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Acute post-ischaemic ventricular changes: role of microcirculatory trouble and myocardial oedema in isolated working rat hearts

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Acute postischaemic ventricular changes are characterized by ventricular wall alterations, involving both ischaemic and non-ischaemic segments of the ventricle. These changes include either myocytic or interstitial structural damage and/or rearrangement. One of the consequences of myocardial ischaemia is the development of interstitial and intracellular oedema, which results in an increase of wall tension, plugging of the microcirculation and ventricular remodeling. In this study we have detected the effects of post-ischaemic microcirculatory permeability changes on ventricular function and myocytic or interstitial structural damage and/or rearrangement.

We have evaluated the effects of reperfusion-induced oedema on acute ventricular changes and mechanical function in isolated working rat hearts. After 15 min. of global ischaemia, hearts were perfused with isotonic Krebs-Henseleit (iKH, 287 mOsm) or with hypertonic KH (hKH, obtained by adding 80 mM sucrose to KH, 320 mOsm) to reduce reperfusion-induced oedema. Acute ventricular changes were evaluated in terms of haemodynamic parameters, enzyme release, heart weight, myocyte damage (immunoperoxidase labelled anti-lactic dehydrogenase antibodies), cellular fibronectin deposition and rearrangement (immunoperoxidase labelled antifibronectin antibodies), endothelial permeability (fluorescein isothiocyanate-albumin immunofluorescence). An ultrastructural examination was also performed to determine cellular (mitochondria, fibrils) and interstitial damage. In isotonic-perfused hearts all functional parameters were significantly reduced. This was concomitant with a significant increase of myocyte damage, endothelium permeability and c-fibronectin deposition in perivascular space. These changes were related to myocardial oedema; hypertonic reperfusion of the hearts resulted in a significant protection.

These data indicate that, in isolated working rat hearts, there is a close relationship between reperfusion-induced oedema and acute ventricular changes, involving myocyte damage and interstitial matrix rearrangement. *J Clin Basic Cardiol* 1999; 2: 267-74.

Key words: reperfusion oedema, reperfusion injury, microvascular permeability, endothelium, fibronectin

Acute postischaemic ventricular changes are characterized by ventricular wall alterations, involving both ischaemic and non-ischaemic segments of the ventricle [1-3]. These changes include either myocytic (necrosis, contraction bands, cellular and mitochondrial swelling) or interstitial (oedema, microcirculation alteration) structural damage and/or rearrangement [1, 4], leading to a loss of contractile function of the ischaemic myocardium. This, in turn, causes a redistribution of cardiac loading and the remaining viable myocardium is called upon to maintain cardiac output. One of the consequence of myocardial ischaemia is the development of interstitial and intracellular oedema, which results in an increase of wall tension, plugging of the microcirculation and ventricular remodeling [4-8].

A clear link between acute and chronic remodeling is still controversial, particularly with regard to the relationship between acute damage and interstitial cell rearrangement. These mechanisms seem to be operating already in the early phases of reperfusion [9-12].

From the hypothesis that reperfusion oedema might play a significant role in the reperfusion damage and in the post-ischaemic left ventricular remodeling, the early mechanism responsible for these events was investigated in order to reduce the postischaemic myocardial damage. In order to classify the relationship between reperfusion oedema and acute postischaemic ventricular changes, we evaluated the effects of: 1) the increase of coronary microvascular permeability caused by ischaemia and 2) the oedema related to the reperfusion on the overall ventricular function and morphology.

Material and methods

Animals and perfusion technique

Using the working heart apparatus [13], isolated hearts (n = 110) of adult male Wistar rats (body weight 250-300 gr.) were perfused with a modified isotonic Krebs-Henseleit solution (iKH: 108 mM NaCl, 25 mM NaHCO₃, 4.8 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 11 mM glucose, 287 mOsm) or hypertonic KH (320 mOsm, hKH, see below), obtained by adding 80 mM sucrose. The buffer was bubbled continuously with 95 % O₂ and 5 % CO₂ maintaining a pH of 7.4. Preload (height of atrial chamber) and afterload (height of aortic chamber) were set at 20 and 72 cm respectively. Aortic and coronary flows were measured collecting aortic chamber overflow and heart chamber effluent into graduated cylinders. Aortic pressure was monitored through a membrane transducer (TNF-R, Viggo-Spectramed, Oxnard, CA) connected to a side arm of the aortic cannula. Heart rate was determined with an epicardial ECG (Cardioline 350/1, Milan, I). Minute work was computed as the product of cardiac output (sum of aortic and coronary flows) and peak systolic aortic pressure.

Experimental protocol

The experimental design is reported in Figure 1. The hearts were split into 2 main groups: *Group A* (NIH = non-ischaemic hearts, n = 60), the majority (n = 40) being subjected to 105 min. of working heart full flow, aerobic perfusion. *Group B* (IH = ischaemic hearts, n = 50), where the hearts were sub-

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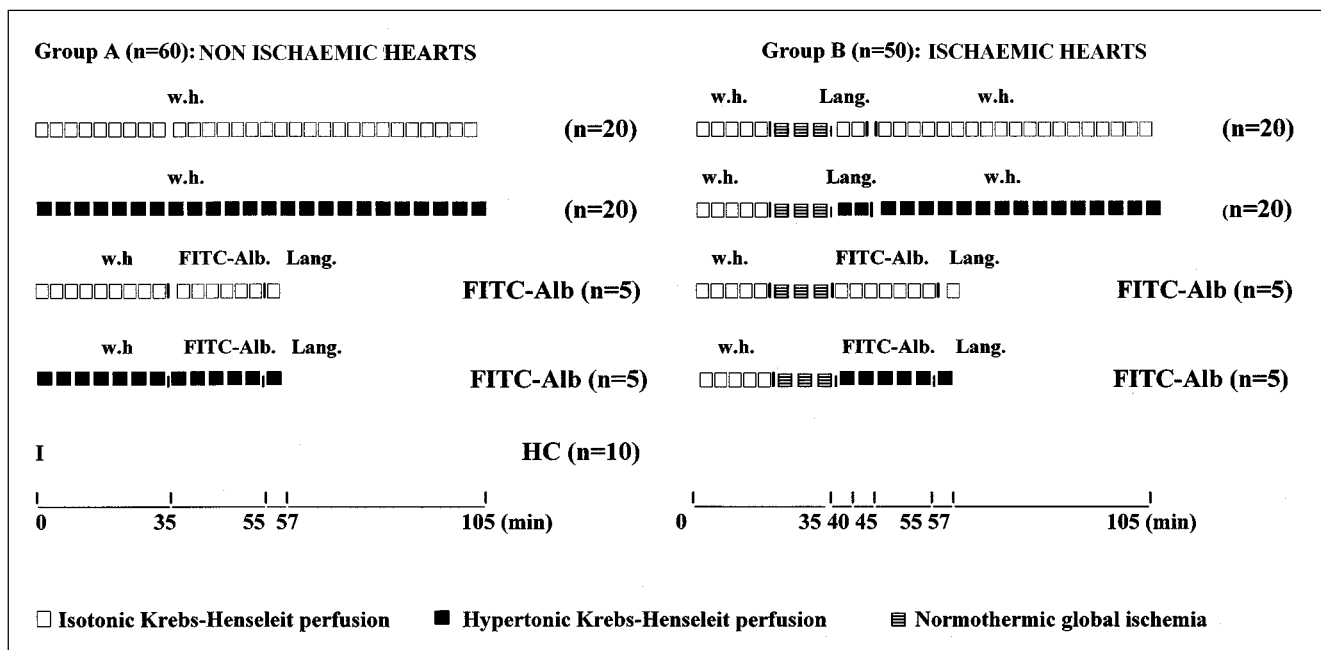


Figure 1. Experimental protocol; FITC-Alb: fluorescein isothiocyanate-albumin; HC: histological control; Lang.: Langendorff reperfusion; w.h.: working heart

mitted to 20 min. of stabilization followed by 15 min. of global ischaemia and 70 min. of reperfusion ($n = 40$), using the Langendorff method for the first 10 min. and working heart technique for the remaining 60 min. In each group the hearts were further divided into subgroups, according to whether the buffer utilized was hyper- (hKH) or isotonic (iKH). In Group A, 20 hearts were perfused aerobically with iKH (Group A, isotonic) and 20 hearts with hKH (Group A, hypertonic). In Group B, 20 hearts were reperused with iKH (Group B, isotonic) and other 20 with hKH (Group B, hypertonic). In this last subgroup, the reperfusion was made with iKH for the first 5 min. and then with hKH buffer. In addition, in Group A, ten hearts were simply washed with iKH and used as histological control (HC).

In order to assess microvascular permeability, additional hearts were perfused with fluorescein isothiocyanate-albumin (FITC-albumin). In Group A, starting at the 35th min., hearts ($n = 5$) were subjected to 20 min. Langendorff perfusion with 75 mg FITC-albumin, dissolved in 200 ml of iKH, followed by two minutes of perfusion with iKH Langendorff to eliminate intravascular fluorescence and reweighted. Other 5 hearts of the same group were submitted to the same procedure, using hKH buffer. In Group B the same protocol was implemented and, after 15 min. of global ischaemia, 5 hearts were reperused with FITC-albumin iKH and 5 hearts with FITC-albumin hKH (after 5 min. of iKH).

Evaluation of ventricular changes

Interstitial changes

- Evaluation of microvascular permeability: Changes in microvascular permeability were determined in the hearts perfused with FITC-albumin by means of fluorescent microscopy. Ventricles were cut transversally into 4–5 blocks. Tissue blocks were immediately embedded in O.C.T. Compound (Miles, Elkhart, IN) medium and stored at -80°C . Tissue blocks were mounted on a specimen holder in a Slee microtome-cryostat maintained at -35°C and oriented so that capillaries and muscle fibers could be cross-sectioned. $10\ \mu\text{m}$ sections were obtained from each tissue block and placed on a

labelled slide, which was prewarmed on a hot plate. Slides were immediately returned to the hot plate for drying, placed in a dark box for at least 1 hour and then viewed and photographed at 40x under fluorescent light. FITC-albumin accumulation was quantified per section using an image analysis system (Image-Pro plus, Media Cybernetics, Silver Spring, MD) and expressed as integrated optical intensity (IOI) units according to Ramirez et al. [14].

- Evaluation of fibronectin deposition: To assess the distribution of fibronectin in the interstitial space tissue samples (8/ subgroup) were investigated by immunohistochemistry using rabbit monoclonal antibodies against cellular fibronectin (c-FN, YLEM, Rome, Italy). The specimens were cut into 4–5 blocks and formalin fixed. Serial step sections (20–30 for each experiment), cut at $5\ \mu\text{m}$ were dewaxed in xylene, hydrated through graded ethanols and washed in 0.01 M phosphate buffer solution (PBS), pH 7.4. Endogenous peroxidase activities were quenched in 0.3 % (v/v) hydrogen peroxidase in absolute methanol for 30 min. Immunoperoxidase staining was performed using streptavidin-biotin system kit for primary rat antibody (YLEM, Rome, Italy). The primary antibody ($200\ \mu\text{l/slide}$) was incubated for 60 min at room temperature in a humidified chamber. Peroxidase reaction was developed in 0.06 % diaminobenzidine tetrahydrochloride (DAB, Sigma, Milan, Italy) in PBS with 0.01 % hydrogen peroxide for 7 min. at room temperature. After haematoxylin counterstaining, the slices were photographed and analysed for the presence and distribution of immunostaining using an image analysis system (Image-Pro plus, Media Cybernetics, Silver Spring, MD). An average of 30 fields in each section were examined at 120x magnification. Results are expressed as follows: area of immunostaining deposition/total area of myocardium $\times 100$. The analysis of the distribution of perivascular (PV) and perimyocytic (PM) c-FN was also performed and data are reported as percentage values (%) / total immunostaining deposition area.

- Evaluation of myocardial oedema

Changes in the weight of the heart: Myocardial reperfusion oedema was estimated as percent gain in heart weight ($n = 32/\text{group}$). The following equation was employed: heart weight before/heart weight after ischaemia-reperfusion $\times 100$. Myocardial water content was derived from wet weight/dry weight ratio (ww/dw ; $n = 8/\text{group}$); the dry weight was obtained after maintaining overnight at 105°C .

Ultrastructural evaluation of interstitial space: Rat myocardial specimens ($n = 8/\text{subgroup}$) were fixed in 2.5 % glutaraldehyde for at least 3 hours. Specimens were post-fixed in 1.33 % osmic acid, dehydrated and impregnated with toluol and then included in Resin Epon 812. Thin and $600\text{ \AA}$ ultrathin sections were obtained and respectively stained with toluidine blue or with both uranyl acetate and lead citrate. An average of 30 fields in each section were examined and photographed at $4,500\times$ magnification. Interstitial space evaluation was performed using the same image analysis system reported before, quantified as interstitial space area/total myocardium area $\times 100$ and data were compared with histological controls.

Myocyte damage

Myocyte injury assessment was based on electron microscopy (see above). Mitochondrial damage was analysed using a mitochondrial score index [15]. Mitochondrial score index valued as an average of 30 fields in each section, based on scores of 0 to 4; 0: normal mitochondria; 1: clearing of matrix density and separation of cristae; 2: clearing of matrix density and disruption of cristae; 3: marked clearing of matrix density and disruption of cristae; 4: disruption of cristae and rupture of inner and outer membranes.

Myocardial necrosis enzyme assay of coronary effluent was also performed before ischaemia (20 min.), during Langendorff (35, 37, 39 min.), and during working heart reperfusion (55, 65, 75, 85, 95, 105 min.). Creatin phosphokinase (CPK) and lactate dehydrogenase (LDH) activities from eluate samples

were determined using enzymatic analysis and commercial kits (Boehringer Mannheim).

Other heart specimens ($n = 8/\text{group}$) were subjected to *in vitro* labelling of myocytes displaying lesions or abnormal sarcolemmal permeability, using rabbit anti-lactate dehydrogenase-H4 (LDH-H4) polyclonal antibodies (Chemicon Int., Temecula, CA) (see cFn evaluation for details). Results are expressed as follows: area of immunostaining deposition/total area of myocardium $\times 100$.

Statistical analysis

The results are expressed as mean $\pm$ SD. Statistical analysis was performed by analysis of variance (ANOVA) after having assessed the data for normal distribution, and, where indicated, by paired *t* tests. Linear correlations were performed by standard techniques [16]. Probability of null hypothesis of $< 5\%$ ($p < 0.05$) was considered statistically significant.

Results

Postischaemic ventricular function

Postischaemic functional response of Group B perfused under iso- or hypertonic conditions compared with that obtained with the same experimental protocol in Group A (non-ischaemic heart) is reported in Figure 2. In Group B, the aortic pressure of the isotonic reperfusion subgroup showed a significant reduction ($p < 0.001$) as compared with the pre-ischaemic value. In the hypertonic Group B, the post-ischaemic reduction of aortic pressure was significantly ($p < 0.001$) lower than that isotonic subgroup. The protective effect of hypertonic reperfusion was more evident when coronary and aortic flows were considered. Particularly, coronary flow (Figure 3) was constantly reduced ($p < 0.001$) in the isotonic Group B with respect to the isotonic Group A, and significantly lower ($p < 0.001$) than hypertonic Group B. As expected, the positive effect of hypertonic reperfusion on ventricular function was also confirmed by minute work analysis (Figure 2, MW).

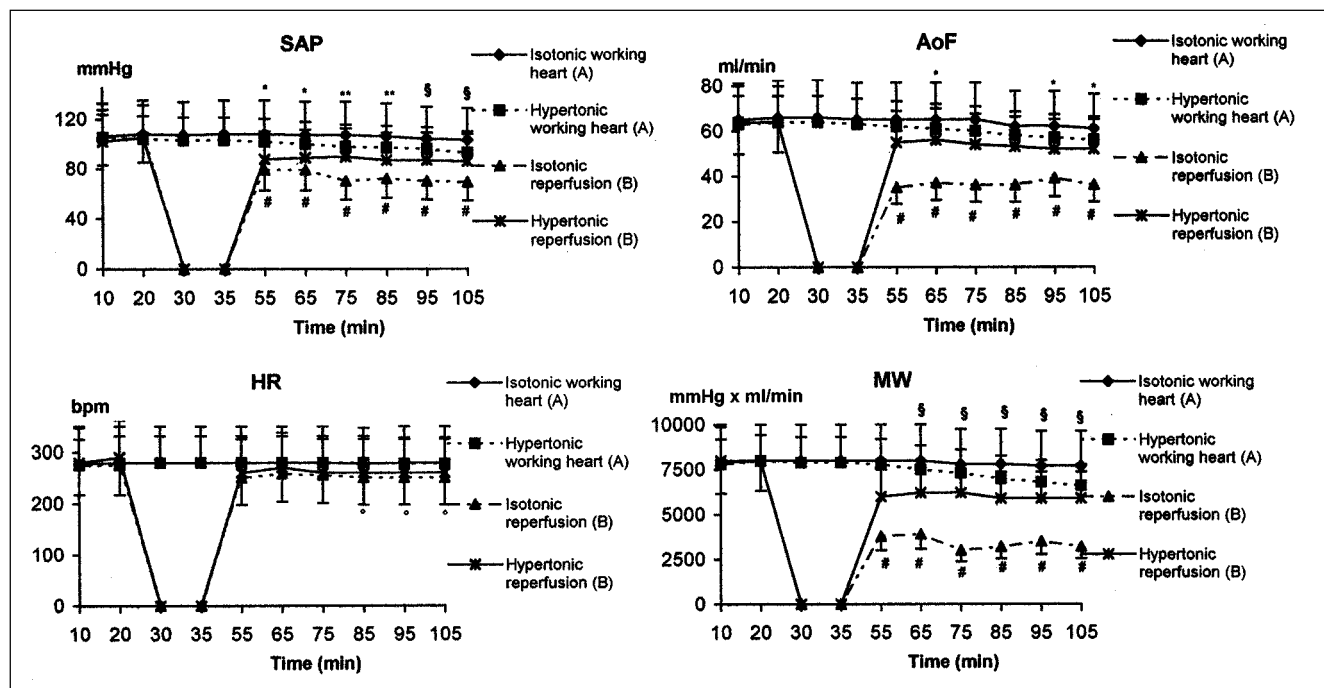


Figure 2. Haemodynamic data in all groups; (A): group A, not-ischaemic hearts; (B): group B, ischaemic hearts; SAP: systolic aortic pressure; AoF: aortic flow; HR: heart rate; MW: minute work; * = $p < 0.05$, ** = $p < 0.003$, § = $p < 0.001$ isotonic vs hypertonic WH, ° = $p < 0.005$, # = $p < 0.001$ isotonic vs hypertonic reperfusion. Data are expressed as mean $\pm$ SD ($n = 20/\text{subgroup}$).

Table 1. Morphological data

	Non-ischaeamic hearts (A)			Ischaemic hearts (B)	
	Histological control	Isotonic working heart	Hypertonic working heart	Isotonic reperfusion	Hypertonic reperfusion
LDH deposition (% area)	4.0 ± 2.0	5.5 ± 3.0	7.5 ± 4.0	38.0 ± 11.0**	11.0 ± 4.5
FITC-albumin diffusion (IOI)	9.0 ± 2.0	11.0 ± 3.0	9.7 ± 3.5	61.0 ± 4.6**	31.0 ± 7.0
cFN deposition (% area)	10.1 ± 4.5	9.7 ± 3.0	9.4 ± 3.2	25.0 ± 9.3**	9.8 ± 4.2
Ultrastructural analysis					
MSI	2.5 ± 0.5	3.4 ± 2.3*	9.3 ± 2.1	147.0 ± 21.0**	35.0 ± 11.0
Interstitial area (%)	9.6 ± 3.5	10.2 ± 2.0*	6.2 ± 1.5	25.2 ± 5.9**	11.0 ± 2.3

* = $p < 0.001$ isotonic vs hypertonic working heart; ** = $p < 0.001$ isotonic vs hypertonic reperfusion

cFN: cellular fibronectin, FITC-albumin: fluorescein isothiocyanate-albumin; IOI: integrated optical intensity; LDH: lactate dehydrogenase; MSI: mitochondrial score index. Data are mean ± SD.

Post-ischaeamic structural ventricular changes

Early interstitial changes

• Vascular microcirculatory changes: After FITC-albumin infusion, in the isotonic Group B a significant increase ($p < 0.001$) of fluorescence in perivascular and perimyocytic spaces with respect to the isotonic Group A was observed (Figure 4 and Table 1). In the hypertonic Group B, FITC-albumin diffusion fluorescence was significantly reduced ($p < 0.001$) vs the isotonic subgroup and limited to perivascular space, remaining, however, significantly higher ($p < 0.001$) when compared to the isotonic Group A.

• Reperfusion oedema: After 15 min. of global ischaemia, the isotonic Group B hearts weighed more ($p < 0.001$) than the isotonic Group A. In the Group B, the hypertonic subgroup weighed significantly less ($p < 0.001$) than the isotonic. These data were further confirmed by ww/dw ratio reported in Table 2, indicating an occurrence of post-ischaeamic reperfusion oedema. These data were supported by the morphological analysis showing acute modifications of the interstitial space between ischaemic and not-ischaeamic isotonic and hypertonic hearts (Table 2). As expected, isotonic Group B showed a significant ($p < 0.001$) increase of interstitial area (interstitial oedema), especially evident in perivascular space. The addition of sucrose to KH (hypertonic reperfusion) resulted in a significant reduction of oedema with a preservation of the interstitial geometry as compared with isotonic Group B.

• Interstitial fibronectin deposition: The isotonic Group B showed an increase ($p < 0.001$) in cFN deposition as compared with the isotonic Group A (Figure 4 and Table 1), with a redistribution in perivascular and perimyocytic spaces. The

same analysis performed in the hypertonic Group B evidenced (Table 1) a significant reduction of the perimyocytic and perivascular cFN deposition as compared with isotonic subgroup (isotonic reperfused hearts: 25 ± 9.3 % area; hypertonic reperfused hearts: 9.8 ± 4.2 % area, $p < 0.001$). No significant changes were observed when the hypertonic Group B were compared with Group A and histological controls.

Myocyte damage

• Release of myocardial necrosis enzymes: The behaviour of enzyme releases (CPK and LDH) in coronary effluent is reported in Figure 5. The hypertonic Group A had a significant increase ($p < 0.001$) of CPK and LDH release as compared to the isotonic subgroup showing an overlapping release in the early phase of the procedure, but with a significant in-

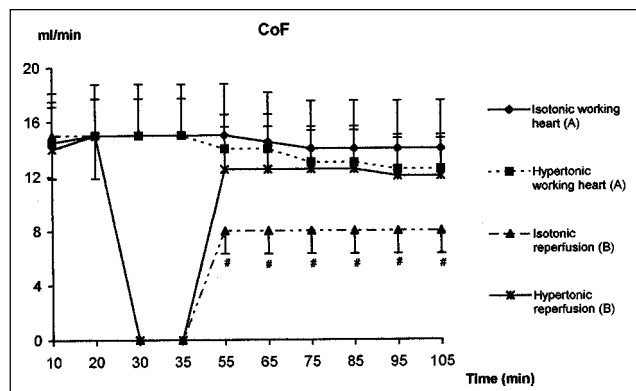


Figure 3. Time course of coronary flow (CoF) in all experimental groups. (A): group A, not-ischaeamic hearts; (B): group B ischaemic hearts; # = $p < 0.001$ isotonic vs hypertonic reperfusion. Data are expressed as mean ± SD (n=20/subgroup).

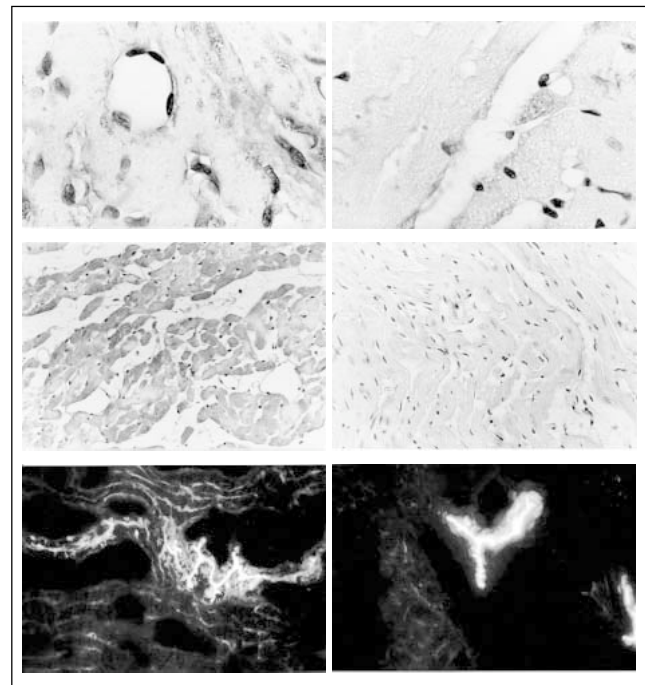


Figure 4. Histological findings. *Left*: ischaemic rat heart subjected to ischaemia and isotonic reperfusion. *Right*: effects of hypertonic reperfusion. *Top*: (x400) tissue specimens investigated by immunohistochemistry using monoclonal antibodies against cellular fibronectin. *Middle*: (x200) tissue specimens investigated by immunohistochemistry using monoclonal antibodies against antilactic dehydrogenase-H4 (LDH-H4) polyclonal antibodies. *Bottom*: (x200) FITC-albumin extravasation (microvascular permeability) in perivascular and perimyocytic space. In hearts submitted to hypertonic reperfusion a significant reduction of cFN and LDH tissue deposition is detected associated with a reduction of FITC-albumin diffusion.

crease ($p < 0.001$) of release of both necrosis enzymes (CPK and LDH) in the late phase in the hypertonic subgroup with respect to the isotonic. In Group B, before ischaemia and during the early reperfusion either the isotonic than the hypertonic subgroup showed a similar release of CPK and LDH: successively, the isotonic subgroup showed a significant ($p < 0.001$) higher CPK and LDH releases with respect to the hypertonic subgroup.

• Tissue LDH deposition and electron microscopy evaluation: The hypertonic Group A had a modest increase of LDH tissue deposition compared to controls and to the isotonic subgroup (Table 1). Instead, the isotonic Group B showed a significant increase in LDH deposition (Figure 4) compared to the hypertonic subgroup and to histological controls (isotonic reperfusion: 38 ± 11 % area; hypertonic reperfusion: 11 ± 4.5 % area; $p < 0.001$).

Morphological evaluation of the isotonic Group B (Figure 6 and Table 1) evidenced signs of ischaemic damage in terms of necrotic areas with disruption of normal interfibril geometry, swollen cells and mitochondria, with severe alterations of the cristae. The damage was more evident than in isotonic Group A and histological controls. In the hypertonic Group

B there is a significant decrease in damage as compared with the isotonic subgroup. In the hypertonic Group A a slight alteration of myofibrils and mitochondria was detected, more evident than the isotonic subgroup (Table 1).

Discussion

Our results suggest that in the isolated working rat heart there is a relationship between postischaemic alterations of microvascular permeability, reperfusion-induced oedema and myocardial damage both at the level of the myocyte and of the extracellular matrix.

Acute interstitial changes

Coronary microvascular permeability alterations and interstitial fluid diffusion

Several experimental studies suggested that ischaemia causes endothelial dysfunction with consequent increase of coronary vessel permeability [17]. The dysfunction depends upon membrane damage, activation of transmembrane transport system and hypercontracture of the endothelial cells. Sumergreen et al. reported that these alterations are not modified by oxygen free radicals but can be ameliorated by calcium channel

Table 2. Heart weight changes

	Not-ischaemic hearts (A)		Ischaemic hearts (B)	
	Isotonic working heart	Hypertonic working heart	Isotonic reperfusion	Hypertonic reperfusion
Wet weight B (gr.)	1.36 ± 0.40	1.35 ± 0.25	1.36 ± 0.30	1.37 ± 0.40
Wet weight E (gr.)	1.47 ± 0.50	1.39 ± 0.30	$1.89 \pm 0.40^{**}$	1.53 ± 0.50
Weight gain (%)	$8.0 \pm 3.0^*$	3.0 ± 1.5	$38.0 \pm 7.0^\#$	12.0 ± 5.2
Ww/dw	3.9 ± 0.4	3.6 ± 0.5	$5.1 \pm 0.3^\$$	4.3 ± 0.3

B: heart weight at beginning of experiments; E: heart weight at the end of experiments; Ww/dw: wet weight/dry weight ratio; * = $p < 0.001$ isotonic vs hypertonic working heart; ** = $p < 0.05$, § = $p < 0.01$, # = $p < 0.001$ isotonic vs hypertonic reperfusion. Data are mean $\pm$ SD.

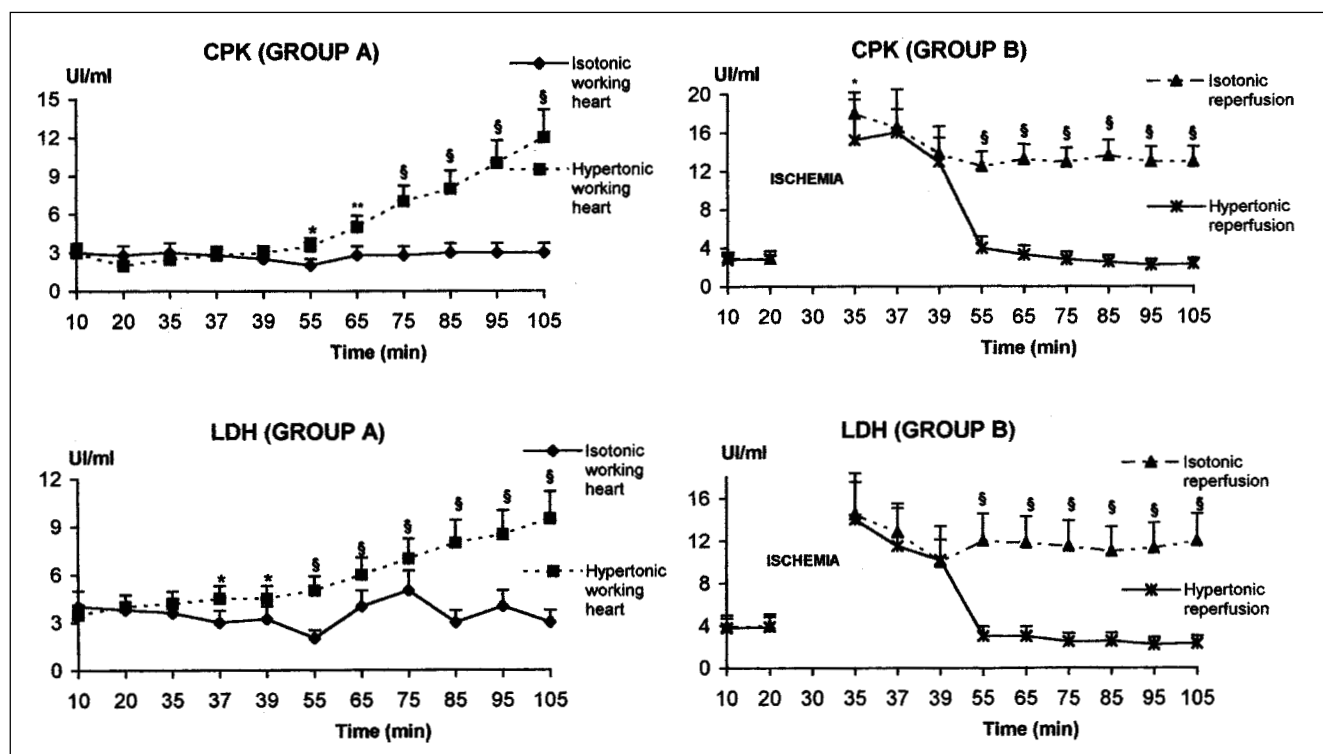


Figure 5. CPK and LDH release in coronary effluent (IU/ml). Group A: not-ischaemic hearts; Group B: ischaemic hearts; * = $p < 0.05$, ** = $p < 0.005$, § = $p < 0.001$. Data are expressed as mean $\pm$ SD ($n=20$ /subgroup).

blockers [18, 19]. In our study FITC-albumin diffusion was used to evaluate both the entity and the localization of fluids: as shown in previous studies from our group, the short ischaemia employed causes a significant increase in permeability, with diffusion of FITC-albumin in perivascular space [20]. With the progression of ischaemia, the alterations expand to perimyocytic space forming caps around single muscle fibers. This process could be one of the casual factors of the functional alteration observed after ischaemia [5, 6, 17, 21].

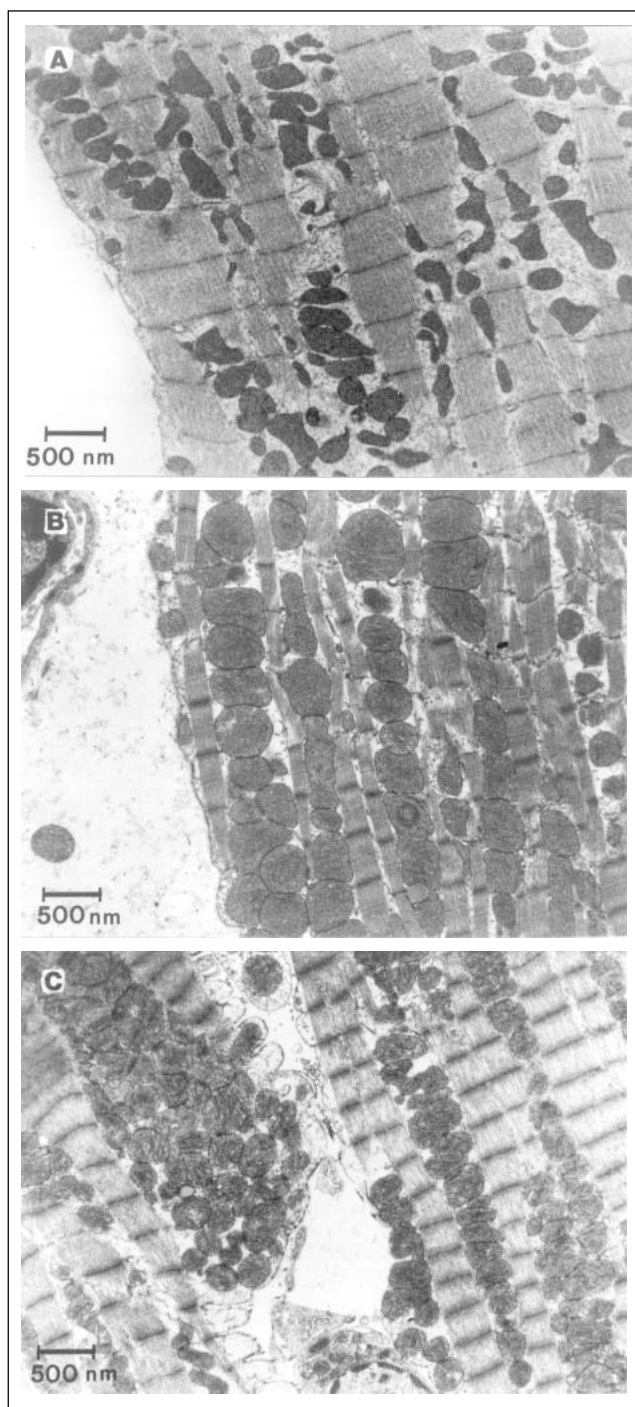


Figure 6. Ultrastructural morphometry (4,500x). A: histological control rat heart; B: ischaemic hearts submitted to isotonic reperfusion, C: ischaemic hearts submitted to hypertonic reperfusion. Global ischaemia time was 15 min. In group B a significant mitochondrial damage and increase of interstitial space is evidenced.

Reperfusion with hypertonic buffer, which strongly influences the osmotic balance, results in a significant reduction of the oedema extension and of reperfusion damage. On the other hand, perfusion with the same hypertonic buffer of the aerobic heart causes functional deterioration associated with increase in myocardial necrosis and enzyme release [20]. This apparent contradiction may depend on interstitial fluid volume and interstitium-vascular lumen osmotic gradient; in *non ischaemic* condition, at physiological interstitium-capillary osmotic load, the perfusion with an hypertonic agent induced, as observed in group A, a progressive interstitial dehydration followed by a progressive increase of coronary resistances, a reduction of coronary flow and an ischaemic suffering of myocardium; in *ischaemic* condition a significant increase of osmotic load is detected and it is responsible for interstitial and intracellular fluid accumulation at reperfusion time; in this condition, hypertonic reperfusion reduces myocardial oedema and its effect on coronary microcirculation. Particularly, in our experimental model we have induced a *global no-flow* ischaemia, which determinates, during ischaemic period, a significant reduction of heart weight and ultrastructural signs of interstitial dehydration (unpublished data). For this reason, we have delayed the hypertonic reperfusion five minutes in order to induce, at reperfusion time, a normalization of interstitial fluid volume; starting after this period hypertonic reperfusion, the excessive increase of interstitial oedema and the following alteration of coronary microcirculation are prevented.

cFN tissue deposition

FN is a dimeric glycoprotein located in extracellular matrix [22–24]. It has a role in cell growth and adhesion, with wound healing and with interstitial remodeling (hypertrophy, healing or reactive interstitial fibrosis). It is involved in early phases of postischaemic changes of extracellular matrix. Furthermore, only 2–3 hours after rat coronary occlusion a plasma FN deposition has been evidenced [10] more evident after 12–24 hours, when cellular synthesis of FN starts. Tissue FN is later substituted, after a few days, with collagen fibers and fibroblast proliferation, resulting in interstitial fibrosis [8]. Moreover, FN modulates tissue RAA system and the synthesis of interstitial growth factors therefore can play a role in interstitial remodeling [8, 9]. In our study we have observed in the early phases of reperfusion a cFN deposition and a redistribution which correlates with reperfusion-induced oedema ($r = 0.836$; $p < 0.001$) in terms of both location and entity. Knowlton et al. have evidenced significant difference in tissue FN expression in reperfused and not reperfused infarctions. A higher expression of FN in reperfused infarctions could be due to interstitial oedema which is more prominent in reperfused hearts [10]. Furthermore a reduction of cFN, seen in postischaemic hearts reperfused with hypertonic solution, does not seem to be due to artifacts caused by interstitial space reduction, since in non-ischaemic hearts perfused with the same solution, the interstitial area reduction (probably due to interstitial dehydration) is not associated with significant variation of cFN deposition. Our data are in agreement with the hypothesis of Weber et al. who report that FN fibrils form a perivascular network around vessels with altered permeability followed by migration and proliferation of fibroblasts around this network and type I and III collagen deposition [25]. In addition our data show that, in the acute state, cellular FN, not only plasma FN, is induced, suggesting a role in the remodeling process.

Myocyte damage

In ischaemic hearts, hypertonic reperfusion evidenced a significant reduction of necrosis enzyme release as compared with isotonic reperfusion, which is correlated with reperfusion myocardial oedema (mean CPK release during reperfusion: $r = 0.67$, $p < 0.01$). In isolated hearts, we have evaluated cell damage using LDH tissue deposition. This technique is capable of identifying myocytes displaying lesions or abnormal sarcolemmal permeability. It allows the limits of the other techniques (ie, triphenyltetrazolium chlorhydrate-staining, TTC) to be bypassed such as identification of damaged but not necrotic myocytes. Reduction of LDH deposition and positive correlation ($r = 0.874$, $p < 0.001$) with myocardial oedema observed in hypertonic reperfused hearts suggests the important role played by myocardial water content in the development of myocytic damage. Our data are in agreement with previous reports [6, 26] describing a reduction of the infarct size using hyperosmotic mannitol. However, other authors [21, 27, 28] failed to demonstrate an effect of mannitol infusion after ischaemia in pigs, dogs and baboons. These differences can be attributed to the different animal models used, the time of ischaemia and degree of osmolarity. In our study ischaemia was capable of reducing ventricular function and inducing cell damage. However, damaged myocytes are still viable as demonstrated by the rapid recovery after hypertonic KH [5].

Ventricular changes and cardiac function

Our results are in agreement with previous reports [4, 6, 21, 29] and they underline the negative effect exerted by reperfusion oedema on cardiac function. This phenomenon can be due to both an increase in myocyte damage and to mechanical alterations. Particularly, we have evidenced an inverse relation between myocardial oedema and coronary flow ($r = -0.65$; $p < 0.01$) and between coronary flow and myocardial damage ($r = -0.726$; $p < 0.001$) in ischaemic hearts. However, there are conflicting data regarding the possible pathophysiological role of myocardial oedema in the extension and progression of ventricular dysfunction [2, 17, 26, 27, 30, 31]. The increase of myocardial tissue water content induces an increase in microcirculatory resistance and extravascular pressure leading to mechanical stress of the surrounding tissue and may participate in the impairment of coronary flow and its distribution. Moreover, impaired oxygen and substrate diffusion to the myocytes may follow a mismatch of capillary to myofiber morpho-functional coupling and an increase in intercapillary distances caused by excessive myocardial tissue accumulation. In addition, an increase of interstitial fluid may induce a significant change of osmotic and oncotic homeostasis between interstitial and intracellular space which may be one of the factors responsible for the observed cellular and mitochondrial alterations. Finally, a possible involvement of reperfusion oedema in "no-reflow phenomenon" cannot be excluded.

Limits of our study

One of the limits of our study is the use of isolated heart reperfused with saline solution and not with whole blood. Thus, the effects of plasma and of the corpuscle elements of blood on reperfusion oedema are not evaluated. This method, however, allows the primitive endothelial alterations in the absence of cardiac load variations to be evaluated. Furthermore it furnishes biochemical data concerning tissue alterations during ischaemia-reperfusion. The use of sucrose as hyperosmotic agent is well recognized as it lacks scavenger effects. In the experimental model reported, the absence of

whole blood largely reduces the importance of the free-radicals on myocardial reperfusion damage and justifies the employment of hypertonic saline solution. However, in the same model [22], the effect of SOD and catalase on the impairment of the reperfusion damage is very slight, evidencing a modest free-radicals tissue and microvascular endothelium production. Moreover, in the isolated working rat heart plasmatic FN, commonly present in the early phases of reperfusion, is virtual. Furthermore, in our study FN is cellular, due to *ex novo* cell synthesis (minimally) or to interstitial redistribution of cFN in extracellular matrix. In order to confirm the hypothesis of a cellular synthesis of FN, Northern and Western blot analysis is necessary.

Conclusions

Our results suggest the importance of postischaemic myocardial interstitial oedema in acute ventricular changes. While the relationship between oedema and ventricular dysfunction was already reported in literature [26, 30], the direct involvement of oedema in cell damage has not been established. Furthermore, our study shows that interstitial oedema can promote, even in the acute phases, a redistribution of FN in the interstitial space. These conclusions contribute to explaining the pathophysiology of acute interstitial remodeling after ischaemia or infarction and demonstrate the importance of microvascular permeability dysfunction due to ischaemia-reperfusion. Another interesting observation is that a single short ischaemic period can trigger interstitial changes. This mechanism, linked with the variations of microvascular permeability observed after a brief ischaemia, is probably involved in the early interstitial fibrotic changes, resulting in the remodeling process observed in ischaemic patients without myocardial infarction. In conclusion, interstitial matrix alterations occur immediately after myocardial ischaemia-reperfusion. Modulation of this process in the acute phases could contribute to improved ventricular function, to reduced fibrotic remodeling and, as a final positive result, to promoting a cardiac repair rather than a cardiac remodeling [25].

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