Sequential hemodynamic assessment in aortic valve insufficiency in rats

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OBJECTIVE: In animal models of aortic insufficiency, the right common carotid artery is typically used to damage valve leaflets; this strategy impedes subsequent assessments of left ventricular hemodynamics. The present study aimed to establish an alternative technique that would allow subsequent left ventricular catheterization to monitor sequential hemodynamics in rats with aortic insufficiency.

METHOD: The right internal and external carotid artery branches were dissected. The internal branch was temporarily occluded. The external branch was also occluded, and the proximal, patent segment was catheterized. Via the catheter, ventricular hemodynamic evaluations were performed before and after incurring leaflet damage. The catheter was removed, and the right external branch was permanently ligated. The temporary right internal carotid occlusion was released, and blood flow was re-established. After four weeks, left ventricular hemodynamic measurements were performed from the right common carotid artery.

RESULTS: Four weeks after the establishment of aortic insufficiency, left ventricular hemodynamic parameters showed a classic chronic hemodynamic pattern, similar to that observed in patients with chronic or compensated aortic insufficiency. Systolic blood pressure was elevated and pulse pressure was increased.

CONCLUSION: This new method of carotid artery catheterization permitted two sequential, distinct hemodynamic measurements, in experimental model of aortic valve insufficiency.

KEYWORDS: aortic insufficiency; hemodynamic; cardiac remodelling.


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INTRODUCTION

Diverse experimental models of volume and pressure overload have been developed to investigate the pathophysiologic mechanisms of cardiac remodelling and dysfunction. The most widely used animal models are myocardial infarction and aortocaval fistulæ for volume overload and aortic coarctation and renovascular hypertension for pressure overload. In contrast, only a paucity of studies have investigated cardiac valve disease, particularly in small animals.

Some studies have shown that structural valve changes can be induced by ergot alkaloids1, serotonin releasing agents2, and dopamine receptor agonists3. The combined use of these drugs in genetically modified animals4 has recently produced some reliable models of valve disease. However, valve disease often involves simultaneous damage to multiple valves; this precludes the determination of specific cardiac structural and functional responses to dysfunction of a single valve.

Two surgical models of aortic valve insufficiency have been previously described for rats. In 1989, Uematsu et al.5 performed selective perforations in the aortic valve, either in the right cusp alone or in both right and left cusps; the perforations were produced by means of a 0.8 mm plastic rod inserted through the right common carotid artery. Hemodynamic parameters were used to estimate the degree of aortic valve insufficiency. In 2002, Arsenault et al.6 produced aortic valve damage by retrograde perforation of the aortic cusps with an 18-gauge epidural catheter. They performed concurrent transthoracic echocardiography to guide the catheter from the right common carotid artery through the proximal aorta and into the left ventricle. This also enabled measurement of the valve regurgitation produced during the procedure. Both methods precluded the subsequent use of the right common carotid artery; thus, it was not possible to make sequential assessments of systemic and left ventricular hemodynamics.

The present study proposes an alternative technique to address this important limitation. We reasoned that catheterization of only the right external branch of the carotid artery would allow us to produce surgical damage to the aortic valve cusps without impeding the sequential assessment of hemodynamics. This innovative technique...
maintains an intact, patent common carotid artery for investigations of left ventricular responses to different strategies. Thus, we could alter the load conditions to the heart and follow ventricular responses in chronic follow-up studies. This approach was expected to obviate the need for expensive, specialized equipment, such as the echocardiograph, and additionally, the need for highly skilled personnel.

**METHODS**

All procedures were carried out in accordance with the norms of the Brazilian College of Animal Research and The Universities Federation for Animal Welfare. Our Institutional Ethical Committee approved the protocol (Faculdade Medicina de Botucatu – UNESP, protocol 715 – CEEA, 12/02/2009).

**Surgical procedure and hemodynamics**

Male Wistar rats, aged 10-12 weeks, were anesthetized with ketamine (100 mg/kg ip) and diazepam (1 mg/kg ip). A midline skin incision was made on the ventral side of the neck. The right salivary glands were moved apart to expose the carotid triangle, formed by the omohyoid muscle medially, the sternocleidomastoid muscle laterally, and the digastic muscle cranially. There, the carotid artery bifurcation was identified. The right internal and external carotid arteries were then carefully dissected and isolated from neighbouring structures with a pair of microsurgical tweezers under a surgical microscope (x13, M900-D.F. Vasconcellos, Brazil). Care was taken to preserve the integrity of the carotid sinus body and associated nerves and vascular supply. The internal branch of the carotid artery was temporarily occluded by tightening a double loop of 6.0 cotton thread. The remaining proximal portion of the patent common carotid artery was perforated with a surgical loop. The artery was subsequently detached from the sinus body with fine microsurgical forceps under a surgical microscope (M900-D.F. Vasconcellos, Brazil). Care was taken to preserve the integrity of the carotid sinus body and associated nerves. The dammed leaflet with a surgical microscope (M900-D.F. Vasconcellos, Brazil) at x10 magnification. The injury to the aortic valve was evaluated by examining the damaged leaflet(s) with a surgical microscope (M900-D.F. Vasconcellos, Brazil) at x10 magnification. The injury to the aortic valve was evaluated by examining the damaged leaflet(s) with a surgical microscope (M900-D.F. Vasconcellos, Brazil) at x10 magnification. The injury to the aortic valve was evaluated by examining the damaged leaflet(s) with a surgical microscope (M900-D.F. Vasconcellos, Brazil) at x10 magnification.

A transverse slice of the heart that included both ventricles was fixed in buffered formalin, embedded in paraffin, and cut into 5 μm sections. Tissue sections were stained with hematoxylin–eosin (HE). Morphometric measurements were performed with a digital image analysis system (Leica Imaging Systems Ltd., Cambridge, UK). Left ventricular geometry included estimating the degree of left ventricular dilatation, based on left ventricular lumen area (mm²); hypertrophy, based on left ventricular wall area (mm²); and relative wall thickness, based on the ratio of the left ventricular wall and lumen areas.

To estimate cardiomyocyte hypertrophy, left ventricular tissue sections were examined at x1000 magnification. Myocyte diameter (μm) was measured through the centre of the oval, central nuclei of longitudinally displayed myocytes. Twenty cardiomyocytes were analysed per heart, mostly in the interventricular septum.

**Statistical analysis**

Continuous data with Gaussian distributions are presented as mean ± standard deviation. Data with skewed distributions are presented as median (interquartile range). Comparisons of baseline, acute AR, and chronic AR hemodynamic data were performed with one-way repeated measures analysis of variance or Friedman repeated measures analysis of variance (complemented by Student Newman Keuls test), as appropriate. Cardiac remodelling was evaluated by comparing these specimens with hearts from 10 unoperated control rats. The Student’s t-test or Mann-Whitney Rank Sum test were used for testing significance. P-values < 0.05 were considered significant. Statistical analyses were performed with Sigma Stat 3.1 (Systat, California, USA).

**RESULTS**

All animals that underwent AR survived through the chronic follow-up.
Hemodynamics

Systemic hemodynamic measurements are summarized in Table 1 and depicted in Figure 2. The SBP and DBP dropped by 37% and 24%, respectively, from baseline immediately after AR. Four weeks later, SBP increased by 49% while DBP only rose 13% when compared to baseline values. Pulse pressure remained unchanged immediately after AR, but significantly increased at 4 weeks, primarily as a result of the elevation in SBP.

LVDEP increased by 61% immediately after AR, but returned to values statistically comparable with baseline measurements at 4 weeks. Left ventricular function was reflected in the pattern of LVEDP changes (Figure 3). Both $\dot{+}dP/dt$ and $\dot{-}dP/dt$ were depressed immediately after AR, but subsequently returned to baseline values at 4 weeks.

Morphometry

At necropsy, all 10 rats exhibited evident damage to the aortic valve, as perforations in 1 to 2 aortic valve cusps. The borders of the valve cusp lesions were thick, with scattered hardened nodules (Figure 4). Compared with controls, AR rats exhibited an 18% increase in cardiac weight index and a 32% increase in myocyte cell diameter. With AR, the left ventricular wall area increased by 57% and the left ventricular lumen was enlarged 33%, but the relative wall thickness was unchanged (Table 2).

**DISCUSSION**

In a rat model of experimental aortic valve insufficiency, we developed a new method of carotid artery catheterization that permitted two sequential, distinct hemodynamic measurements, separated by a four-week interval.

**Table 1 - Hemodynamic variables determined at before (baseline), immediately after surgery for aortic valve insufficiency (acute AR) and at the end of a 28-day follow-up (chronic AR).**

<table>
<thead>
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<th>Baseline</th>
<th>Acute AR</th>
<th>Chronic AR</th>
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<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>119 (116-123)</td>
<td>91 (88-92)</td>
<td>181 (174-186)*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76 ± 8.4</td>
<td>48 ± 5.2*</td>
<td>86 ± 7.9**</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>43 ± 5.8</td>
<td>42 ± 7.2</td>
<td>91 ± 7.2*</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>6.9 ± 1.6</td>
<td>11.1 ± 5.0*</td>
<td>9.2 ± 0.2</td>
</tr>
<tr>
<td>$\dot{+}dP/dt$ (mmHg/s)</td>
<td>8901 (7758-10264)</td>
<td>5559 (4759-6210)*</td>
<td>8755 (8522-8970)*</td>
</tr>
<tr>
<td>$\dot{-}dP/dt$ (mmHg/s)</td>
<td>6959 (5616-7398)</td>
<td>3756 (2968-4141)*</td>
<td>4617 (4398-4981)*</td>
</tr>
<tr>
<td>CDP (mmHg)</td>
<td>71 ± 12.5</td>
<td>37 ± 9.4*</td>
<td>77 ± 7.8*</td>
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SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEDP, left ventricular end diastolic pressure; $\dot{+}dP/dt$, maximum positive first derivative of left ventricular pressure; $\dot{-}dP/dt$, maximum negative first derivative of left ventricular pressure. CDP: coronary driving pressure. Friedman repeated measure analysis of variance, complemented by Student Newman Keuls test; *P < 0.05 vs. baseline. **P < 0.05 vs acute AR.
Recent investigations have shown that echocardiography appeared to be effective for studying experimental aortic insufficiency. However, in addition to cost, echocardiography requires specialized professional skills to acquire and analyze the images; moreover, it only provides indirect hemodynamic data. Other methods of cardiac imaging, including magnetic resonance, implicate significantly higher cost and more laborious data analysis. Alternatively, the present study introduced a simple, feasible, catheterization method for performing sequential LV hemodynamic measurements. This technique is expected to be particularly useful when morphometry and hemodynamics are performed in parallel.

In fact, our results reproduced the classic hemodynamic pattern found in patients with chronic or compensated aortic insufficiency. This included an elevation in SBP with a resultant increase in pulse pressure. In our rat AR model, following immediate LV dysfunction and blood pressure instability, LV remodelling occurred, representing an effective cardiac adaptation to both pressure and volume overloads. This remodelling resulted in restoration and preservation of...

**Table 2 - Morphometric and histological measurements determined after 4 weeks of aortic valve insufficiency (AR).**

<table>
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<tr>
<th></th>
<th>Control</th>
<th>AR</th>
<th>p-value</th>
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<tr>
<td>Cardiac weight index (g/kg)</td>
<td>3.01(2.93-3.11)</td>
<td>3.49(3.06-4.00)</td>
<td>0.0232</td>
</tr>
<tr>
<td>Myocyte diameter (um)</td>
<td>11.29 ± 1.30</td>
<td>13.99 ± 0.94</td>
<td>&lt;0.0001</td>
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<tr>
<td>LV lumen area (mm²)</td>
<td>0.06 ± 0.02</td>
<td>0.08 ± 0.01</td>
<td>0.0015</td>
</tr>
<tr>
<td>LV wall area (mm²)</td>
<td>0.30 ± 0.05</td>
<td>0.58 ± 0.08</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Relative wall thickness</td>
<td>4.90(3.58-6.45)</td>
<td>6.60(6.48-7.10)</td>
<td>0.0155</td>
</tr>
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Mean ± SD or Median(25%-75%); LV: left ventricular
LV function that lasted at least 4 weeks after aortic insufficiency. Accordingly, morphometric analyses demonstrated that eccentric LV hypertrophy developed with proportional enlargements in the LV cavity and walls. Indeed, both cardiac weight and myocyte diameter increased, LV lumen and wall areas were enlarged, and relative wall thickness remained unchanged. These alterations occurred in parallel with the changes in hemodynamic values, which indicated that the LV adapted to preserve function.

It remains unknown whether antihypertensive drugs, such as angiotensin-converting enzyme inhibitors, β-adrenoceptor antagonists (β-blockers), or nifedipine, might be effective in preventing pathological remodelling following aortic insufficiency. It has not been determined which antihypertensive treatment might be best in this setting, or to what extent blood pressure should be reduced to avoid further reductions in myocardial perfusion pressure. We previously showed, in experimental models of MI and aortocaval fistula, that coronary driving pressure was an important factor in subendocardial remodelling. Thus, reducing DBP at the expense of coronary driving pressure might impede myocardial perfusion, particularly to the subendocardium, which could result in pathological remodelling.

To investigate the effects of different antihypertensive treatments on LV remodelling following AR, it will be necessary to follow changes in coronary perfusion pressure by performing sequential hemodynamic measurements.

In conclusion, we demonstrated a new technique that permits sequential measurements of LV hemodynamics separated by a four-week time interval. This technique will enable determinations of the time course of LV maladaptation following aortic insufficiency. It will facilitate investigations that aim to test strategies for preventing and treating pathological remodelling by offering a low cost approach that does not require specialized professional skills, such as those associated with cardiac imaging methods.

RESUMO

OBJETIVOS: No modelo experimental de insuficiência arterial, a artéria carótida comum direita é utilizada para danificar os folhetos valvares, o que impede avaliações hemodinâmicas subsequentes do ventrículo esquerdo. O objetivo deste estudo foi estabelecer um modelo alternativo de insuficiência aórtica em ratos, que permitisse a cateterização ventricular esquerda para monitorizar a hemodinâmica em dois momentos distintos.

MÉTODOS: Os ramos da artéria carótida interna e externa direitas foram dissecadas. O ramo interno foi temporariamente ocultado. O ramo externo foi monitorizado a hemodinâmica em dois momentos distintos.

RESULTADOS: Quatro semanas após o estabelecimento de insuficiência aórtica, os parâmetros hemodinâmicos do ventrículo esquerdo mostraram um padrão clássico de hemodinâmica crônica, similar ao observado em pacientes com insuficiência aórtica crônica. A pressão arterial sistólica foi elevada e pressão de pulso foi aumentada.

CONCLUSÃO: Este novo método de categorização da artéria carótida permite duas medidas hemodinâmicas sequenciais distintas e caracteriza bem o modelo de insuficiência aórtica em ratos.

REFERENCES