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(Article begins on next page)

CARDIO 01488

The hypertrophied myocardium and coronary disease. Structural changes in patients submitted to aortocoronary bypass surgery

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Seventeen patients with coronary disease submitted to myocardial revascularization were studied. Ten patients had a hypertrophied ventricle, and 7 had normal ventricular mass. Myocardial biopsies were obtained before ischemia and at the time of reperfusion and were assessed for: volume fraction of fibrous tissue, myocyte diameter, morphometric mitochondrial studies and ultrastructural changes. The volume fraction of fibrous tissue in patients with hypertrophied ventricle was 1.9 ± 0.04 , and in patients with normal ventricular mass was 0.9 ± 0.01 ($p < 0.05$). The diameter of the myocyte was $23 \pm 0.3 \mu\text{m}$ and $18 \pm 1.2 \mu\text{m}$ for patients with hypertrophied and normal ventricular mass, respectively ($p < 0.01$). The value of volumetric density for pre-ischemia samples in patients with a hypertrophied ventricle was 23 ± 2.2 and in patients with normal ventricular mass was 35 ± 2.7 ($p < 0.02$). Grades 3 and 4 of damaged mitochondria were significantly increased in reperfusion samples from patients with a hypertrophied ventricle compared to pre-ischemia samples. Collagen growth was increased in hypertrophied hearts which were also more sensitive to the ischemia/reperfusion mechanism.

Key words: Cardiac ultrastructure; Myocardial hypertrophy; Ischemic heart disease

Introduction

Numerous researchers have demonstrated in animal models that the hypertrophied left ventricle is more vulnerable to the ischemia reperfusion mechanism than the normal ventricle [1-5].

Although the increased susceptibility of the hypertrophied ventricle to ischemic injury in cardiac surgery has long been recognized [6], very few works have specifically studied the structural changes of the human hypertrophied heart [7,8]. Ferrans [9] must be credited for the most extensive investigation in the structural aspects of human cardiac hypertrophy. However, these observations were performed in a heterogeneous group of patients and did not analyze ischemic or reper-

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fusion changes. Furthermore, there is no knowledge up to now concerning the structural modifications as well as the tolerance to the ischemia/reperfusion mechanism which occurs in the human hypertrophied left ventricle associated with coronary heart disease. The present study attempts to characterize structural changes occurring in the hearts of patients with ventricular hypertrophy and coronary disease who were submitted to coronary bypass surgery.

Materials and Methods

This prospective trial involves 10 patients with coronary heart disease and left ventricular hypertrophy, and 7 with coronary heart disease and normal ventricular mass. To ensure a homogeneous population, patients with associated valve disease were excluded from this protocol. Within the hypertrophied group 8/10 patients had a history of chronic sustained hypertension. In the remaining 2 patients the cause of the left ventricular hypertrophy was undetermined.

One patient with a hypertrophied heart, had grade III unstable angina (New York Heart Classification), and the remaining patients with hypertrophied hearts had grades III-IV stable angina. One patient with normal ventricular mass had grade III unstable angina and the remaining patients with normal ventricular mass had grade III stable angina. Evidence of previous myocardial infarction was observed in 2 patients with a hypertrophied ventricle and in 3 with normal left ventricular mass. Patients who had akinetic or dyskinetic areas in the ventriculogram were excluded from this protocol.

The left ventricular mass (LVM) was assessed measuring the right anterior oblique projection of the ventriculogram according to the formula:

$$\text{LVM} = 0.9 \times \text{LV volume} + 15$$

using a Digisonics (Houston, Texas) computer. The value obtained was divided by the body surface area of the patient and expressed as left ventricular mass index [10]. Previous authors have demonstrated a good correlation of this method

TABLE I
Clinical characteristics.

	HVM	NVM
Age	55.9 ± 0.8	59.4 ± 2
Ejection fraction (%)	51.3 ± 2.5	55 ± 3
LVM (g/m ²)	191 ± 13	109 ± 7 *
Ischemia (min)	52 ± 5	63.5 ± 7
Number of grafts	2.9 ± 0.2	3.2 ± 0.3

LVM = left ventricular mass; HVM = hypertrophied ventricular mass; NVM = normal ventricular mass. * $p < 0.01$.

with measurements of left ventricular mass at autopsy [11]. The left ventricular mass was considered hypertrophic when it was greater than 120 g/m². The difference of mean values between the two groups was statistically significant and is detailed in Table 1.

Surgical technique

Cardiopulmonary bypass was instituted and the perfusate was cooled to 28°C. Myocardial protection was achieved using blood cardioplegia supplemented with mannitol (40 mmol/l) and potassium (20 mEq/l). The infusion was administered at 10 to 12°C and the pH at this temperature was 7.4. An initial dose of 500 ml was administered and repeated every 30 min. Topical cooling was maintained throughout the ischemic period. Ejection fraction, aortic cross-clamping time and the number of grafts performed are depicted in Table 1.

Myocardial biopsies were obtained before the aorta was cross-clamped and after 10 min of reperfusion, and they were designated as pre-ischemia and reperfusion samples, respectively. The specimens were obtained from the apex of the left ventricle within an area of normal appearance, using a Travenol Tru-cut biopsy needle and immediately placed in cold 3% glutaraldehyde in 0.1 mol/l of cacodylate buffer (pH 7.4). Written informed consent for myocardial biopsies was obtained from all patients.

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Light microscopy studies

The samples measured 2 to 3 mm in greatest dimension and a total of four biopsies were obtained from each patient. The largest specimen was routinely processed for light microscopy by embedding in paraffin. Ribbons of sections (4 mm in thickness) were serially cut and mounted so that each slide contained 3 to 4 sections. These serial slides were consecutively stained with hematoxylin-eosin and Mallory Trichrome. For the morphometric assessment of fibrosis, the Mallory Trichrome was used. Each tissue section was examined under a grid containing 121 points and the volume fraction of fibrosis was established (see below). The area of tissue analyzed ranged from 1 to 5 mm². If the specimen for light microscopy was small (1 mm or less in its greatest dimension), a portion of the specimen for electron microscopy was also processed. The biopsy samples were coded, and all slides were blindly examined by two observers (J.M. and R.B.).

Cell damage was graded as follows: 1+ less than one focus of cell damage per field ($\times 400$); 2+ one to two foci; 3+ three to five foci; and 4+ more than five foci of cell damage. Cell damage was classified histologically into acute and healing phases, and each phase was graded separately. Acute cell damage was defined as myocytolysis (vacuolar changes), or frank cell necrosis associated with an inflammatory infiltrate consisting of mononuclear cells and polymorphonuclear leukocytes. Healing cell damage was defined as myofiber cell loss and replacement by granulation tissue associated with a mononuclear cell infiltrate. Healed cell damage was defined as focal fibrosis lacking an inflammatory cell infiltrate, and was graded as fibrosis.

The average diameter of myocytes was measured by means of a micrometer. These measurements were made from cross- and oblique sections at a magnification of $400\times$. A micrometer fitted to the eye piece of a binocular microscope that was calibrated with a standard micrometer mounted on a glass slide was used. The average diameter from the range of each case was obtained, and a score for each of the two types of patients was applied. The volume fraction of fi-

brosis was measured in randomly selected sections without respect for the direction of sectioning in 20 fields at $400\times$ and was taken as the number of points of the grid falling on fibrosis divided by the points falling on total tissue. Sarcomere with contraction band formation was not considered for these determinations.

Ultrastructural studies

Tissues for transmission electron microscopy were fixed in 1% osmium tetroxide, dehydrated and embedded in Epon. Blocks were selected from different depths of the biopsy specimen, and from each block, sections of 1 μm thick were cut, stained with 1% toluidine borax, and examined by light microscopy to select appropriate areas for thin sectioning. Several ultrathin sections were obtained from each block, mounted in copper grids and stained with uranyl acetate and lead citrate. A total of 130 micrographs were taken at $5000\times$ and $10,000\times$ magnification with a Jeol JEM-100C, Japan electron microscope.

Semiquantitative analysis. Mitochondria, glycogen depletion, myofibrillar edema and overall cell morphology were assessed according to the criteria of Kloner and co-workers [12]. A score of 0 through 4 was applied with 0 considered normal and 4 representing irreversible organelle or cell damage. The overall cell morphology score assessed depletion of glycogen stores, myofibrillar and mitochondrial edema.

The mitochondrial damage was graded on a scale of 0 to 4, with 0 considered normal and 4 representing irreversible damage (massive swelling and architectural disruption with rupture of inner and outer mitochondrial membranes) [12]. When a point on the grid fell on a mitochondrion, it was assigned a numerical value of 0 through 4 depending on its morphologic degree of damage. The studies were performed in a blind fashion by two observers. The average obtained from the observers was expressed for each grade as a percentage from the total number of mitochondria counted per sample. Approximately 300 mitochondria were scored for each specimen.

Quantitative studies. Pre-ischemia and reperfusion specimens from the hypertrophied and normal ventricles were analyzed applying the mathematic formulae described by Weibel and co-workers [13] for the following quantitative determinations.

Volumetric density. This index determined the percentage of cytoplasm volume corresponding to mitochondria. The organelles were examined under a grid containing 100 points and applying the formula:

Volumetric density

$$= \frac{\text{number of checked mitochondria}}{\text{total number of points}},$$

where the number of checked mitochondria is the number of points within mitochondrial profiles, and total number of points refers to the total number of points distributed in the cytoplasm.

Numerical density. This index determines the number of mitochondria in the unit tissue volume and was obtained using the same grid as described above and applying the following formula:

$$\text{Numerical density} = \frac{N}{\beta \sqrt{\text{volumetric density}}},$$

where N is the number of mitochondrial profiles per unit of area measured, and β is an estimated mitochondrial coefficient of 2.25. According to Weibel and co-workers [13], this followed from an estimation of mean axial ratios of elliptic profiles which was 1.8 for mitochondria. Assuming in first approximation, an ellipsoidal shape for mitochondria, it could be roughly estimated that these organelles should be about 4 times as long as they are thick.

The average volume of an individual mitochondrion was estimated by dividing the volumetric density by the numerical density.

Statistical analysis

The paired t -test was used to determine the significance of differences for ventricular mass,

volume fraction of fibrous tissue, myocyte diameter, quantitative determinations of mitochondria and semiquantitative determinations of mitochondrial damage.

The Wilcoxon analysis was used to compare the scores of edema and glycogen content.

Results

Clinical outcome

All patients survived the surgical procedure and were eventually discharged between the 6th and 10th postoperative day. Three patients with a hypertrophied ventricle developed perioperative myocardial infarction as measured by the appearance of new Q waves and an ST shift on the electrocardiogram and by three-fold increases of serum creatine kinase values. In one of them, the myocardial infarction was associated with left ventricular insufficiency which required inotropic support with a major dose of dopamine hydrochloride (more than 5 g/kg/min). A second patient with hypertrophied ventricular mass also required transitory inotropic support. The postoperative recovery in the patients with normal ventricular mass was uneventful.

Light microscopy

Qualitative studies. The most remarkable findings in the biopsy specimens from the patients with a hypertrophied heart were a substantial rise in myocyte diameter and moderate to severe interstitial fibrosis (Fig. 1). Nuclear abnormalities were also more frequent among these patients. In the patients with normal ventricular mass the fibrous tissue was scarcely present between bundles of myocytes. Cell damage was relatively infrequent in both groups.

Semiquantitative studies. The volume fraction of fibrous tissue in the patients with hypertrophied hearts was $1.9 \pm 0.04\%$ and in those with normal ventricular mass was $0.9 \pm 0.01\%$ ($p < 0.05$). The myocyte diameter in patients with hypertrophied ventricles was $23 \pm 0.3 \mu\text{m}$, and in patients with normal ventricular mass was $18 \pm 1.2 \mu\text{m}$ ($p < 0.01$). The hypertrophy score was $2.2 \pm$



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Ultrastructure

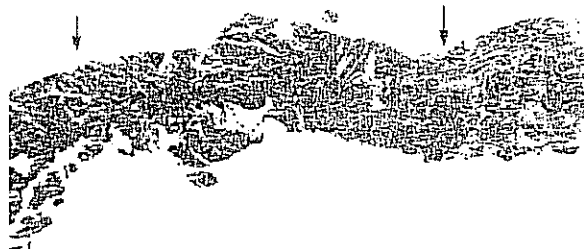


Fig. 1. Extensive fibrosis in a patient with hypertrophied ventricle. The arrows show the areas of fibrosis. Trichrome of Mallory ($\times 100$).

0.1, and 0.66 ± 3 ($p < 0.05$) for the patients with hypertrophied and normal ventricles, respectively.

Qualitative studies. Pre-ischemia biopsy specimens in the hypertrophic group showed slightly disorganized sarcomeres with disruption in some areas. Some myelin figures were seen within the mitochondria and in the sarcoplasm. Reperfusion samples had a heterogeneous pattern with normal or moderately swollen mitochondria and myofibrillar disruption in some areas, and moderate to massive mitochondrial swelling in other sections (Fig. 2).

Pre-ischemia biopsy specimens in the patients with normal ventricular mass showed well preserved architecture in most samples. In some cases, foci of mitochondria showing mild to moderate swelling were observed. Also, a mild degree of intramyofibrillar and/or intermyofibrillar edema was detected. Morphological changes in

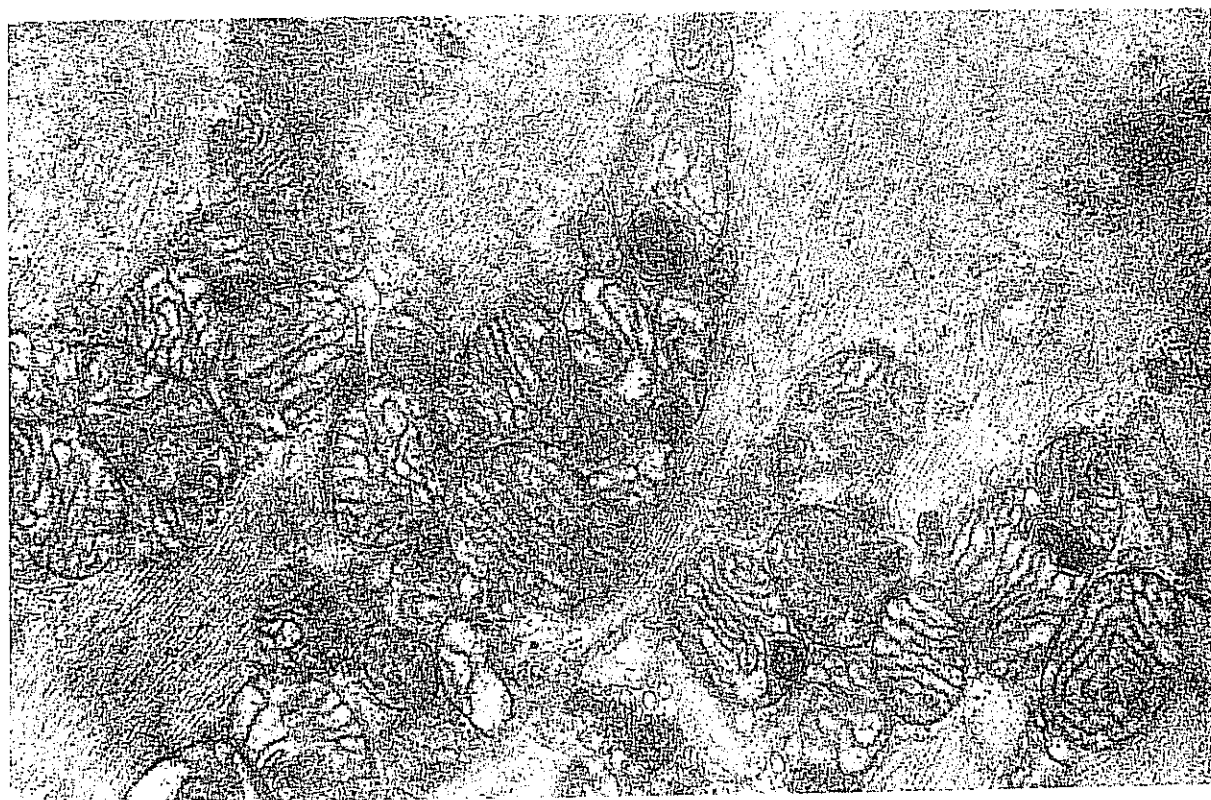


Fig. 2. Postischemic biopsy specimen in a patient with hypertrophied ventricle. Massive swelling of mitochondria, disruption of cristae, rupture of mitochondrial membrane and intramitochondrial myelin figures are observed ($\times 10,000$).

the reperfusion specimens from this group showed some areas with disruption of myofibrils (Fig. 3). Both pre-ischemia and reperfusion specimens showed loss of glycogen granules.

Semiquantitative studies. No significant differences were observed in overall edema, interstitial edema, and glycogen concentration between pre-ischemia and reperfusion samples in both groups. The values are listed in Table 2.

The percentages for grades 0 to 4 of damaged mitochondria for pre-ischemia and reperfusion samples in both groups are given in Table 3. In the patients with a hypertrophied ventricle significant statistical differences were observed for grades 3 and 4 (severe) damaged mitochondria between pre-ischemia and reperfusion samples. In the patients with normal ventricular mass, the differences between pre-ischemia and reperfusion samples were not statistically significant.

TABLE 2

Overall edema, interstitial edema and glycogen concentration.

Overall edema	Interstitial edema	Glycogen concentration
Hypertrophied ventricular mass		
A 1.1 ± 0.1	1.4 ± 0.1	1.9 ± 0.2
B 1.3 ± 0.1	1.3 ± 0.1	2 ± 0.2
Normal ventricular mass		
A 2.2 ± 0.2	1.8 ± 0.2	2.1 ± 0.3
B 1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.2

A = pre-ischemia; B = reperfusion.

Quantitative studies. The values for volumetric density of mitochondria in the patients with a hypertrophied heart were 23 ± 2 and 26 ± 4 for pre-ischemia and reperfusion samples, respectively. For those with normal ventricular mass

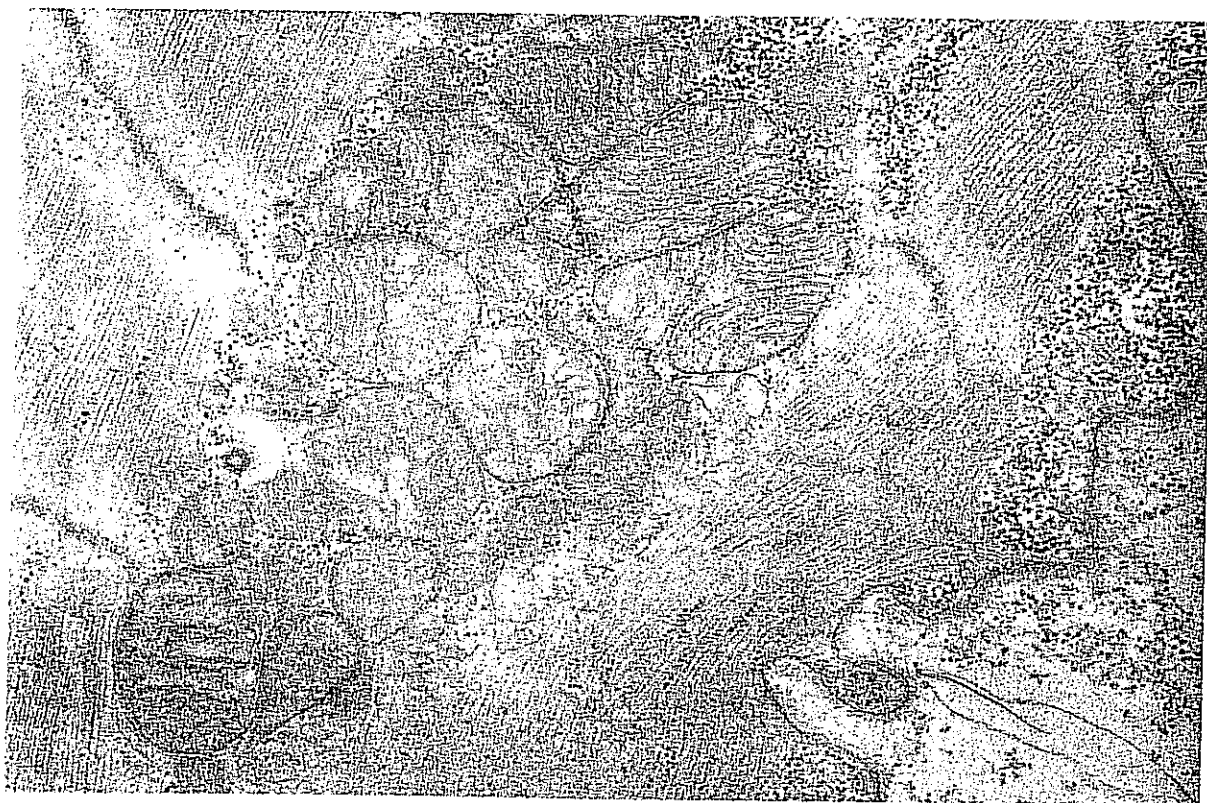


Fig. 3. Postischemia biopsy specimen in a patient with normal ventricular mass. The mitochondria have intact membranes and tightly packed cristae. Glycogen content is preserved ($\times 10,000$).

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TABLE 3
Grading of mitochondrial damage.

		Mitochondrial grade (%)				
		0	1	2	3	4
Hypertrophied ventricular mass						
A	75.6 ± 3.7	12 ± 2.6	5.6 ± 1.5	2.8 ± 0.6	3 ± 0.8	
B	56.4 ± 11	9.4 ± 2	11.6 ± 3.7	10.5 ± 4	12 ± 4.7	
Normal ventricular mass						
A	66 ± 9	9 ± 1.7	11.8 ± 3.6	6 ± 2.6	7 ± 3.7	
B	59.8 ± 8	10.6 ± 4	17.6 ± 5	7 ± 1.7	5 ± 1.4	

* $p < 0.05$. A = pre-ischemia; B = reperfusion.

these values were 35 ± 3 and 31 ± 3 . The difference for pre-ischemia values between both groups was statistically significant ($p < 0.02$). The numerical density of mitochondria in the patients with hypertrophied hearts was 52.9 ± 6 and 48.8 ± 6 for pre-ischemia and reperfusion samples, respectively. For those with normal ventricular mass these values were 41.3 ± 4 and 50 ± 1 (NS). The values for individual mitochondrial volume in the cases with hypertrophied hearts were 4.3 ± 0.6 , and 5.2 ± 1 for pre-ischemia and reperfusion samples, respectively. For the cases with normal ventricular mass the respective values for pre-ischemia and reperfusion samples were 4.8 ± 0.7 and 5.9 ± 1 (NS).

Discussion

The results of this study indicate that the hearts of patients with coronary disease and left ventricular hypertrophy developed a higher percentage of fibrosis and myocardial cell enlargement than coronary patients without hypertrophic ventricles. A decreased volumetric density corresponding to mitochondria was also observed. These differences were statistically significant. According to the grading of mitochondrial damage, our results indicate that a significant increase in mitochondrial damage is observed in the reperfusion samples for the patients with hypertrophied hearts compared to those with normal ventricular mass. In our experience, mitochondria were the structures most affected by the ischemia and reperfusion.

It has been postulated that the hypertrophied ventricle is more susceptible to ischemia and that these patients have an increased surgical risk [6-8,14]. The development of myocardial hypertrophy reduces maximal coronary vasodilator capacity. This mechanism, associated with a restricted proliferative capillary development that does not keep pace with contractile protein synthesis, affects coronary circulation [15-17]. As a consequence of this new arrangement, the degree of hypoxia increases particularly in the subendocardial layer. With associated coronary disease, as was the case in our experience, a further risk should be expected.

Fibrosis in the pressure overloaded left ventricle

Myocardial hypoxia serves as a stimulus for collagen synthesis which consists in a reactive fibrosis that accumulates mainly in the interstitium. The range of collagen volume fraction has been studied through transmural or postmortem biopsies in normal and pressure overloaded human myocardium, namely congenital and acquired coarctation of the aorta, aortic stenosis, and in postmortem hypertensive and hypertensive diabetic hearts [18,19]. In these reports the percentage of fibrosis varied widely but it was always greater than in normal hearts. In our experience, the patients with hypertrophied hearts had a mean percentage of fibrosis which was significantly higher than in the patients with normal ventricular mass. These values were similar to those observed in increased workload conditions.

It has been shown that collagen growth increases myocardial stiffness and reduces chamber compliance [20,21]. These alterations in the contractile performance of the ventricle may be enhanced when the heart is submitted to the ischemia/reperfusion mechanism as it occurs in open heart surgery. Therefore, the hypertrophied myocardium with coronary disease has several factors that may jeopardize the postoperative outcome of these patients. In our experience, 3 patients from the hypertrophied group developed perioperative myocardial infarction and one of them required inotropic support.

Sarcomere and mitochondrial changes

Another structural finding in the hypertrophied group was a significant increase of the diameter of the myocyte. This may be produced on the basis of protein generation of the myofibrillar component. This growth exceeds that of mitochondrial synthesis and this was shown by a significant decrease of the volumetric density of mitochondria in the pre-ischemia samples of the patients with hypertrophied ventricle which represented the volume fraction of tissue occupied by these structures. These observations agree with previous reports that showed an increased myofibrillar/mitochondrial ratio in the hypertrophied hearts [22]. The values for individual mitochondrial volume were lower in the patients with hypertrophied ventricular mass than in patients with normal ventricular mass, suggesting that the reduced volumetric density was secondary to a decreased size of the organelles with minimal changes between pre-ischemia and reperfusion samples in both groups. These differences were not statistically significant.

We may conclude from these observations, that these patients develop similar ultrastructural changes to those seen in other types of pathology with increased workload. These changes were: accumulation of collagen, increased size of the myocyte and increased myofibrillar/mitochondrial ratio. These modifications were demonstrated by quantitative and semiquantitative methods.

Regarding the structural changes of the human hypertrophied myocardium submitted to ischemia, only two works are available in the literature and none of them analyze the association of ventricular hypertrophy with coronary disease [7,8]. Warner and co-workers [8], performed semiquantitative analyses of morphologic changes in patients operated for aortic valve replacement and observed significant alterations during the periods of ischemic arrest and reperfusion. In our experience, the patients had ventricular hypertrophy associated with coronary heart disease, and therefore significant morphologic alterations should be expected. In this regard, the most striking feature was the mitochondrial damage

observed in the reperfusion samples from the patients with an enlarged ventricular mass, which showed increased grades 3 and 4 of severely damaged mitochondria compared to the pre-ischemia biopsies with wide variations between patients. On the other hand, the patients with normal ventricular mass did not show severe mitochondrial damage and all showed a homogeneous preservation of these structures. Other parameters such as edema and glycogen concentration were not significantly different between groups.

Adequacy of myocardial protective techniques

In a previous study we showed that supplementing the cardioplegic solution with mannitol resulted in a significant reduction of early postoperative arrhythmias and a better preservation of the ultrastructure [23]. One of the properties of mannitol appears to be related to its capacity as a scavenger of the hydroxyl radical [24]. This was demonstrated by chemiluminescence, a method which we have widely applied for both in vitro and in vivo determinations of lipoperoxidative processes [25-28]. In the present work we supplemented blood cardioplegia with mannitol. Blood cardioplegia has shown to be superior to crystalloid solutions [29-32], and the benefits of both mannitol and blood cardioplegia were particularly observed in our patients with normal ventricular mass in whom an adequate preservation of the ultrastructure in the reperfusion samples was achieved. However, we failed to obtain similar results in the hypertrophied hearts.

Several alternatives may be taken into consideration to improve the protection in the hypertrophied heart with coronary disease. (1) The cardioplegic solution should be more frequently or even continuously administered as postulated by other authors [33]. (2) The administration of warm blood cardioplegic reperfusate just before the aorta is unclamped appears to neutralize cellular acidosis, optimizes the rate of metabolic repair and minimizes calcium paradox [33]. (3) Substrate enrichment of the blood with glutamate or aspartate replaces Krebs cycle intermediate utilized during ischemia [34]. (4) The use of combined

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antegrade/retrograde cardioplegia ensures good distribution particularly in the subendocardial layer [35,36]. (5) An increased tolerance of the heart to a prolonged period of ischemia when it was preceded by one or more brief episodes of ischemia has been recently observed [37,38]. This procedure called "preconditioning" remains in the research field and the response of the hypertrophied myocardium to this technique is still unknown. (6) Finally, the addition of oxygen-free radical scavengers to the cardioplegic solutions should also be considered. Although several issues still remain unresolved, there is no doubt that oxygen-free radicals are important contributors to myocardial injury during the reperfusion period [39,40]. In our experience the use of mannitol [23], deferoxamine [28], and the preoperative administration of vitamins A and E [41] ameliorated reperfusion damage and decreased lipoperoxidative stress. On the basis of our results we conclude that the patients with hypertrophied myocardium and coronary disease have several structural abnormalities characterized by a restricted blood supply, collagen proliferation, and alteration of the myofibrillar/mitochondrial ratio. These changes are similar to those observed in hearts with aortic stenosis or other conditions with increased workload. The association of coronary disease probably increases the sensibility to ischemic damage and therefore, myocardial protection techniques should be enhanced in these patients.

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