SCIENTIFIC LETTERS

Dilated cardiomyopathy and *Chlamydia pneumoniae* infection

Dilated cardiomyopathy (DCM), characterised by a severe dysfunction of the heart muscle, often results from a myocarditis, which could be caused by a variety of organisms or mediated by autoimmune responses to the exposure of cardiac specific antigen, such as myosin, after cardiomyocyte damage. DCM of humans can be experimentally reproduced in susceptible mouse strains by immunisation with purified cardiac myosin.[1] Moreover, chlamydia infection may mediate heart disease through an antigenic mimicry between the chlamydia outer membrane protein and muscle specific α myosin of the heart.[2] The aim of this study was to investigate the prevalence of *Chlamydia pneumoniae* infection by measuring the serum IgG and IgA antibodies against *C pneumoniae* in patients with DCM.

Twenty six consecutive patients with DCM, aged 56.9 (15.5) years, diagnosed according to the World Health Organization/International Society and Federation of Cardiology criteria,[3] were enrolled. Twenty eight healthy subjects, mean (SD) age 57.4 (13.7) years, matched for age and sex, were used as the controls; they were chosen from the subjects undergoing coronary angiography during the same period and not showing any significant coronary stenosis, valvar disease or other cardiovascular diseases. None of the patients had a clinical history of specific heart muscle disease that may have caused left ventricular dysfunction nor any systemic and endocrine diseases. All patients were classified into New York Heart Association (NYHA) functional class I–IV based on the presence or absence of dyspnoea during their daily activities. Routine ECGs, chest radiographs, two dimensional echocardiograms, and coronary angiography were performed on all the patients.

Serum concentrations of anti-*C pneumoniae* specific IgG and IgA antibodies were measured using a new enzyme linked immunosorbent assay (ELISA) method that uses *C pneumoniae* specific outer membrane protein as the antigen (Hitachi Chemical Co, Tokyo, Japan). Recent investigations have shown that the ELISA methods is more sensitive and specific than immunofluorescence used previously for detecting the antibodies to *C pneumoniae*, and it may be the preferred tool for diagnosing chlamydia infections in routine clinical practice.[4] Briefly, specimens, diluted 210-fold for IgG and 21-fold for IgA, were added to microtitre plates packaged by specific antigen and the optical density was measured at 405 nm after incubation. The correction coefficient and the cut-off point were determined by the mean absorption of the positive and negative controls, then the cut-off values of the samples were calculated using the formula of "sample's absorption" (correction coefficient/cut-off point). The seropositivity of both IgG and IgA antibodies was defined as the following criteria: cut-off value < 1.10 for negative (−), ⩾ 1.10 positive (+), respectively. Data were expressed as the mean (SD) or percentage deviation and the analysis was performed using the Statistical Analysis System (SAS). Differences between the two groups were assessed with the unpaired Student's *t* test for continuous variables and χ^2 test for categorical variables. Significance was established at the $p < 0.05$ level.

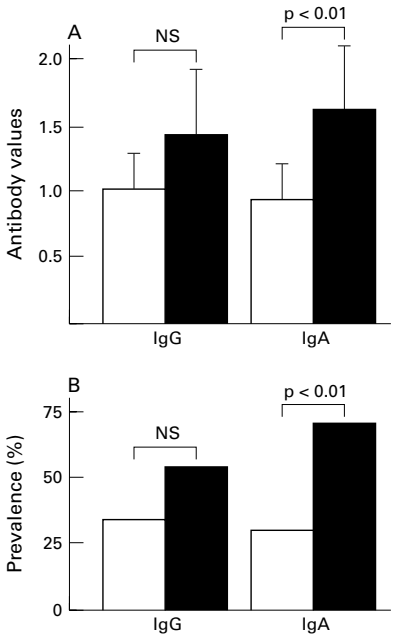

Figure 1 (A) Values and (B) prevalence of Chlamydia pneumoniae IgG and IgA antibodies in dilated cardiomyopathy patients (n = 26) (black bars) and controls (n=28) (white bars). NS, not significant.

The patients with DCM were characterised by the clinical manifestations of left ventricular dysfunction or congestive heart failure, such as tachycardia (88.5 (31.0) *v* 70.1 (10.8) beats/min, $p < 0.05$), increased cardiothoracic ratio (58.6 (7.5) *v* 49.9 (5.6), $p < 0.001$), and higher NYHA score (2.3 (0.7) *v* 1.3 (0.5), $p < 0.001$). Moreover, the serum concentrations of acute phase proteins, which reflect the degree of inflammation, such as C-reactive protein (1.8 (3.5) *v* 0.1 (0.2) mg/dl, $p < 0.05$) and fibrinogen (382.2 (112.5) *v* 278.4 (51.6) mg/dl, $p < 0.001$), were significantly higher in DCM subjects compared with controls. The echocardiographic data also suggested left ventricular functional impairment in patients with DCM, such as left ventricular diastolic dimension (64.8 (10.1) mm *v* 46.5 (4.4) mm, $p < 0.001$) and left ventricular systolic dimension (55.2 (10.0) mm *v* 29.3 (5.2) mm, $p < 0.001$). As shown in fig 1, after adjusting for age and sex, the mean value of IgA antibody against *C pneumoniae* in the controls were significantly different from the DCM patients (0.9 (0.6) *v* 1.6 (1.0), $p < 0.01$). However, no significant difference in IgG value was observed between the two groups (1.0 (0.6) *v* 1.4 (0.9)). The prevalence of IgA antibody was detected in 17/26 (65.4%) of DCM patients and in 8/28 (28.6%) of the controls ($p < 0.01$), but no significant difference was observed for IgG prevalence between the two groups (14/26 (53.8%) *v* 10/28 (35.7%)).

DCM could be mediated by autoimmune responses to the cardiac α myosin heavy chain, by which inflammatory heart disease in humans can be experimentally reproduced in susceptible mouse strains.[5] A recent study has proved that a peptide from the murine cardiac α myosin heavy chains that has sequence homology to the 60 kD cysteine-rich outer membrane protein of chlamydia was shown to induce autoimmune inflammatory heart disease in mice, suggesting chlamydia mediated heart disease is induced by antigenic mimicry of a heart muscle specific protein.[2] On the other hand, *C pneumoniae*, previously known as TWAR-strain, is an obligate intracellular parasite and tends to cause persistent infection. Several cases of myocarditis caused by *C pneumoniae* infection have been reported and TWAR was recently identified by polymerase chain reaction in the myocarditis heart.

Our study showed that there was a higher value and more frequent seropositivity of IgA antibody against *C pneumoniae* in patients with DCM, indicating that the prevalence of persistent infection with *C pneumoniae* was higher in patients with DCM because an increased IgA antibody often reflects an active or persistent infection, but the level IgG antibody reflects only a previous or past infection with *C pneumoniae* infection.[5] Although the role of *C pneumoniae* infection in the pathogenesis of DCM was not fully elucidated in this study, it could be speculated that *C pneumoniae* infection associates with DCM by at least three mechanisms as follows: causing chronic and persistent inflammation of the heart muscle; damaging the infected cardiomyocytes and exposing myocardial myosin to the immune system; and inducing an autoimmune response by its antigenic mimicry with a cardiac myosin peptide.

Our study provides the first clinical evidence that DCM in humans could be associated with persistent *C pneumoniae* infection. Since the infection is treatable, an attempt at early diagnosis and effective antibiotic treatment against *C pneumoniae* might be considered in patients with cardiomyopathy.

H SONG
H TASAKI
A YASHIRO
K YAMASHITA
T TOYOKAWA
Y NAGAI
H TAKATSU
H TANIGUCHI*
Y NAKASHIMA
2nd Department of Internal Medicine,
**Department of Microbiology*
School of Medicine,
University of Occupational and Environmental Health, Kitakyushu, Japan

Correspondence to: Dr Hiromi Tasaki, 2nd Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan: h-tasaki@med.uoeh-u.ac.jp

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β Blockers enhance early diastolic filling in ischaemic heart disease: a radionuclide assessment

There is accumulating evidence that β blockers improve symptoms and prognosis in patients with ischaemic heart disease (IHD).[1] Impaired filling of the left ventricle is often found in association with IHD.[2] Improvement in diastolic left ventricular function may be an important mechanism of action of β blockers. This study investigated the effect of β adrenergic blockade on diastolic filling in patients with IHD.

We carried out a retrospective analysis of left ventricular filling in 265 consecutive patients attending our department for routine clinical evaluation of chest pain. All patients underwent our standard imaging protocol of thallium-201 myocardial perfusion imaging and list mode radionuclide ventriculography (RNVG). Left ventricular filling was assessed using RNVG, which has been previously validated for the assessment of cardiac diastolic function.[3 4] It has also been extensively used in the assessment of left ventricular filling in various conditions.[4]

Background corrected left ventricular activity–time curves were generated using a fixed left ventricular and background region of interest. Left ventricular ejection fraction (LVEF) was calculated in the usual manner. The normal lower limit of LVEF with this technique is 40% in our institution. The percentage of left ventricular filling in the first third of the diastolic period was defined as the first third filling fraction (1/3FF), expressed as a percentage of the stroke volume. The previously described heart rate adjusted first third filling fraction index (which is equal to 1/3FF/R-R interval) was also used.[5]

Values are expressed as mean (SD). Spearman's correlation coefficient (r_s) was used for correlation between variables. Non-parametric Kruskal-Wallis statistic was used for data comparison between three groups. Individual pairs of parameters were further compared by Dunn's formula. A probability value of $p < 0.05$ was considered significant.

All patients were in sinus rhythm; valve incompetence was ruled out on the grounds of history, physical examination, and echocardiography. According to patients' medication and the results of the radionuclide tests the following three groups of patients were formed: (1) a control group of 32 individuals receiving no systematic cardioactive medication and with normal myocardial perfusion and normal left ventricular function; (2) a group of 148 patients receiving no systematic cardioactive medication and with abnormal perfusion; (3) a group of 85 patients on β blockers and with abnormal perfusion. Seventy one of these patients were receiving atenolol, six were on propranolol, four on metoprolol, and four on bisoprolol.

There was no significant difference in age, and resting diastolic and systolic blood pressure between the groups of patients studied. LVEF was not correlated to either the first third filling fraction or the heart rate adjusting first third filling fraction. LVEF was significantly lower in patients with IHD, as assessed by thallium scintigraphy, receiving no medication (38.9 (11.0) compared to the control group (44.8 (6.3), $p < 0.001$). In patients on β blockers the LVEF (38.1 (9.5)) was also significantly reduced in comparison with the control group ($p < 0.001$). No significant difference in LVEF was found between the two groups of patients with IHD.

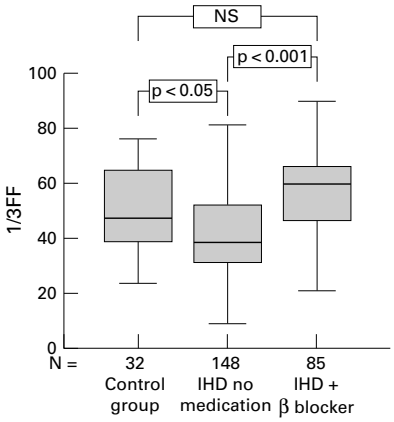

Figure 1 Values of the first third filling fraction (1/3FF) in the three groups of patients.

Finally, the R-R interval (in ms) was significantly prolonged in patients with IHD on β blockers (972 (143)) compared to the control group (853 (125), $p < 0.005$) and the patients with IHD receiving no cardioactive medication (819 (136), $p < 0.001$); no significant difference in the R-R interval was found between the latter two groups of patients.

The first third filling fraction was significantly reduced in patients with IHD receiving no medication (41.1 (14.9)) in comparison to the control group (49.4 (14.5), $p < 0.05$) and to patients with IHD on β blockers (56.5 (15.2), $p < 0.001$); no significant difference was found between the latter two groups. Figure 1 shows the comparison of the first third filling fraction in the three groups of patients.

The heart rate adjusted first third filling fraction index was also significantly reduced in patients with IHD receiving no medication (49.7 (15.3)) in comparison to the control group (57.6 (13.3), $p < 0.02$) and to patients with IHD on β blockers (58.0 (13.8), $p < 0.001$); no significant difference was found between the latter two groups. These findings imply that the early filling enhancement that β blockers exert in patients with IHD may not be a function of suppressed heart rate alone.

Apart from LVEF, no other variables that may influence diastolic indices were significantly different between the control group and those patients with IHD receiving no systematic cardioactive medication. However, the first third filling fraction did not depend on LVEF. R-R interval was significantly prolonged in the group of patients with IHD on β blockers compared to both the control group and the patients with IHD receiving no medication. Again, the heart rate adjusted first third filling fraction radionuclide ventriculography index allowed for the adjustment of the heart rate effect on left ventricular filling.

The present study suggests that in patients with IHD on β blocker treatment resting diastolic function is restored to normal levels, and is significantly above that of a group of patients with IHD receiving no cardioactive medication. A decrease in the myocardial oxygen requirement, and hence prevention of myocardial ischaemia, may be the most important mechanism involved. A reduction in systemic blood pressure might also be expected to improve diastolic function. However, only 17.5% of patients included in the β blocker group gave a history of hypertension, and no significant difference in blood pressure was found between the groups of patients studied.

This study is consistent with other work suggesting that diastolic dysfunction is associated with ischaemic heart disease. A limitation of this present paper is that coronary angiography was not carried out on all patients; however thallium scintigraphy was used to carry out a functional assessment of the extent of ischaemic heart disease. Moreover, it suggests that this significant resting filling impairment can be restored to normal levels using β blockade.

E MORALIDIS
G McCURRACH
W MARTIN
I HUTTON
Department of Medical Cardiology, Glasgow Royal Infirmary, Glasgow, UK

Correspondence to: Dr G McCurrach, Glasgow Royal Infirmary, 10 Alexander Parade, Glasgow G31 2ER, UK; gpca07@udcf.gla.ac.uk

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Long term treatment with enalapril reduces plasma concentrations of macrophage colony stimulating factor in patients with coronary artery disease

Previous studies both in vivo and in vitro showed that angiotensin converting enzyme (ACE) inhibitors exert antiatherogenic effects. Long term blockade of ACE significantly reduces atherosclerosis related events in patients with coronary artery disease.[1] These results suggest that this class of drugs may present antiatherogenic properties; however, the mechanism for it remains to be fully elucidated. Macrophage colony stimulating factor (M-CSF, one of the major inflammatory cytokines) and transforming growth factor β (TGF-β, one of the major anti-inflammatory cytokines) have been shown to play a key role in the pathogenesis of atherosclerosis. Indeed, we and others have previously shown that plasma concentrations of M-CSF are increased while those of TGF-β are decreased in patients with coronary artery disease, and that the ratio of plasma concentrations of M-CSF and those of TGF-β well correlates with the severity of coronary atherosclerosis.[2 3] However, no study has ever examined the effect of ACE inhibitors on the cytokine system in patients with coronary artery disease. We thus examined whether or not long term blockade of ACE improves the altered plasma concentrations of M-CSF and those of TGF-β.

This study was approved by the human research committee of our institute. Informed consent to participate in this study

Table 1 Patient demographics

	Control group	*Enalapril group*	*p Value*
Number	15	15	
Age (years)	66.7 (1.8)	62.7 (2.3)	0.179
Sex (male/female)	13/2	13/2	1.000
Hypertension	7	8	1.000
Current smoker	1	3	0.591
Total cholesterol (mmol/l)	4.93 (0.21)	5.28 (0.23)	0.266
Blood pressure			
Before			
Systolic (mmHg)	126.7 (4.0)	135.9 (5.0)	0.161
Diastolic (mmHg)	70.6 (2.4)	82.3 (2.3)	0.020
After 16 weeks			
Systolic (mmHg)	127.8 (5.8)	130.5 (5.9)	0.754
Diastolic (mmHg)	70.1 (3.7)	78.8 (3.5)	0.100
Medications			
Aspirin	12	14	0.591
β Blockers	7	8	1.000
Calcium channel blockers	10	13	0.388
Nitrates	8	4	0.264
Statins	6	6	1.000

Results are presented as mean (SEM).

was obtained from all patients. Thirty consecutive patients were randomly divided into two groups; one group received 2.5 mg/day of enalapril for the first eight weeks, followed by 5.0 mg/day for the next eight weeks, while another group served as a control. All patients were proved to have significant coronary artery disease by coronary angiography. Exclusion criteria included acute coronary events, recent coronary angioplasty or bypass surgery within three months, renal or liver diseases, any malignant tumours, or any inflammatory diseases. There was no significant difference in the prevalence of coronary risk factors or that of medications, including aspirin (table 1). Blood pressure was fairly controlled with other medications in both groups before the enalapril treatment, and there was no significant difference in systolic blood pressure between the two groups, while diastolic blood pressure was significantly higher in the enalapril group than in the control group. However, enalapril did not significantly lower blood pressure in both groups (table 1). Fasting venous blood samples were collected before treatment, and eight and 16 weeks after in the treated group, and before and 16 weeks after the observation period in the control group. Plasma concentrations of M-CSF and TGF-β were measured by an enzyme linked immunosorbent assay.[2]

In the enalapril group, mean (SEM) plasma concentrations of M-CSF tended to decrease at the 2.5 mg dose (from 361.9 (46.4) pg/ml at baseline to 316.7 (50.6) pg/ml) and were significantly decreased at the 5.0 mg dose (284.3 (39.5) pg/ml, $p < 0.01$), whereas they remained unchanged in the control group (361.4 (40.1) pg/ml at baseline and 393.7 (39.8) pg/ml at 16 weeks) (fig 1). By contrast, plasma concentrations of TGF-β remained unchanged in both groups: 6.6 (0.5), 6.8 (0.6), and 6.3 (0.8) pg/ml at baseline, eight, and 16 weeks in the enalapril group, respectively, and 7.2 (1.2) and 7.7 (1.3) pg/ml at baseline and 16 weeks in the control group, respectively.

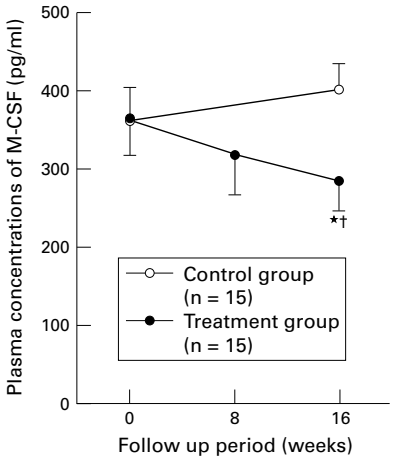

*Figure 1 Reduction of plasma concentrations of M-CSF by long term treatment with enalapril. The long term treatment with enalapril (2.5 mg daily for the first eight weeks and 5.0 mg daily for the next eight weeks) significantly reduced plasma concentrations of M-CSF in the treated group but not in the control group. Results are presented as mean (SEM). *p < 0.05 v 0 week, †p < 0.01 v control group (by Wilcoxon matched pairs, signed ranks test).*

Macrophages synthesise and release various cytokines that influence vascular tone, plaque stability, and thrombogenesis. M-CSF is a multifunctional inflammatory cytokine that regulates differentiation, proliferation, and survival of monocytes and macrophages. Furthermore, M-CSF deficiency significantly reduces atherogenesis in a mouse model of atherosclerosis,[4] whereas its overexpression accelerates atherogenesis caused by increased monocyte recruitment and enhanced smooth muscle migration and proliferation.[5] Thus, it is highly possible that the decrease in M-CSF plasma concentrations by long term blockade of ACE may suppress the progression of atherosclerosis, thus contributing, at least in part, to the reduction of cardiovascular events in patients with coronary artery disease.[1] By contrast, plasma concentrations of TGF-β was not significantly changed by the long term blockade of ACE, suggesting that this anti-inflammatory cytokine may not play a major role in the antiatherogenic effect of ACE inhibitors.

KENJI SADAMATSU
HIROAKI SHIMOKAWA*
HIDEKI TASHIRO
KUNIHIKO YAMAMOTO
Division of Cardiology, St Mary's Hospital, Kurume, Japan
**Department of Cardiovascular Medicine, Kyushu University, Graduate School of Medical Sciences, Fukuoka, Japan*

Correspondence to: Dr Hiroaki Shimokawa, Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan; shimo@cardiol.med.kyushu-u.ac.jp

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