ACE Inhibition and Target Organ Protection

Milan
June 1997

ACE Inhibition and Target Organ Protection

Proceedings of the Symposium held at Milan on Thursday, June 12 1997

General Editors:
Alistair S. Hall, *Institute for Cardiovascular Research, University of Leeds, UK*
Thomas Unger, Department of Pharmacology
Christian Albrechts University of Kiel, Germany

Published as a service to medicine by
Euromed Communications Ltd
on behalf of
Hoechst Marion Roussel
Route 202-206, P.O. Box 6800
Bridgewater, New Jersey 08807-0800 USA

ISBN 1 899015 25 6

Printed and bound in the United Kingdom by Warwick Printing Company Ltd.

Ultrasonic Detection of Fibrous Tissue in Left Ventricular Hypertrophy

Michele Ciulla, Roberta Paliotti and Fabio Magrini*
*Centro di Fisiologia Clinica e Ipertensione – Istituto di Clinica Medica Generale e Terapia Medica, Ospedale Maggiore - University of Milan, Italy. *Cattedra di Medicina Interna, C.L.O. – University of Cagliari, Italy*

Myocardial collagen matrix remodelling

The cardiac interstitium is composed of non-myocyte cells, including cardiac fibroblasts, and a structural fibrillar protein matrix which governs the architecture of the myocardium. The matrix is responsible for the support of myocytes and capillaries and contains mainly fibrillar collagen of type I and a lower content of collagen type III.[1] Because of its tensile properties collagen represents a major determinant of myocardial stiffness.

Collagen turnover is regulated by cardiac fibroblasts that express the genetic message for fibrillar collagen in the heart. Myocardial fibrosis is an abnormal increase in collagen content that involves a growth or an altered metabolism of cardiac fibroblasts; this process can occur on the basis of a reactive or reparative process in response to several signals[2] (**Table 1**). The progressive accumulation of collagen alters myocardial and vascular structure and function (remodelling) and has been recognized in several experimental models of hypertension, myocardial infarction and heart failure.

Several lines of evidence have suggested that a marked increase in collagen network represents a risk factor for the performance of the coronary microcirculation,[3] the systolic-diastolic function[4] or the electric stability of the heart.[5] However it is not completely established which signals are involved, what is the time course and finally if a reversal of the fibroblast activation process is possible. The main reason for this

Table 1: Signals activating and deactivating cardiac fibroblasts

Signals	**Time course**
Natural ageing	years
Increased work-load	months-years
Tissue damage	days-weeks
Hormonal effectors	
Activators	**Deactivators or Inhibitors**
Angiotensin II	Bradykinin
Aldosterone	Prostaglandin, PGE_2
Deoxycorticosterone-DOC	Triiodothyronine
Endothelin – ET1 and ET2	Nitric Oxide, EDRF

(Modified from reference 2.)

lack of information is that the detection and the quantification of collagen accumulation requires the use of invasive techniques such as endomyocardial biopsy. Since collagen deposition, due to its marked acoustic impedance, has an important influence on the echo texture image from the myocardium,[6] various approaches have been proposed for the non-invasive evaluation of the pathophysiological state of cardiac muscle with ultrasound.[7] Therefore the possibility of performing non-invasive myocardial characterization may be clinically relevant, in particular in hypertensive patients, in whom control of target organ damage is considered a desirable goal of therapy.

Ultrasonic Myocardial Characterization: Physical basis and experimental evidence

The identification of abnormalities in the pathophysiological state of tissues based on the analysis of returning ultrasounds is currently defined as ultrasonic tissue characterization. This approach is based on the hypothesis that ultrasound attenuation is related to the physical properties of individual tissues. The conventional echo image depicts, at a relative low resolution, the echo reflections from the various components of the myocardium, such as muscle fibres, capillaries and collagen strands, acting as diffuse reflectors. The ultrasound reflection process is specific for each tissue[8] with great differences between different tissues (**Figure 1**).

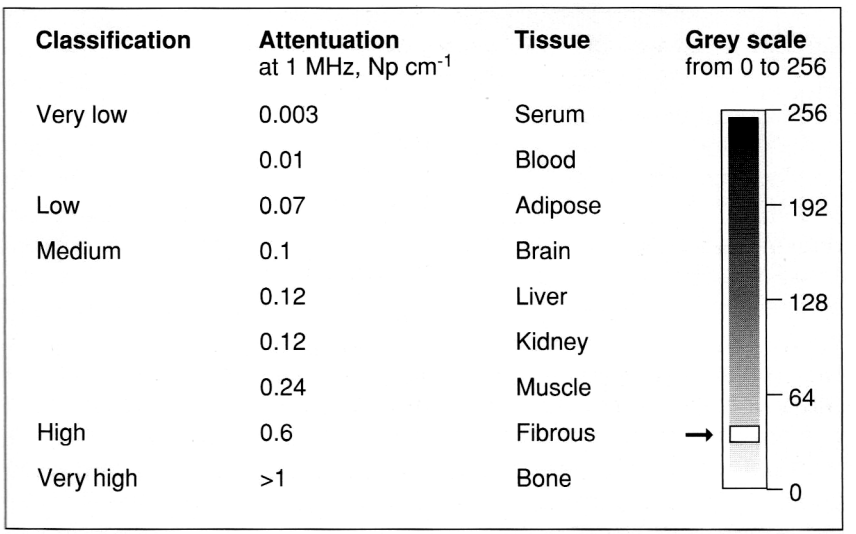

Figure 1. Attenuation properties in different tissues. Fibrous tissue shows an attenuation factor that is about 60 times higher than the value of blood. The scale represented on the right corresponds to the conventional echo grey-scale (low echoreflectivity = black, high echoreflectivity = white).

When the regular tridimensional architecture of the myocardium is altered, the acoustic properties of the tissue are changed and the interactions of echoes within the tissue produce a non-homogeneous pattern that has been observed in several pathological conditions. The possibility of evaluating ventricular wall structure by analyzing the echo image pattern has therefore been suggested. Because of the marked acoustic impedance of fibrous tissue (low water content, acoustic impedance about 10,000 times higher than the impedance of surrounding tissues)[9] that is responsible for an increase in echoreflectivity, collagen content has been proposed as a marker of tissue structure integrity. Various approaches have been used to quantitatively assess collagen content with ultrasounds[3] by analyzing tissue echoreflectivity. Methods have included grey scale or backscatter analysis of returning echoes[10,11,12]. Although backscatter analysis provides a more independent approach from instrumentation settings and from the operator in comparison with the grey scale method, it is a more complex approach and the potential of grey-scale analysis has been shown to reproduce the information of backscatter in many conditions[13,14].

Experimental and clinical studies have demonstrated the possibility to assess non-invasively the ultrastructural properties of tissues by ultrasounds. Picano *et al.* have studied the fibrotic heart in different pathological conditions, involving a structural cardiac remodelling (i.e. hypertension and myocardial infarction),[15] by comparing the backscatter and the histologically assessed collagen content, *in vivo* and *in vitro.* The results indicated collagen accumulation was the major determinant of the cardiac echoreflectivity. Similar results have been found by Mimbs and coauthors[16] with the analysis of the echo-attenuation. In dogs, after ligation of the left anterior descending coronary artery, they have demonstrated a good correlation between the infarct size (determined by the depletion of the creatine kinase) and the ultrasound attenuation, indicating that the regional infarction is associated with changes in echoreflectivity, related to collagen accumulation [17].

In patients with left ventricular chronic disease (valvular, congenital and coronary artery disease) Shaw *et al.* [18] have compared the analysis of the pixel intensity of echo images with the collagen content. In this study a good correlation between the echo and the histological pattern have been found.

In a group of hypertensive patients with mild to moderate left ventricular hypertrophy without left ventricular dysfunction Gigli *et al.*[19] have demonstrated that the integrated backscatter is not altered, suggesting that collagen growth is not necessarily associated with the increase in left ventricular wall thickness. Similar findings have been observed in athletes with left ventricular hypertrophy[20]. Lucarini *et al.*[21] reported no variations in integrated backscatter despite the regression of cardiac hypertrophy following antihypertensive treatment.

In a recent study[22] conducted in a group of 9 hypertensive patients with left ventricular hypertrophy (LVMI > 125 g/m^2) and biopsy proven different degrees of myocardial fibrosis (collagen volume fraction %, CVF = 4.3±1.6; normal values < 2%) using a color-scale analysis of echocardiographic images approach we have found a significant correlation (r = 0.72) between the pixel grey/color level frequency distribution and CVF. Collagen content appears to be the major determinant of regional echo intensity, its increase resulting in a progressively wider asymmetrical left shift of the myocardial color histogram (**Figure 2**).

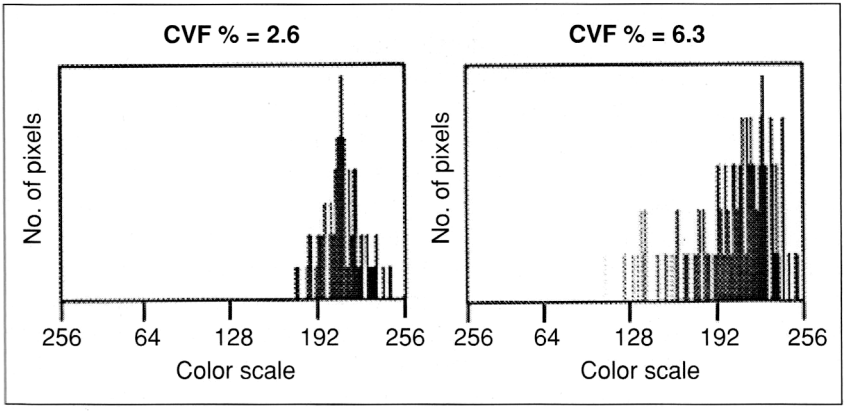

Figure 2. Color histograms derived respectively from a patient with a low (left panel) and a high (right panel) Collagen Volume Fraction (CVF). Normal CVF values are up to 2%.

Left Ventricular Hypertrophy (LVH) and Myocardial Fibrosis

It is well known that in hypertension the risk of cardiovascular events is increased in the presence of left ventricular hypertrophy; several studies have reported a progressive accumulation of fibrillar collagen[23,24] in patients with hypertensive heart disease. While the hypertrophic growth of the cardiac myocytes leads to an increment in myocardial mass, the non-myocytes growth is reflected in a structural remodelling of the cardiac interstitium. The involvement of this non-myocyte component is not a uniform accompaniment to myocytes hypertrophy, therefore the growth of myocyte and non-myocyte cells are probably independent processes and have a different regulatory control mechanism.[25] In agreement with this hypothesis in patients with mild to moderate hypertension and left ventricular hypertrophy (LVMI > 125 g/m^2) we report an increase in estimated collagen content, without a significant correlation with left ventricular mass, confirming that LVH and myocardial fibrosis are independent processes.[22] On the contrary, in exercise training athletes with LVH, Lattanzi[20] reports a normal integrated backscatter, indicative of a normal collagen pattern, suggesting that collagen growth is not associated with the increase in left ventricular wall thickness.

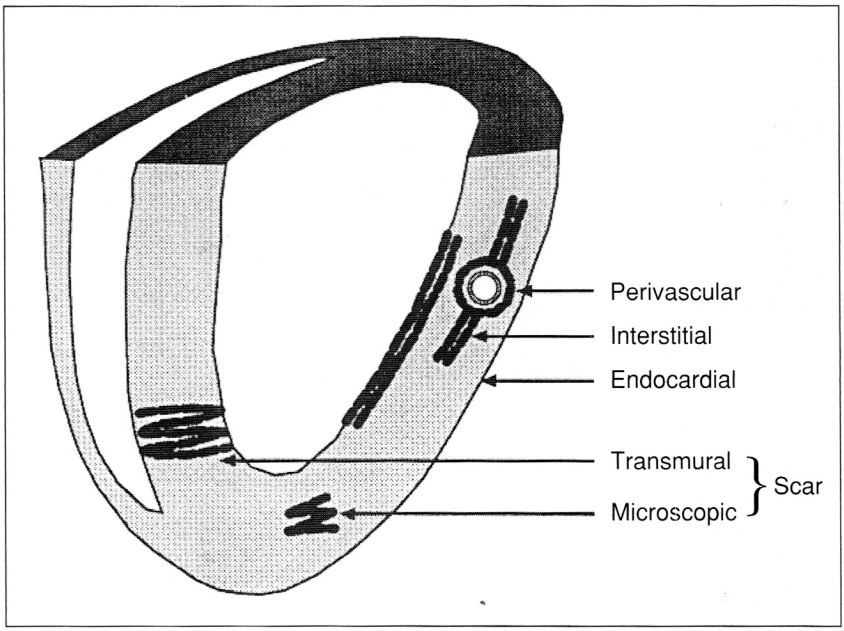

Figure 3. Fibrous tissue location within left ventricular wall. In hypertensive patients with Left Ventricular Hypertrophy interstitial and perivascular location is more common, microscopic scars are also reported as result of focal necrosis process involving myocytes.

The results obtained in athletes support the concept that the remodelling of the myocardium in normal conditions is an adaptative process to the increased work load and is accompained by a proportionate growth of myocytes and non-myocytes; conversely an unbalanced growth between myocytes and non-myocyte cells accounts for an heterogeneity in myocardial structure and could be considered responsible for the pathological remodelling of the heart seen in hypertension.[26]

In hypertension, an increase in fibrillar collagen around myocytes and intramyocardial coronary arteries has been described (**Figure 3**) and has been held responsible for abnormal myocardial stiffness that could explain the early impairment in diastolic function commonly observed in hypertension. A vascular remodelling, accompanied by a marked increase in periarteriolar fibrosis, contributes to the increased total volume density of interstitial fibrosis[3] (**Figure 4**). Some authors report a change in microvascular permeability with leakage of

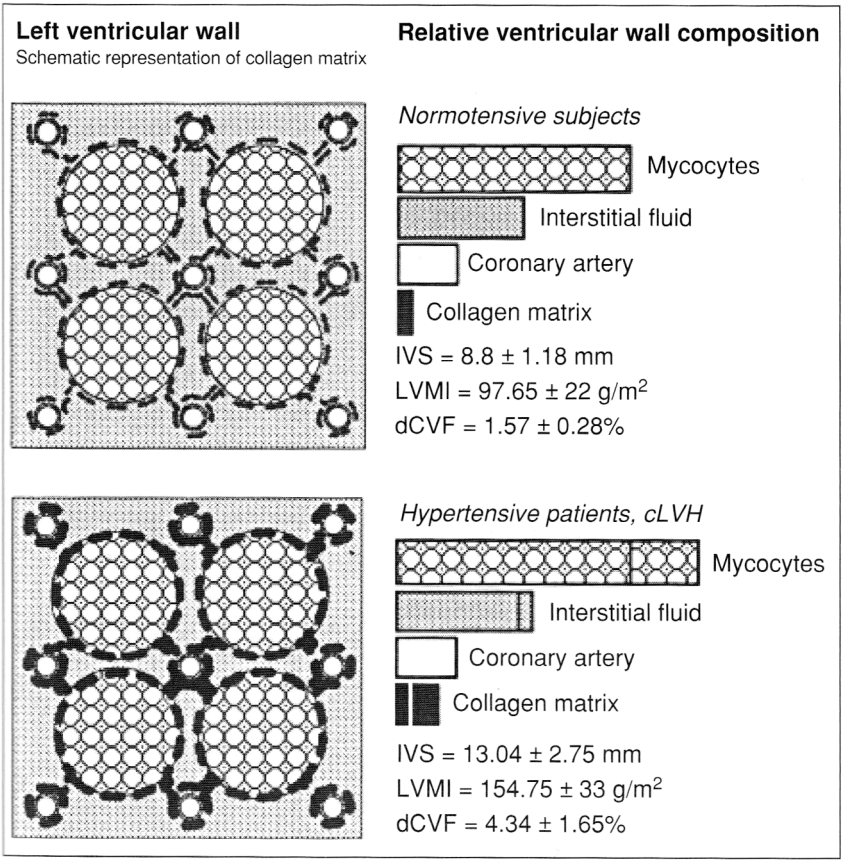

Figure 4. Schematic representation of collagen matrix in left ventricular wall and relative wall composition in Normotensive subjects (top panels) and in Hypertensive patients with concentric LVH (cLVH) (bottom panels). (reference 22).

macromolecules;[27] this oedema formation could be responsible for the impaired ventricular performance either by increasing myocardial stiffness or by altering oxygen diffusion distances.

While considering the factors that could promote a reactive fibrosis in hypertensive hearts, we must consider not only the pressure overload that is responsible for the hypertrophy of cardiac myocytes, but also trophic factors of whom the renin angiotensin system is one of the best

candidates.[27] Finally, pathological hypertrophy is to be considered as an heterogeneous process in which not only the quantity of myocardium is increased, but its quality or structure is altered. The disproportionate non-myocardial cells growth produces a distortion in myocardial structure that affects the specific ultrasound properties of the tissue resulting in a high refractile ultrasonic pattern that could be considered a marker of tissue integrity. Further information, from studies on the effects of antihypertensive therapy on tissue echoreflectivity, is needed in order to document whether the fibrosis process could be reversed.

References

1. Weber KT. Cardiac interstitium in health and disease: the fibrillar collagen network. 1989. *J Am Coll Cardiol* **13:** 1637–1652.
2. Brilla CG, Maisch B and Weber KT. 1992. Myocardial collagen matrix remodelling in arterial hypertension. *European Heart Journal* **13** (supplement D): 24–32.
3. Schwartzkopff B, Motz W, Frenzel H *et al.* 1993. Structural and functional alterations of the intramyocardial coronary arterioles in patients with arterial hypertension. *Circulation* **88:** 993–1003.
4. Weber KT, Sun Y, Guarda E. 1994. Structural remodelling in hypertensive heart disease and the role of hormones. *Hypertension* **23** (part 2):869–877.
5. Cameron JS, Myerburg RJ, Wong SS *et al.* 1983. Electrophysiologic consequences of chronic experimentally induced left ventricular pressure overload. *JACC* **2**(3): 481–487.
6. Kossoff G, Gossett WJ, Carpenter DA *et al.* 1976. Principles and classification of soft tissues by grey scale echography. *Ultrasound Med Biol* **2:** 89–105.
7. Miller JG, Perez JE and Sobel BE. 1985. Ultrasonic characterization of myocardium. *Progress in Cardiovascular Diseases* **27**(2): 85–110.
8. Ultrasonic Tissue Characterization. 1976. NBS Special Publication, U.S. Government Printing Office ed. Washington n° 453.
9. Price RR, Jones TB, Goddard J, Everette James A. 1980. Basic concepts of ultrasonic tissue characterization. *Radiologic Clinics of North America* **18**(1): 21–30.
10. Marini C, Ghelardini G, Picano E, *et al.* 1993. Effects of coronary blood flow on myocardial gray level amplitude in two dimensional echocardiography: an experimental study. *Cardiovasc Res* **27:** 279–283.
11. Sagar KB, Pelc LR, Rhyne TL *et al.* 1991. Estimation of myocardial infarct size with ultrasonic tissue characterization. *Circulation* **83:**1419–1428.
12. Chandrasekaran K, Aylward PE, Fleagle SR, *et al.* 1989. Feasibility of identifying amyloid and hypertrophic cardiomyopathy with the use of computerized quantitative texture analysis of clinical echocardiographic data. *J Am Coll Cardiol.* **13**(4):832–40.
13. Perez JE, Miller JG, Barzilai B, *et al.* 1988. Progress in quantitative ultrasonic characterization of myocardium: from the laboratory to the bedside. *J Am Soc Echo* **1** (4):294–305.
14. Olshansky B, Collins SM, Skorton DJ, Prasad N.V. 1984. Variation of left ventricular myocardial gray level on two-dimensional echocardiograms as a result of cardiac contraction. *Circulation* **70**(6): 972–977.
15. Picano E, Pelosi G, Marzilli M, *et al.* 1990. *In vivo* quantitative ultrasonic evaluation of myocardial fibrosis in humans. *Circulation* **81:** 58–64.

16. Mimbs JW, Yuhas DE, Miller JG *et al.* 1977. Detection of myocardial infarction *in vitro* based on altered attenuation of ultrasound. *Circ Res* **41:**192–198.
17. Mimbs JW, O'Donnell M, Bauwens D *et al.* 1980. The dependence of ultrasonic attenuation and backscatter on collagen content in dog and rabbit hearts. *Circ Res* **47:** 48–58.
18. Shaw TRD, Logan-Sinclair RB, Surin C, *et al.* 1984. Relation between regional echo intensity and myocardial connective tissue in chronic left ventricular disease. *Br Heart J* **51:** 46–53.
19. Gigli G, Lattanzi F, Lucarini AR, *et al.* 1993. Normal ultrasonic myocardial reflectivity in hypertensive patients. A tissue characterization study. *Hypertension.* **21:** 329–334.
20. Lattanzi F, Di Bello V, Picano E *et al.* 1992. Normal ultrasonic myocardial reflectivity in athletes with increased left ventricular mass. A tissue characterization study. *Circulation* **85:** 1828–1834.
21. Lucarini AR, Gigli G, Lattanzi F *et al.* 1994. Regression of hypertensive myocardial hypertrophy does not affect ultrasonic myocardial reflectivity: a tissue characterization study. *J Hypertens* **12:** 73–79.
22. Ciulla M, Paliotti R, Hess DB *et al.* 1997. Echocardiographic patterns of myocardial fibrosis in hypertensive patients: endomyocardial biopsy versus ultrasonic tissue characterization. *J Am Soc Echo* **10**(6): 657–664.
23. Pearlman ES, Weber KT, Janicki JS *et al.* 1982. Muscle fiber orientation and connective tissue content in the hypertrophied human heart. *Lab Invest* **46** (2):158–164.
24. Huysman JA, Vliegen HW, Van-der-Laarse A, Eulderink F. 1989. Changes in nonmyocyte tissue composition associated with pressure overload of hypertrophic human hearts. *Pathol Res Pract* **184:** 577–581.
25. Weber KT and Brilla CG. 1991. Pathological hypertrophy and cardiac interstitium. *Circulation* **83:**1894–1865.
26. Brilla CG, Reams GP, Maisch B and Weber KT. 1993. Renin-Angiotensin system and myocardial fibrosis in hypertension: regulation of the myocardial collagen matrix. *Eur Heart J* **14**(suppl J): 57–61.
27. Laine GA. 1988. Microvascular changes in the heart during chronic arterial hypertension. *Circulation Research* **62:** 953–960.