Comparison of in vivo and in vitro haemodynamic function in experimental heart failure: use of echocardiography

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Abstract

Objective: To compare in vivo and in vitro haemodynamic performance in two models of experimental cardiac failure. To validate echocardiography as a means of non-invasive assessment of left ventricular dysfunction in rabbits. Methods: Cardiac failure was induced by doxorubicin injection (1-1.25 mg · kg⁻¹ twice weekly for 8 weeks (n = 16)) or coronary ligation (n = 12), with 12 controls. Left ventricular diastolic dimension and ejection fraction were assessed in vivo by echocardiography. The doxorubicin-treated and ligation hearts were subdivided by ejection fraction > 0.40 or ≤ 0.40 into non-failing and failing groups. Thermocililution cardiac output was measured in vivo at baseline and after a fluid load. Basal cardiac output and peak cardiac output achieved by increased preload were measured in vitro in the working heart mode. Results: The mean ejection fractions in the doxorubicin-treated and ligation groups were significantly (P < 0.001) lower than in controls, but there was wide inter-individual variability ranging from normal to severely impaired function [mean ± s.d. (range) controls 0.65 ± 0.03 (0.59-0.72), doxorubicin 0.45 ± 0.11 (0.30-0.67), ligation 0.42 ± 0.12 (0.25-0.65)]. Basal and peak cardiac outputs in vivo and in vitro were significantly lower in the doxorubicin and coronary ligation groups than in controls, although there was a wider scatter of values in the pathological groups. Among the doxorubicin and coronary ligation groups, hearts with ejection fractions ≤ 0.40 demonstrated significantly impaired haemodynamic function compared with those with ejection fractions > 0.40. There were significant correlations between ejection fraction and all indices of haemodynamic function in vivo and in vitro. Conclusions: Simple non-invasive measurement of ejection fraction allowed improved characterization of haemodynamic responses in vivo and in vitro. Individual assessment of animals by echocardiography will improve interpretation of cellular or molecular studies in experimental heart failure by relating observed abnormalities to the degree of global cardiac dysfunction.

Keywords: Heart failure; Hemodynamics; Cardiomyopathy; Echocardiography; Myocardial infarction; Doxorubicin

1. Introduction

Despite recent therapeutic advances such as the use of angiotensin converting enzyme inhibitors, advanced congestive cardiac failure still carries a dismal prognosis [1,2]. New insights are needed into the mechanisms responsible for the gradual decline in cardiac function which characterizes the transition from compensated left ventricular dysfunction to overt cardiac failure. Elucidation of the chronic cardiac responses to injury requires the use of animal models of cardiac failure in addition to clinical studies. Experimental heart failure may be induced by a variety of techniques [3,4] including pressure overload (e.g. hypertension, aortic constriction), volume overload (e.g. valvular regurgitation, arterovenous fistulae) or rapid pacing. However, models of cardiac failure due to chronic myocardial infarction or primary myocardial failure are of greater clinical relevance in view of the role of these conditions as the major causes of cardiac failure in man [3].

Experimental studies into the mechanisms responsible for cardiac failure are increasingly performed at the cellular or subcellular level. Such studies, comparing “control” and “heart failure” animals, are predicated on the assumption that the intervention used to induce cardiac failure

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results in a uniform response in all animals. Should this not be the case, the power of any study to identify mechanisms responsible for cardiac contractile failure will be compromised unless the extent of cardiac functional impairment is documented in each animal.

The purpose of the present study was to characterize the extent and variability of haemodynamic abnormalities in two models of cardiac failure in the rabbit. A particular aim was to investigate the use of the simple non-invasive technique of echocardiography in the prediction of cardiac function in vivo and in vitro, both under basal conditions and in response to haemodynamic stress.

2. Methods

The investigation was performed in accordance with the Animals (Scientific Procedures) Act 1986.

2.1. Doxorubicin cardiomyopathy

A well documented regimen was used for the induction of heart failure due to doxorubicin toxicity [6-8]. Adult male New Zealand White rabbits weighing 2.5-3.5 kg received doxorubicin via a marginal ear vein in a dose of 1-1.25 mg · kg⁻¹ twice weekly for 8 weeks. Control rabbits received 0.9% saline in equivolumetric doses over the same period. Echocardiographic and haemodynamic studies were performed at 10 weeks or later to allow resolution of any direct haemodynamic effects of doxorubicin.

2.2. Coronary ligation / chronic infarction

Adult New Zealand White rabbits (2.5-3.5 kg) received premedication with intramuscular fentanyl/fluanisone 0.4 ml · kg⁻¹ (Hypnorm, Jansen Pharmaceuticals). Anaesthesia was induced with midazolam (1-2 mg · kg⁻¹) given via an indwelling cannula in the marginal ear vein. The rabbit was intubated and ventilated using a Harvard small animal ventilator with a mixture of nitrous oxide, oxygen and halothane in a 1:1:1 ratio at a tidal volume of 20 ml and a frequency of 30 min⁻¹. A left thoracotomy was performed through the 4th intercostal space. Quinidine hydrochloride 10 mg · kg⁻¹ (Sigma Pharmaceuticals) was administered intravenously 15 min prior to coronary artery ligation to reduce the incidence of ventricular fibrillation. The major ventricular branch of the left coronary artery was ligated halfway between its origin and the cardiac apex. The rabbit coronary system has a poor collateral circulation and the area of ischaemic myocardium quickly became apparent giving an approximate idea of the area affected. Ventricular fibrillation occurred in approximately 30% of cases, usually 8 to 12 min following occlusion. Defibrillation was undertaken with a 2.0 joule epicardial shock. When an acceptable area of infarction (approximately 20% of the left ventricle) had been produced and the animal was haemodynamically and electrically stable, the thoracotomy was closed. The animals received 20 ml of isotonic saline subcutaneously to replace perioperative losses and were given intramuscular antibiotics for 48 h. Postoperative analgesia with buprenorphine 0.03 mg · kg⁻¹ every 8 h [9] was continued for the first 3-4 days, combined with convalescence in a warm clean environment with adequate monitoring of any distress. Echocardiographic and haemodynamic assessment were undertaken 8 weeks after myocardial infarction.

2.3. Echocardiographic assessment of left ventricular size and function

Echocardiographic examination was performed under light fentanyl/fluanisone sedation 0.3 ml · kg⁻¹. The animal was placed prone on a table with an area removed so that the ultrasound probe could be brought from below and placed on a shaved area of the anterior chest wall. Imaging was performed using a Toshiba 5 MHz short focus, wide angle phased array neonatal transducer and a Toshiba SSH160A echocardiograph with 2-dimensional real time and M mode acquisition and on-line cineloop computer analysis facilities. The best acoustic window was from the right parasternal position which gave long and short axis views of the left ventricle equivalent to the left parasternal window in the human. Left ventricular size was assessed by measurement of the maximum left ventricular end-diastolic internal diameter (LVIDd) at the level just below the tips of the mitral valve. The transducer was then rotated 90° to obtain a transverse short axis sector image. The end-diastolic and end-systolic frames were captured, the endocardial border, excluding the papillary muscles, was traced onto the screen, and the enclosed area was automatically calculated. The ejection fraction (area) was calculated as (end-diastolic area - end-systolic area/end-diastolic area).

All echocardiographic images were acquired and analysed by a single experienced operator. A day-to-day variability study was performed to determine reproducibility of image acquisition and analysis. Seven animals were examined two times approximately 3 days apart. Twenty-four randomly chosen end-diastolic and end-systolic frames were coded and analysed blind by one observer. The coefficient of variance and maximum difference between measurements on the same animal were 1.5% and < 6% for LVIDd and 2.5% and < 6% for ejection fraction respectively.

2.4. Measurement of thermodilution cardiac output

Thermistor catheters were made by removing the thermistor beads from commercially available Swan Ganz thermodilution catheters (Ecosse Medical Ltd) and remounting them in the tips of polythene cannulae (1.0 mm
The thermistors were calibrated with a Wheatstone bridge amplifier connected to a BBC computer, which was programmed to calculate the cardiac output by integrating the area under the temperature (i.e. voltage) time curve, taking into account calibration and biological constants [7]. Under anaesthesia, the injectate and thermistor catheters were implanted into the left ventricle and descending aorta respectively via the carotid and femoral arteries. Measurements were made 24 h after catheter implantation with the animals fully conscious and unrestrained. Repeated measurements of cardiac output were undertaken by the rapid injection of 1 ml of saline of known temperature into the left ventricle. A previous study [10] and preliminary experiments had shown that injection of 1 ml over 2 s into the left ventricle gave highly reproducible results with < 10% variation between measurements.

Rabbits are unable to exercise reproducibly, so cardiac reserve was determined by volume expansion of the circulation with an infusion of 50 ml isotonic saline over 1 min via the left ventricular cannula. The baseline and maximum cardiac outputs were converted to an index value by dividing by the body weight in kg.

2.5. In vitro haemodynamic study

2.5.1. Perfusion technique

After the performance of the in vivo cardiac output measurements the rabbits were injected with heparin 2000 i.u. intravenously and then given an overdose of sodium pentobarbitone. The heart was removed and placed in a beaker containing oxygenated Tyrode’s buffer chilled to 4°C and retrograde perfusion by the Langendorff technique [11] was commenced. One of the pulmonary veins was cannulated with the left atrial cannula while the other pulmonary veins were ligated. After equilibration and baseline measurement of coronary flow, perfusion in the working heart mode [12] was initiated by opening the left atrial inflow and allowing left ventricular ejection. The height of the left atrial reservoir (preload) was set at 10 cm H$_2$O, with an aortic column of 75 cm H$_2$O (afterload) against which the left ventricle ejected. Epicardial temperature was monitored during the procedure with an electronic temperature probe. The water-jacketed glass apparatus was heated and thermostatically controlled to maintain the temperature of the epicardium at 34–35°C. The perfusion medium was a modified Tyrode’s buffer solution pH 7.40, equilibrated with O$_2$:CO$_2$ (95:5). The final concentrations in this buffer were (μM): Na$^+$ 142, K$^+$ 4.0, Ca$^{2+}$ 1.8, Mg$^{2+}$ 1.0, H$_2$PO$_4^-$ 0.4, HCO$_3^-$ 28, glucose 11.0. The perfusion fluid was renewed every hour during the procedure to ensure an adequate glucose concentration. The output of working hearts was measured using a calibrated low resistance flow meter inserted between the aortic elasticity chamber and bubble trap. Coronary sinus effluent was measured by collection over a timed period. Total cardiac output is the sum of aortic flow and coronary sinus flow.

Left ventricular pressure was recorded from a 21-gauge short plastic cannula inserted by direct puncture, and attached via stiff wide bore plastic tubing to a Gould pressure transducer and the Mingograph 7 inkjet recorder. The frequency response of the system was adequate to record undamped peak systolic and end-diastolic pressures accurately. At the heart rates used in the study, and particularly in the failing hearts, left ventricular end-diastolic pressure exceeded the height of the left atrial reservoir above the heart. The former value was used as an index of preload.

Samples of coronary effluent were collected during the experiment for later estimation of lactate concentration using a commercially available enzymatic method (Boehringer Mannheim Diagnostica).

2.5.2. Experimental protocol

The heart was paced from the right atrium at a cycle length of 300 ms. Baseline working heart conditions were established using the lowest preload necessary to achieve a stable baseline aortic forward flow of ≥ 80 ml/min against an afterload of 75 cm H$_2$O. Peak cardiac output in vitro was determined by progressive increase of the height of the left atrial reservoir keeping afterload constant. LV systolic pressure, LV end-diastolic pressure, forward aortic flow and coronary sinus flow were measured after a 2 min period of equilibration at each level of load. Peak cardiac output was defined as the maximum stable value which could be maintained for ≥ 5 min. To ensure stability of the preparation, haemodynamic measurements were repeated after return to baseline working heart mode.

2.6. Histological assessment of myocardial damage

After the in vitro studies, the hearts were immediately placed in 10% neutral buffered formalin for subsequent histological assessment. The ventricles were sectioned at four levels parallel to the base of the heart, passing through the right and left ventricles and septum at each level. Each of the 4 blocks was embedded in paraffin wax, and 4 μm sections were cut and stained with haematoxylin and eosin and Martius scarlet blue for examination by low and high power light microscopy.

2.7. Doxorubicin cardiomyopathy

The hearts were examined by a pathologist blinded to the treatment regimen. A semiquantitative scoring system modified from that described by Jaenke [13] was devised to assess the degree of myocyte vacuolation or atrophy and interstitial fibrosis caused by the doxorubicin. This score represented the relative distribution and amount of cardiac tissue involved at each level of the heart examined and is shown below:

- 0 = normal, no damage.
• 1 = single myocytes or a small group involved at one level only.
• 2 = small groups affected at one or more levels.
• 3 = large area or confluent areas at one level.
• 4 = large groups or confluent areas at more than one level.
• 5 = circumferential involvement at one or more levels.

2.8. Coronary ligation

A qualitative assessment was made of the transmural extent of infarction, the degree of homogeneity of the infarct and the sharpness of the border zone. Quantitative measurement of infarct size was undertaken by planimetry with the microscope linked to a computer allowing the infarcted and non-infarcted areas of the left ventricle to be traced in outline at each of the 4 levels sectioned. The size of infarction was expressed as a percentage of total planimetered area.

2.9. Statistical methods

Continuous data are presented as mean (± standard deviation). Student's two sample paired or unpaired t tests were applied for normally distributed data and the non-parametric method of Mann Whitney was used for skewed data. A chi-squared test for discontinuous variables was used with a Fisher exact correction introduced for small numbers where the observed or expected frequency was less than 5. Analysis of variance was applied for multiple comparisons of related data between groups (parametric ANOVA for normal data and Kruskal-Wallis analysis of variance for non-parametric data). When a significant difference was indicated by the F statistic the Newman Keuls multiple comparison test was applied to identify differences in mean values. For continuous comparison between grouped data, correlation coefficients (r) were estimated for the degree of association between two variables. If these were found to be significant, then scatter plots of the two sets of data were made and simple regression analysis was performed. A value of P < 0.05 was taken as statistically significant except where otherwise stated.

3. Results

3.1. Echocardiography

Data were available on 16 doxorubicin-treated hearts, 8 saline-treated controls, 12 coronary ligation hearts and 4 sham-operated controls. There was no statistically significant difference in any parameter between the saline-treated controls and sham-operated controls, and the results were pooled (n = 12). The doxorubicin group and coronary ligation groups had significantly lower mean ejection fraction areas, 0.45 ± 0.11 and 0.42 ± 0.12 respectively, than the control group, 0.65 ± 0.03 < 0.001. The scatter of ejection fraction values was wider in the doxorubicin and ligation groups than in controls (Table 1) and indicated a range from normal to severely depressed left ventricular function. An arbitrary value of ≤ 0.40 for ejection fraction area was used to define those with significant left ventricu-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>In vivo and pathological data: all animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF</td>
<td>LVIDd (mm)</td>
</tr>
<tr>
<td></td>
<td>Basal CI (ml·min⁻¹·kg⁻¹)</td>
</tr>
<tr>
<td></td>
<td>Peak CI (ml·min⁻¹·kg⁻¹)</td>
</tr>
<tr>
<td></td>
<td>ΔCI (ml·min⁻¹·kg⁻¹)</td>
</tr>
<tr>
<td></td>
<td>Histological grade</td>
</tr>
<tr>
<td></td>
<td>Infarct area (%)</td>
</tr>
<tr>
<td></td>
<td>Liver wt/Body wt (%)</td>
</tr>
<tr>
<td>Controls (n = 12)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.65</td>
</tr>
<tr>
<td>s.d.</td>
<td>0.03</td>
</tr>
<tr>
<td>Range</td>
<td>0.59–0.72</td>
</tr>
<tr>
<td>Doxorubicin (n = 16)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.45 ††</td>
</tr>
<tr>
<td>s.d.</td>
<td>0.11</td>
</tr>
<tr>
<td>Range</td>
<td>0.30–0.67</td>
</tr>
<tr>
<td>Coronary ligation (n = 12)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.42 ††</td>
</tr>
<tr>
<td>s.d.</td>
<td>0.12</td>
</tr>
<tr>
<td>Range</td>
<td>0.25–0.65</td>
</tr>
</tbody>
</table>

EF — in vivo ejection fraction.
LVIDd — Echocardiographic left ventricular internal dimension (diastole).
CI — Thermodilution cardiac index.
Histol — Histologic score of doxorubicin cardiomyopathy (see text for details).
† Median value.
†† P < 0.01; †‡ P < 0.001; Controls vs Doxorubicin or Coronary ligation.
lar dysfunction or heart failure. This resulted in 7 doxorubicin-treated and 6 coronary ligation animals being designated as having significant left ventricular dysfunction.

The doxorubicin and coronary ligation groups had significantly more dilated left ventricles than the control group ($P < 0.001$), but there was considerable overlap between the control and pathological groups (Table 1).

### 3.2. In vivo cardiac index

The mean basal cardiac indices of the doxorubicin-treated and coronary ligation animals were significantly lower than in the control group ($P < 0.001$, Table 1). In addition, there were subnormal increases in mean cardiac index in response to volume loading in the doxorubicin-treated and coronary ligation groups ($P < 0.01$). The consequent mean peak cardiac indices in the two pathological groups were significantly lower ($P < 0.01$) than in controls.

Despite the significant changes in the grouped data, there was considerable overlap in the ranges of basal and peak cardiac index between experimental groups, indicating a heterogeneous response to doxorubicin administration or coronary ligation. When haemodynamic data in the doxorubicin and coronary ligation groups were subdivided according to ejection fractions $> 0.40$ and $< 0.40$ (Table 2), there were significantly lower values for basal ($P < 0.005$) and peak ($P < 0.001$) cardiac index in the animals with impaired ejection fraction. There was no overlap between the basal or peak cardiac index values in normals and in animals with ejection fractions $\leq 0.40$ (Tables 1 and 2).

### 3.3. Clinical and post-mortem signs of heart failure

Indirect evidence of right heart failure was obtained by comparison of the liver weight/body ratios in the experimental groups. Mean values were significantly greater ($P < 0.01$) in the doxorubicin and coronary ligation groups (Table 1) than in controls, and significantly greater in the subgroups with ejection fraction $\leq 0.40$ compared with $> 0.40$ (Table 2), $P < 0.001$. Other signs consistent with fluid retention included pleural effusion, ascites, and scrotal oedema. These were noted in 6/7 doxorubicin-treated and 4/6 coronary ligation animals with ejection fractions $\leq 0.40$. The prevalence of signs of congestion in animals with ejection fractions $> 0.40$ was 0/12, 0/9 and 0/6 in the control, doxorubicin and coronary ligation groups respectively. There was no biochemical evidence of significant renal impairment or hypoalbuminaemia in any animal.

### 3.4. Myocardial histology

The changes in the doxorubicin-treated animals consisted predominantly of myocyte cytoplasmic vacuolation, myocyte necrosis with evidence of fibrotic around individ-

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**Table 2**

<table>
<thead>
<tr>
<th>EF</th>
<th>LVIDd (mm)</th>
<th>Basal CI (ml·min$^{-1}$·kg$^{-1}$)</th>
<th>Peak CI (ml·min$^{-1}$·kg$^{-1}$)</th>
<th>DCI (ml·min$^{-1}$·kg$^{-1}$)</th>
<th>Histological grade</th>
<th>Infarct area (%)</th>
<th>Liver wt/Body wt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF &gt; 0.4</td>
<td>9</td>
<td>0.53 (±0.07)</td>
<td>14.7 (±1.4)</td>
<td>255 (±57)</td>
<td>347 (±91)</td>
<td>92 (±49)</td>
<td>1* (±0.23)</td>
</tr>
<tr>
<td>EF ≤ 0.4</td>
<td>7</td>
<td>0.34 (±0.05)</td>
<td>18.0 (±1.5)</td>
<td>162 (±28)</td>
<td>200 (±38)</td>
<td>38 (±21)</td>
<td>3* (±0.18)</td>
</tr>
<tr>
<td>Coronary ligation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF &gt; 0.4</td>
<td>6</td>
<td>0.51 (±0.08)</td>
<td>15.4 (±0.9)</td>
<td>288 (±53)</td>
<td>404 (±70)</td>
<td>115 (±16)</td>
<td>16 (±4)</td>
</tr>
<tr>
<td>EF ≤ 0.4</td>
<td>6</td>
<td>0.31 (±0.04)</td>
<td>19.0 (±1.6)</td>
<td>208 (±26)</td>
<td>250 (±42)</td>
<td>42 (±18)</td>
<td>35 (±8)</td>
</tr>
</tbody>
</table>

*EF — in vivo ejection fraction.

LVIDd — Echocardiographic left ventricular internal dimension (diastole).

CI — Thermodilution cardiac index.

Histol — Histologic score of doxorubicin cardiomyopathy (see text for details).

* $P < 0.01$; ** $P < 0.001$; EF > 0.4 vs EF ≤ 0.4.

* Median value.
Fig. 1. Scatter plot of correlation between echocardiographic ejection fraction and histological score in doxorubicin-treated animals (closed circles). See text for details. Ejection fraction values in control animals (open circles) are included for comparison and have been allocated a histological score of zero. Regression equation: $y = 10.2x + 6.8$, $r = 0.93$, $P < 0.001$.

ual fibres and areas of fibrous scarring. The degree of involvement was variable ranging from mild patchy areas of fibrosis (Grade 1) to widespread replacement of myocardial fibres (Grade 4). There was no evidence of myocyte necrosis or fibrosis in the control group. A significant correlation was found between histological score and ejection fraction ($r = -0.93$, $P < 0.001$) (Fig. 1).

The infarct area in the coronary ligation animals was nearly always transmural and sharply demarcated, extending up to 75–100% circumferentially in sections below the level of the ligated vessel [14]. The total infarct area ranged from 11 to 47% and there were significant correlations between infarct size and left ventricular impairment as assessed by ejection fraction ($r = -0.94$, $P < 0.001$) (Fig. 2) and left ventricular dimension ($r = -0.89$, $P < 0.001$).

### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Basal CO (ml·min⁻¹)</th>
<th>Basal CSF (ml·min⁻¹)</th>
<th>Basal LVSP (mmHg)</th>
<th>Basal LVEDP (mmHg)</th>
<th>Peak CO (ml·min⁻¹)</th>
<th>Peak CSF (ml·min⁻¹)</th>
<th>Peak LVSP (mmHg)</th>
<th>Peak LVEDP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>170 ± 20</td>
<td>68 ± 10</td>
<td>93 ± 10</td>
<td>0 ± 0</td>
<td>307 ± 31</td>
<td>82 ± 10</td>
<td>102 ± 14</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>Doxorubicin EF &gt; 0.4</td>
<td>9</td>
<td>158 ± 9</td>
<td>69 ± 6</td>
<td>95 ± 9</td>
<td>2 ± 0</td>
<td>291 ± 30</td>
<td>81 ± 9</td>
<td>106 ± 15</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Doxorubicin EF ≤ 0.4</td>
<td>7</td>
<td>146 ± 13</td>
<td>71 ± 8</td>
<td>85 ± 5</td>
<td>6 ± 3</td>
<td>215 ± 18</td>
<td>83 ± 5</td>
<td>94 ± 8</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>Ligation EF &gt; 0.4</td>
<td>6</td>
<td>163 ± 15</td>
<td>67 ± 7</td>
<td>90 ± 11</td>
<td>0 ± 2</td>
<td>256 ± 28</td>
<td>76 ± 7</td>
<td>98 ± 12</td>
<td>5 ± 7</td>
</tr>
<tr>
<td>Ligation EF ≤ 0.4</td>
<td>6</td>
<td>132 ± 10</td>
<td>59 ± 5</td>
<td>78 ± 7</td>
<td>2 ± 2</td>
<td>203 ± 29</td>
<td>70 ± 10</td>
<td>90 ± 12</td>
<td>19 ± 7</td>
</tr>
</tbody>
</table>

Values are mean (s.d.).

* $P < 0.05$; ** $P < 0.01$; Increased load vs baseline.

1 $P < 0.05$; 2 $P < 0.01$; EF > 0.4 vs EF ≤ 0.4.

CO, cardiac output; CSF, coronary sinus flow; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure.

Fig. 2. Scatter plot of correlation between echocardiographic ejection fraction and infarct size as % of left ventricle in coronary ligation animals (open squares). Ejection fraction values in control animals (open circles) are included for comparison and have been allocated an infarct size of zero. Regression equation: $y = -97x + 64$, $r = 0.94$, $P < 0.001$. 

3.5. In vitro haemodynamics

#### 3.5.1. Baseline working heart

Mean in vitro cardiac output in normal hearts in baseline working heart mode was $170 ± 21$ ml·min⁻¹ compared with $146 ± 25$ ml·min⁻¹ and $147 ± 27$ ml·min⁻¹ in the doxorubicin and coronary ligation groups respectively ($P < 0.05$). The hearts with in vivo ejection fractions $> 0.40$ in both doxorubicin and coronary ligation...
groups achieved comparable basal cardiac outputs to the controls with essentially similar levels of left ventricular systolic and end-diastolic pressure (Table 3). In contrast, the mean cardiac output and left ventricular systolic pressure in the hearts with ejection fractions ≤ 0.40 were lower, and were achieved at higher filling pressures. coronary sinus flow was not significantly different between groups, but as a percentage of total cardiac output was higher in the failing hearts than in non-failing or normal hearts.

3.5.2. Peak cardiac output

The peak cardiac output and associated coronary sinus flow, left ventricular systolic and end-diastolic pressures are presented in Table 3. The peak output in normal hearts was 307 ± 31 ml·min⁻¹, an increase of 138 ± 28 ml·min⁻¹ (81%) over baseline. The non-failing hearts in the doxorubicin group achieved comparable increases of 120 ± 21 ml·min⁻¹ (70%) but the increase in the non-failing coronary ligation group, 93 ± 31 ml·min⁻¹ (57%), was significantly less than in normals (P < 0.01). There was no significant difference in LVEDP between the normal and non-failing ligation group, but an increase was seen in the doxorubicin group. The increases in cardiac output in the failing hearts from the doxorubicin and coronary ligation groups, 69 ± 17 ml·min⁻¹ (47%) and 73 ± 20 ml·min⁻¹ (55%) respectively were significantly poorer than in the non-failing (P < 0.05) or control hearts (P < 0.01) respectively. The LVEDP at increased preload was significantly greater in both failing heart groups than in non-failing hearts or controls.

There was no significant rise in coronary sinus lactate concentrations during baseline perfusion or with acute load changes in any group.

3.6. Correlation of in vivo and in vitro data

In order to assess the degree of association between the various haemodynamic measurements, a correlation matrix comparing the indices of cardiac function in vivo and in vitro was determined and is presented in Table 4. For the purposes of this analysis, the control, doxorubicin and coronary ligation data have been pooled. In view of the
multiple comparisons, a value of \( P < 0.01 \) was taken as significant. The echocardiographic indices of ejection fraction and LVIDd correlated significantly with all other indices of in vivo and in vitro function with the exception of a nonsignificant correlation between LVIDd and change in cardiac output in vitro. Scatter plots illustrating the relationship between ejection fraction and peak cardiac index in vivo and in vitro are illustrated in Fig. 3.

4. Discussion

4.1. Variability of left ventricular dysfunction

The results of the present study demonstrate that both coronary ligation and doxorubicin administration produced markedly variable degrees of left ventricular dysfunction in individual animals. The extent of histological damage and effects on left ventricular function were quite variable, resulting in a spectrum of cardiac function ranging from normal to severely depressed, with evidence of fluid retention in the latter suggesting the presence of overt heart failure in addition to ventricular dysfunction. The doxorubicin regime adopted is well documented [13] and has been used by many research groups as a model of cardiac failure in rabbits [6–8,15]. However, the results from the present study suggest that the model is not as reproducible as was previously reported. The basis of the heterogeneity of response was not explored in this study, but may relate to variability in plasma levels and/or interindividual differences in myocardial sensitivity to doxorubicin.

Coronary ligation in the rabbit provided an experimental model of left ventricular dysfunction whose severity was closely related to the extent of infarction (Fig. 2). There was a close correlation between the infarct size, the depression of ejection fraction and the degree of left ventricular dilatation. The total mortality was approximately 35% including anaesthetic and cardiac deaths. The procedure is not without problems, it is technically demanding and there is a learning curve for anaesthesia, optimal ligation site and surgical skill. The degree and range of haemodynamic disturbance were similar to those of the doxorubicin model. The variability in infarct size may be attributable to differences in the site of ligation, the proportion of the left ventricle supplied by the marginal artery or the extent of collateral flow. Regardless of the underlying reasons for the variability in degree of left ventricular dysfunction in the doxorubicin and coronary ligation models, it is clear that simple dichotomy of the animals into "control" and "heart failure" would result in a considerable overlap in the degree of cardiac dysfunction which may confound the interpretation of studies on isolated myocytes or subcellular mechanisms. In contrast, simple noninvasive measurement by echocardiography provided a powerful means of characterizing the haemodynamic performance of the hearts both in vivo and in vitro.

The thermodilution method in animals has shown good agreement with other methods of blood flow measurement with only minor differences in flow estimates [16]. Reliable calculation of left ventricular volumes and ejection fraction from two-dimensional echocardiograms has been shown in human studies [17,18]. The close correlation seen in the present study between echocardiographic left ventricular dimension or ejection fraction and cardiac output suggests that echocardiography is a valid non-invasive technique for the assessment of left ventricular dysfunction in the rabbit. The use of a 5 mHz transducer gave adequate resolution for measurement of intracardiac chamber dimensions, although a higher frequency transducer would be necessary to measure wall thickness accurately.

4.2. Limitations of the study

In this study we attempted to characterize haemodynamic behaviour in two models of cardiac failure. Current clinical concepts suggest that prognosis and global cardiac function in patients with heart failure are best assessed by measurement of oxygen consumption at peak exercise in conjunction with ejection fraction [19]. Measurements during peak exercise are impractical in the rabbit, and we therefore chose peak cardiac output in vivo and in vitro as a measure of cardiac reserve. We used only a single fluid challenge in vivo because of concerns about inducing acute pulmonary oedema with repeated fluid challenges, while in vitro it was possible to increase preload progressively until the peak stable cardiac output was achieved. Further increases in preload resulted either in no additional increase in cardiac output, to arrhythmias or to acute cardiac dilatation and a fall in forward aortic flow. Despite different techniques for determination of peak cardiac output in vivo and in vitro, it is noteworthy that there was a significant correlation between the values of peak cardiac output under the two conditions, despite the absence of correlation between basal values (Table 4).

The function in vitro of hearts with chronic myocardial infarction was similar to the doxorubicin-treated hearts with respect to global changes in stroke volume, cardiac output, end-diastolic and -systolic pressure at baseline and in response to acutely increased preload. However, regional wall motion changes are likely to be operating in the infarcted hearts in a way not relevant to the doxorubicin-treated hearts. These regional wall motion abnormalities make the pressure/volume changes within the left ventricle more complex. There may be regional stretch occurring during diastole and systole at border zones with dysynchronous or akinetic segments. There will also be regional compensatory hypertrophy of surviving myocardium in response to chronically increased stress [20] and this will alter the wall stress and compliance at rest and in response to increased loading conditions. In the working heart preparation paced at a fixed cycle length, a change in cardiac output represents a change in forward
stroke volume. It was not possible to determine in the present study whether changes in stroke volume were due to alterations in end-diastolic volume, end-systolic volume or a combination of the two, as left ventricular dimensions and volumes were not measured in vitro. Furthermore, the peak cardiac output in the failing hearts may have been compromised by the development of mitral regurgitation. It would require studies involving two-dimensional and Doppler echocardiography during perfusion on the working heart apparatus to clarify these questions.

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References


