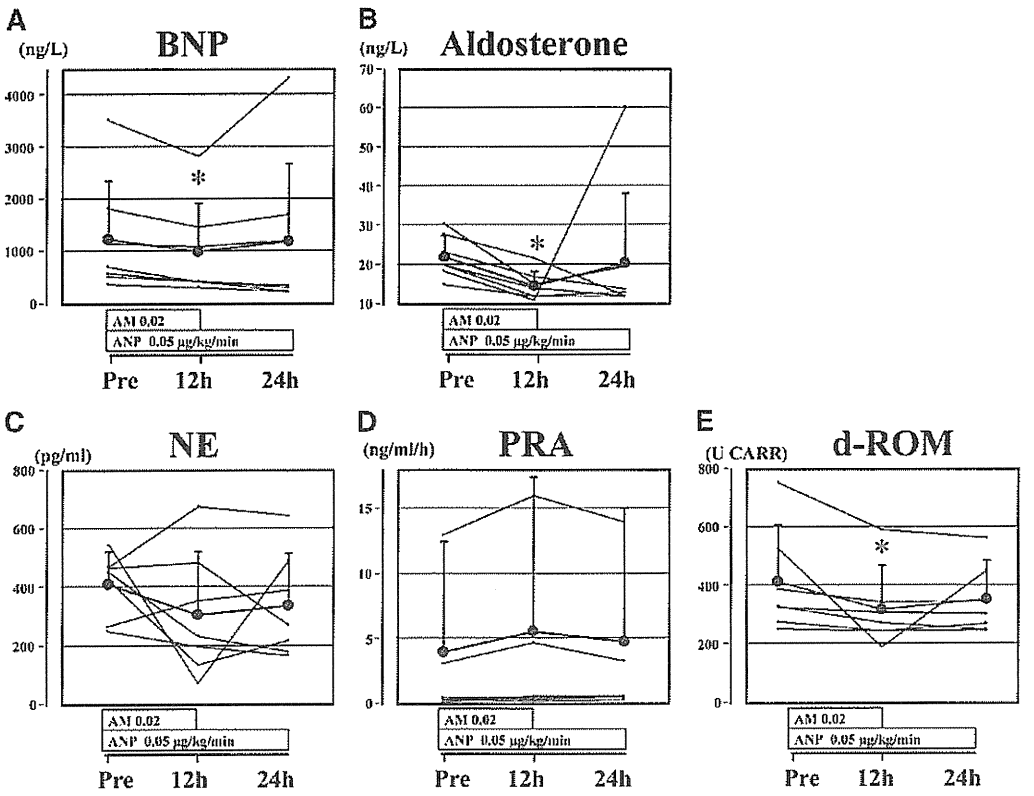

Figure 5. Hormonal [(A) BNP, (B) aldosterone, (C) NE and (D) PRA] and (E) oxidative stress marker (d-ROM) changes during the infusion of AM+hANP and hANP therapy. Data are mean±SD. *P<0.05 vs value at time 0. Abbreviations see in Figure 1.

with the baseline level (data not shown).

Discussion

In our small pilot trial, the administration of combined AM+hANP in patients with ADHF was effective in reducing MAP, SVR, mPA, PAR, PCWP, BNP, aldosterone and d-ROM, and in increasing CI, UV, $U_{Na}V$, $U_{cAMP}V$ and $U_{cGMP}V$. These results suggest that long-term AM+hANP infusion may be an effective treatment for patients with ADHF in a real clinical setting due to the reduction of systemic and pulmonary vascular resistance, positive inotropic effects, inhibitory action of aldosterone secretion and oxidative stress production.

In the present study, AM+hANP therapy significantly decreased SVR, MAP, PAR and mPA without changing HR. Previous studies have demonstrated that AM directly dilated vascular smooth muscle cells in a cAMP-dependent manner[13] A further study also showed that AM dilates vessels via a cGMP cascade with the production of nitric oxide[14,15] In the present study, plasma cAMP levels did not increase, maybe due to the low dose of AM ($0.02\,\mu g \cdot kg^{-1} \cdot min^{-1}$), because our previous studies showed that higher doses of AM ($0.05\,\mu g \cdot kg^{-1} \cdot min^{-1}$) increased plasma cAMP levels in patients with chronic heart failure and pulmonary hypertension[10,16] Thus, whether plasma cAMP increased or not appeared to depend on the dose of AM used. HR did not increase in the present study. Previous studies have demonstrated that AM induced an increase of HR[10] This discrepancy of the results between two studies may be explained by the two reasons: (1) a low dose of AM ($0.02\,\mu g \cdot kg^{-1} \cdot min^{-1}$) was used in the present study compared with the previous study ($0.05\,\mu g \cdot kg^{-1} \cdot min^{-1}$); and (2) hANP was concominantly used in the present study. Because hANP is known to exert a sympathoinhibitory action in heart failure, AM-induced reflex-mediated sympathetic activation may be blunted. Thus, a combination of AM+hANP would aid HR stability through mutual effects[17]

In the present study, AM+hANP therapy increased CI. Because AM+hANP therapy reduced SVR and PAR significantly, the observed increase of CI may be in part due to a reduction of afterload. In addition, several reports demonstrated that AM has positive inotropic effects. Szokodi et al reported that AM enhances cardiac contractility via cAMP-independent mechanisms, including a Ca^{2+} release from intracellular ryanodine- and thapsigargin-sensitive Ca^{2+} stores, activation of protein kinase C and Ca^{2+} influx through L-type Ca^{2+} channels[18] In agreement with these findings, Nagaya et al demonstrated that intravenous AM enhances left ventricular myocardial contraction and improves left ventricular relaxation without increasing myocardial oxygen consumption in patients with left ventricular dysfunction[19] Thus, positive inotropic effects from AM, not mediated via the cAMP/PKA pathway, may be useful in the treatment for ADHF.

Interestingly, AM+hANP therapy reduced BNP, aldosterone and d-ROM levels. The reduction of BNP is considered to be due to hemodynamic improvement, including reduction of SVR, PAR and PCWP[20] Interestingly, AM+hANP therapy reduced plasma aldosterone levels. Previous studies demonstrated that AM inhibits aldosterone production induced by angiotensin II, potassium and Ca^{2+} ion-

ophores in dispersed zona glomerulosa cells[2,3] In vivo, AM prevents increased plasma aldosterone levels induced through the infusion of angiotensin II, a sodium-deficient diet or bilateral nephrectomy[21,22] These findings suggest that AM may have a role in inhibiting aldosterone secretion from zona glomerulosa cells. In addition, intravenous infusion of AM reduced aldosterone levels in humans[10,11,16] Thus, it is possible that AM directly inhibits aldosterone secretion in heart failure. Several lines of evidence show that AM has antioxidative effects[2,3] A recent study has demonstrated that angiotensin II-induced reactive oxygen species production through the activation of NADPH oxidase was significantly attenuated by AM in a concentration-dependent manner[23] Increased oxidative stress plays a major role in the pathogenesis of heart failure[24,25] Thus, the inhibitory effects of AM on aldosterone secretion and production of reactive oxidative species may be useful in the treatment for heart failure.

AM+hANP therapy appeared to increase UV, $U_{Na}V$, $U_{cAMP}V$ and $U_{cGMP}V$, whereas these variables did not change significantly after switching to hANP monotherapy. This suggests that the observed renal effects of AM+hANP therapy may be mainly due to a hANP effect. Many studies have demonstrated that AM has renal vasodilatory, natriuretic and diuretic actions[2] We also reported previously that intravenous infusion of AM ($0.05\,\mu g \cdot kg^{-1} \cdot min^{-1}$) increases GFR, UV and $U_{Na}V$ in rat and human heart failure[10,11,16] The possible reasons why an obvious renal effect from AM was not observed may be due to: (1) the low dose of AM ($0.02\,\mu g \cdot kg^{-1} \cdot min^{-1}$) used; and (2) the severe intensity of heart failure in the present study.

Thus, the present study indicated that the combination of AM and hANP would be good for: (1) potential strong preload and afterload reduction; (2) HR stability through mutual suppression of an AM-induced-HR-increase with hANP and a hANP-induced-HR-decrease with AM; and (3) neurohumoral changes.

We have the following limitations of the present study: (1) the study had a small number of cases and did not have enough subjects to detect a statistical difference in all time-points between baseline values and AM+hANP therapy with regard to hemodynamic parameters; (2) a fixed dose of AM was used regardless of the severity of heart failure, thus the response to the AM+hANP therapy might be blunted in severe ADHF patients; (3) subjects with different heart failure etiologies were included in the present study; and (4) patients with relatively different severities of heart failure were included. Despite these heterogeneities, AM+hANP therapy could show some beneficial hemodynamic and hormonal effects in ADHF.

In summary, we evaluated the effect of AM+hANP therapy in a small pilot study of patients with ADHF. The administration of AM+hANP was associated with a reduction in SVR, PAR, PCWP, mPA, MAP, aldosterone, BNP and d-ROM, and with an increase in CI, UV, $U_{Na}V$, $U_{cAMP}V$ and $U_{cGMP}V$. These data are preliminary and require confirmation in a larger clinical trial.

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