Treatment with spironolactone for 24 weeks decreases the level of matrix metalloproteinases and improves cardiac function in patients with chronic heart failure of ischemic etiology

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MJ Li, CX Huang, E Okello, T Yanhong, S Mohamed. Treatment with spironolactone for 24 weeks decreases the level of matrix metalloproteinases and improves cardiac function in patients with chronic heart failure of ischemic etiology. *Can J Cardiol* 2009; 25(9):523-526.

BACKGROUND: The myocardial extracellular matrix is believed to be central to the remodelling that takes place following myocardial infarction. The contribution of markers of collagen metabolism to this process remains less well understood. The present study examined the contribution of some of the markers of collagen metabolism in cardiac remodelling, as well as the effect of spironolactone on the remodelling process.

OBJECTIVES: To investigate the pathological contribution of markers of collagen metabolism, including matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), type I collagen carboxyterminal telopeptide (ICTP) and procollagen type I carboxyterminal propeptide (PICP), in cardiac remodelling following ischemic cardiomyopathy, and to examine the pharmacoregulatory effects of spironolactone on collagen metabolism.

METHOD: Eighty-six consecutive patients (62 men and 24 women) with chronic heart failure of ischemic etiology (patient group) and 25 age-matched controls were enrolled in the study. The subjects in the patient group were randomly assigned into a spironolactone or nonspironolactone group. Plasma levels of MMP-9, TIMP-1, ICTP and PICP were measured using ELISA and radioimmunoassay techniques. Furthermore, left ventricular diastolic diameter and ejection fraction were assessed using two-dimensional and Doppler echocardiography.

RESULTS: The plasma concentrations of MMP-9, TIMP-1 and the MMP-9 to TIMP-1 ratio, as well as ICTP, were significantly increased in the patient group. The PICP to ICTP ratio in the patient group was significantly lower than that in the age-matched control subjects. After a follow-up period of 24 weeks, the PICP to ICTP ratio increased, and MMP-9, TIMP-1 and the MMP-9 to TIMP-1 ratio decreased in the spironolactone subgroup.

CONCLUSIONS: Biomarkers of collagen degradation were elevated and correlated with depressed heart function; spironolactone may partially reverse the dysregulation in collagen metabolism.

Key Words: ICM; ICTP; MMPs; PICP; Spironolactone; TIMPs

Un traitement de 24 semaines à la spironolactone réduit le taux de métalloprotéinases matricielles et améliore la fonction cardiaque chez des patients atteints d'une insuffisance cardiaque chronique d'étiologie ischémique

HISTORIQUE : On pense que la matrice extracellulaire myocardique est centrale au remodelage qui se produit après un infarctus du myocarde. L'apport des marqueurs du métabolisme du collagène à ce processus est moins bien compris. La présente étude porte sur l'apport de certains marqueurs du métabolisme du collagène dans le remodelage cardiaque ainsi que sur l'effet de la spironolactone sur le processus de remodelage.

OBJECTIFS : Explorer l'apport pathologique des marqueurs du métabolisme du collagène, y compris les métalloprotéinases matricielles (MMP), les inhibiteurs tissulaires de métalloprotéinases (TIMP), le télopeptide carboxyterminal du collagène de type 1 (ICTP) et le propeptide carboxyterminal du procollagène de type 1 (PICP), dans le remodelage cardiaque après une myocardiopathie ischémique, et examiner les effets pharmacorégulateurs de la spironolactone sur le métabolisme du collagène.

MÉTHODOLOGIE : Quatre-vingt-six patients consécutifs (62 hommes et 24 femmes) atteints d'insuffisance cardiaque chronique d'étiologie ischémique (groupe de patients) et 25 sujets témoins appariés selon l'âge ont participé à l'étude. Les sujets faisant partie du groupe de patients ont été répartis au hasard entre un groupe prenant de la spironolactone et un groupe n'en prenant pas. Les auteurs ont mesuré les taux plasmatiques de MMP-9, de TIMP-1, d'ICTP et de PICP au moyen des techniques ELISA et de dosage radio-immunologique. Ils ont également évalué le diamètre diastolique et la fraction d'éjection ventriculaires gauches au moyen de l'échocardiographie bidimensionnelle et de Doppler.

RÉSULTATS : Les concentrations plasmatiques de MMP-9 et de TIMP-1, le ratio de MMP-9 par rapport au TIMP-1 et l'ICTP avaient beaucoup augmenté dans le groupe de patients. Le ratio de PICP par rapport à l'ICTP du groupe de patients était considérablement plus faible qu'au sein du groupe de sujets témoins appariés selon l'âge. Après un suivi de 24 semaines, le ratio de PICP par rapport à l'ICTP avait augmenté et le MMP-9, le TIMP-1 et le ratio de MMP-9 par rapport au TIMP-1 avaient diminué au sein du sous-groupe prenant de la spironolactone.

CONCLUSIONS : Les biomarqueurs de dégradation du collagène étaient élevés et corrélés à une dépression de la fonction cardiaque. La spironolactone peut supprimer partiellement la dysrégulation du métabolisme du collagène.

The myocardial extracellular matrix (ECM) plays an integral role in the myocardial architecture as well as normal cardiac function (1-4).

Matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that play an essential role in the degradation of ECMs. Changes in expression and activity of several MMPs have been identified in

failing myocardium, and pharmacological inhibition of some MMPs has resulted in the suppression of heart failure (5).

Clinical and experimental studies have demonstrated the pathophysiological significance of ECM remodelling in the progression of congestive heart failure (CHF) in cardiomyopathies, although

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Received for publication October 29, 2007. Accepted May 24, 2008

TABLE 1
Biochemical parameters determined in heart failure (HF) patients and control subjects at baseline

Parameters	HF patients (n=86)	Control subjects (n=25)
MMP-9, ng/L	121.3±31.4**	27.2±7.8
TIMP-1, ng/L	2930.7±201.6**	1486.6±88.7
MMP-9:TIMP-1	0.0376±0.0163**	0.0183±0.0964
PICP, µg/L	80.8±31.2	65.9±18.9
ICTP, µg/L	4.5±0.8*	2.4±0.7
PICP:ICTP	18.65±8.33**	27.4±12.65

Data presented as mean ± SD. **P*<0.05; ***P*<0.01 between the two groups. ICTP Type I collagen carboxyterminal telopeptide; MMP Matrix metalloproteinase; PICP Procollagen type I carboxyterminal propeptide; TIMP Tissue inhibitor of metalloproteinases

the altered profile of the end products of collagen metabolism remain less well established (3,4,6-8).

Recent investigations indicate that CHF produces a relatively specific change in the spectrum of biomarkers of collagen metabolism and collagen types (7-10). However, MMP species are not uniformly upregulated in heart failure; rather, this is dependent on different heart failure etiologies and different stimuli. MMP-2 and MMP-3 have been shown to become upregulated only in human nonischemic dilated cardiomyopathy, whereas MMP-9 is upregulated and MMP-1 is downregulated in both nonischemic and ischemic dilated cardiomyopathy (11).

The first objective of the present study was to test the hypothesis that the presence of abnormal serological biomarkers of collagen metabolism is related to cardiac dysfunction in patients with heart failure due to ischemic etiology. The second objective was to evaluate the effect of pharmacological intervention on collagen metabolism in the remodelling process by administration of spironolactone, a potassium-sparing diuretic that has been shown to prolong life in chronic heart failure.

METHODS

Patients

Eighty-six patients (62 men and 24 women) 45 to 74 years of age (mean [± SD] age 59.1±15.3 years) and 25 healthy aged-matched controls (18 men and seven women) 41 to 76 years of age (mean 51.5±8.1 years) participated in the study.

Study protocol

The patient population consisted of consecutive patients with chronic heart failure, with various systolic and diastolic dysfunctions due to documented ischemic etiology. The diagnosis was based on clinical and pathological examination.

Inclusion criteria consisted of a New York Heart Association classification of 2 or greater (degree of CHF) and an average ejection fraction (EF) of 45% or less; a ratio of peak flow velocity at rapid filling of the left ventricle to that at atrial contraction (E/A) lower than 1 and pulmonary congestion on a chest radiograph; elective coronary angiogram with two or more stenotic vessel lesions exceeding 75%; and a positive exercise test.

After the initial evaluation, the 86 patients were randomly assigned, using a double-blind protocol, into either a spironolactone group (n=58) or a nonspironolactone group (n=28) in a 2:1 ratio using central allocation software. Both the spironolactone and nonspironolactone groups took a standard therapy including metoprolol (12.5 mg/day to 25 mg/day), enalapril (10 mg/day), digoxin (0.125 mg/day to 0.25 mg/day), acetylsalicylic acid (100 mg once daily) and a statin as stable principal treatment.

The spironolactone group had a mean left ventricular end diastolic dimension (LVEDD) of 62±5.3 mm and an EF of 41±3.9%. This group was administered oral spironolactone, at a daily dose of

TABLE 2
Baseline and 24-week follow-up characteristics of the spironolactone* group and the nonspironolactone† group

	Spironolactone (n=58)		Nonspironolactone (n=28)	
	Baseline	24 weeks	Baseline	24 weeks
Age, years	59.8±10.2		58.2±9.4	
Men:women	42:16		20:8	
E/A	0.828±0.07	1.027±0.09†	0.859±0.08	0.891±0.07
LVEDDI	32.3±5.9	27.2±4.7‡	31.9±7.1	30.8±5.1
LVEF, %	41.2±3.9	50.7±5.3‡	42.4±4.7	44.1±5.1
BP, mmHg	87.7±7.7	93.9±10.6‡	88.2±9.3	91.8±10.7§
MMP-9:TIMP-1	0.0385±0.0057	0.025±0.003‡	0.0357±0.009	0.0324±0.0030
PICP/ICTP	17.9±2.1	24.1±2.8‡	19.2±2.3	20.3±2.7

Every patient had at least one risk factor, including hypertension, hyperlipidemia and diabetes mellitus. Data presented as mean ± SD unless otherwise specified. *Standard treatment plus spironolactone; †Standard treatment alone; ‡*P*<0.01 versus values before treatment with spironolactone; §*P*<0.05 versus values before treatment. A Peak flow velocity at atrial contraction; BP Blood pressure; E Peak flow velocity at rapid filling of the left ventricle; ICTP Type I collagen carboxyterminal telopeptide; LVEDDI Left ventricular end diastolic dimension index; LVEF Left ventricular ejection fraction; MMP Matrix metalloproteinase; PICP Procollagen type I carboxyterminal propeptide; TIMP Tissue inhibitor of metalloproteinases

25 mg to 50 mg (12.5 mg to 25 mg, twice per day) for 24 weeks. The dosage was scaled up to 50 mg once daily if the patient still showed clinical progression of heart failure without evidence of hyperkalemia. If hyperkalemia developed at any time, the dose was scaled down to 25 mg daily. Follow-up laboratory measurements, including serum potassium and creatinine concentration, were monitored every four weeks for all cases in this group. The age, sex ratio, risk factors including hypertension, hyperlipidemia and diabetes mellitus, and parameters from echocardiography were all matched between the above two groups (Tables 1 and 2).

In addition, 25 healthy subjects (18 men and seven women) 41 to 76 years of age (mean 51.5±8.1 years) with no history of heart disease, including myocardial infarction, valvular failure and systemic hypertension, were enrolled. All of these subjects underwent routine cardiac examinations, including electrocardiography, chest x-ray and echocardiography to exclude coronary artery diseases and heart failure irrespective of its etiology.

Exclusion criteria consisted of conditions that could precipitate or aggravate collagen turnover, including pregnancy, thyroid diseases, tumours, inflammatory disease, other organic heart disease, systemic hypertension, bone and rheumatic diseases, recent trauma (less than two weeks previously) or surgery (less than six months previously), chronic liver or kidney insufficiency, diabetes mellitus and pulmonary fibrosis, and users of drugs such as aldosterone receptor antagonists and glucocorticoids.

The study protocol was approved by the research and ethics committee of the Renmin Hospital of Wuhan University (Wuhan, People's Republic of China). All subjects gave written informed consent.

Laboratory analyses

Three blood samples from peripheral veins were obtained from all patients in a fasting state in the morning at baseline, pretreatment and 24 weeks following treatment. In the age-matched control group, only the baseline blood sample was obtained. Then, all samples were centrifuged at 3000 g for 10 min at 4°C. Aliquots of 200 µL of plasma were then immediately stored at -80°C until assay.

Experimental protocol

MMP-1 and MMP-9 were measured using ELISA kits (Amersham Pharmacia Biotech, Sweden), as previously described by Wilson et al

(5), and MMP-9/tissue inhibitor of metalloproteinases (TIMP)-1 was used to evaluate collagen metabolism.

The biomarkers procollagen I carboxyterminal propeptide (PICP) and type I collagen carboxyterminal telopeptide (ICTP) were quantified by radioimmunoassay kits (Orion Diagnostica, Finland). The PICP to ICTP ratio was used to estimate the balance between collagen I synthesis and degradation. All measurements were performed in duplicate and the average was obtained.

Echocardiography

All subjects underwent two-dimensional real-time echocardiography and Doppler echocardiographic examination (Hewlett-Packard Sonos 5500; Hewlett-Packard, USA). All performances represented three average measurements on five consecutive cardiac cycles and were analyzed blindly. The LVEDD index (LVEDDI) was measured and calculated by two-dimensional echocardiography. Using Doppler echocardiography, E/A and EF of the left ventricle were quantified.

Statistical analysis

All data were expressed as mean $\pm$ SD. The unpaired Student's *t* test was used to compare continuous variables between groups, and the paired *t* test was used for within-group comparison. Correlation analysis between clinical and biomarkers of collagen metabolism were calculated by Spearman's rank correlation test. $P < 0.05$ was considered to be statistically significant. All statistical calculations were performed using SPSS (SPSS Inc, USA) version 12.0.

RESULTS

A total of 111 participants (86 patients and 25 healthy age-matched controls) completed the study.

The changes in the levels of biomarkers of collagen metabolism and their relationships in patients and controls

The plasma concentrations of MMP-9, TIMP-1 and the MMP-9 to TIMP-1 ratio were analyzed. The serum levels of cleaved peptides of collagen metabolism (ie, PICP, ICTP and the PICP to ICTP ratio) were measured at the same time point. All the parameters were compared between heart failure patients and the control subjects. The basic levels of MMP-9, TIMP-1, MMP-9 to TIMP-1 ratio, ICTP and PICP to ICTP ratio were significantly higher than those in the age-matched control subjects (Table 1).

The effects of spironolactone on the cardiac function and biochemical parameters

After 24 weeks of treatment, the spironolactone group showed significant improvements in cardiac function parameters including E/A, left ventricular EF (LVEF), LVEDDI and mean blood pressure (Table 2), as well as changes in the level of biochemical parameters. The plasma levels of MMP-9, TIMP-1 and ICTP decreased significantly compared with the pretreatment levels ($P < 0.05$; Table 3). The MMP-9 to TIMP-1 ratio was also significantly reduced (Table 2). In the non-spironolactone group, there was no significant difference in the plasma concentrations of MMP-9, TIMP-1, PICP and ICTP before and after 24 weeks of standard treatment.

In the spironolactone group, the PICP to ICTP ratio increased significantly after 24 weeks of treatment (Table 2; $P < 0.01$) but the two ratio indexes did not change significantly in the nonspironolactone group ($P > 0.05$).

Correlations between serum level of collagen turnover and echocardiographic parameters

Echocardiographic parameters, which measure the diastolic function of the left ventricle, were found to have significant correlations with turnover of type I collagen fiber. E/A was significantly correlated with MMP-9 to TIMP-1 ratio ($r = 0.712$, $P = 0.018$). LVEDDI had significant correlations with PICP and ICTP, but none of these parameters were related to the PICP to ICTP ratio. Furthermore, LVEDDI also correlated with the MMP-9 to TIMP-1 ratio ($r = 0.312$, $P = 0.03$).

TABLE 3
Telopeptide profiles in heart failure patients at baseline and 24 weeks after treatment with spironolactone

	Baseline	24 weeks
TIMP-1	2898 $\pm$ 257.5	2290.8 $\pm$ 240.9*
MMP-9	123.8 $\pm$ 6.3	79.2 $\pm$ 6.9*
PICP	78.3 $\pm$ 6.4	72.7 $\pm$ 8.1
ICTP	4.6 $\pm$ 0.27	3.1 $\pm$ 0.38*

Data presented as mean $\pm$ SD. * $P < 0.05$ versus baseline values. ICTP Type I collagen carboxyterminal telopeptide; MMP Matrix metalloproteinase; PICP Procollagen type I carboxyterminal propeptide; TIMP Tissue inhibitor of metalloproteinases

LVEF, a pivotal parameter of cardiac systolic function, correlated with TIMP-1 (Table 2).

No significant correlation was evident when EF was compared with PICP, ICTP and the PICP to ICTP ratio.

DISCUSSION

Chronic heart failure of ischemic etiology is associated with diffuse changes in the architecture and composition of the ECM in animal heart failure models and ischemic cardiomyopathy (ICM) patients (6,7). There has been considerable interest in the mechanism and extent of activation of the local MMP proteolytic system in human ischemic myocardium (7,8). Using ELISA, Spinale et al (4) found MMP-9 levels significantly increased and MMP-1 decreased markedly in tissue from explanted hearts in end-stage ICM patients.

Using a gene mutation or knockout animal model, MMP-9 has been shown to be closely related to chronic coronary artery ligation-induced left ventricular enlargement and collagen accumulation (11). We know that any imbalance between collagenase and its inhibitors leads to a change in the constitution of the ECM and, subsequently, cardiac remodelling. In our study, there were elevated plasma levels of MMP-9 and TIMP-1, and an especially increased MMP-9 to TIMP-1 ratio in the ischemic failing myocardium, implying a broken equilibrium characterized by a dominance of type I collagen degradation over its synthesis, which is in accordance with the report by Wilson et al (5). Matched with these phenomena, the degradation products (ICTP) of type I collagen, which is one of the substrates of MMP-9, should be enhanced concurrently. As shown in the present study, the ICTP in the patient group was almost double that in normal controls due to the elevated breakdown of type I collagen in the ECM of ischemic myocardial tissue (Table 1).

TIMP-1 increased synchronously with MMP-9. This could be interpreted as a consequence of a feedback regulation mechanism that antagonizes increased MMP-9 concentration. The diastolic functions assessed by E/A were clearly impaired in our patients. This may be explained by the slightly increased concentration of PICP and highly elevated TIMP-1 level, which probably aggravated myocardial stiffness, mainly by facilitating interstitial fibrosis in myocardial tissue (2).

In the spironolactone group, we found that after 24 weeks of therapy, the E/A increased significantly, indicating that the cardiac diastolic function could be restored significantly – almost to the normal range – by spironolactone. More importantly, the systolic function was also improved significantly, as shown by the LVEF.

The Randomized Aldactone Evaluation Study (RALES) (12) showed that high serum levels of markers of cardiac collagen synthesis were significantly associated with poor outcome, and decreased during spironolactone therapy in patients with ICM, indicating that spironolactone may improve cardiac function by inhibiting cardiac fibrosis (12-16). In our study, the biomarkers of metabolism of type I collagen also regressed toward normal after 24 weeks of spironolactone administration. A possible explanation is that the collagen phenotypic distribution may be partially altered after an appropriate period of spironolactone intervention.

Limitations

Our study has several limitations. First, while the area of collagen metabolism with its turnover products is a wide and complex system, the design of our study examined only a small area. Our observations are therefore only confirmatory of events that take place in this complex system.

Second, in assessing cardiac function, we used echocardiographic E/A and LVDDI to assess diastolic function and LVEF to assess systolic function. If we had used parameters such as tissue Doppler imaging, the assessment of the structural alteration in cardiac musculature may have been interesting.

CONCLUSION

We have confirmed earlier findings that serological markers of collagen metabolism for cardiac ECM turnover are elevated in the peripheral venous blood of patients with chronic heart failure of ischemic etiology. Additionally, we have elucidated a regulatory system and mechanism of collagen metabolism existing in the ECM of ischemic myocardial tissue that is responsible for the cardiac fibrosis and dysfunction that can be mostly reversed by spironolactone administration. Further investigations are necessary to characterize a wider profile of biomarkers of collagen turnover.

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