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ORIGINAL RESEARCH

Sequential hemodynamic assessment in aortic valve insufficiency in rats

Marcia Kiyomi Koike,[I] Beatriz Bojikian Matsubara,[II] Luiz Shiguero Matsubara,[II] Clovis de Carvalho Frimm[I]

[I]Universidade de São Paulo – Faculdade de Medicina, Clinica Medica [II]Universidade Estadual Paulista Julio de Mesquita Filho, Faculdade de Medicina (Botucatu), Clinica Medica

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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Email: mkoike2011@gmail.com

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 fistulae 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 alkaloids[1], serotonin releasing agents[2], and dopamine receptor agonists[3]. The combined use of these drugs in genetically modified animals[4] 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

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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 digastric 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 ($\times$13, 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 nylon thread around the vessel to cut off the flow. The distal external branch was permanently occluded by tying it closed with a 4.0 cotton thread. The remaining proximal portion of the external carotid artery was patent; there, a polyvinyl catheter (OD 0.8 mm, ID 0.5 mm) was introduced and advanced into the left ventricular cavity to measure left ventricular hemodynamics. The surgical procedures are depicted in Figure 1. The left femoral artery was cannulated with a similar polyvinyl catheter to measure systemic pre-procedural (baseline) and post-procedural hemodynamics.

Surgical aortic valve insufficiency (AR, $n = 10$) was performed through the catheter in the carotid external branch. First, the catheter was pulled back from the left ventricular cavity and positioned above the aortic valve leaflets. Then, a metal guide (0.26 mm) was inserted through the catheter and positioned at the aortic valve level. The guide was carefully moved back and forth, twice, to perforate the valve leaflets; then, it was withdrawn. Successful surgical procedure was confirmed by recording a 30% fall in systemic diastolic pressure[7]. Then, the right external carotid catheter was again advanced into the left ventricular cavity to measure post-procedural left ventricular hemodynamics (acute AR). Next, the catheter was removed, and the right external carotid artery was permanently ligated. Finally, the internal carotid artery occlusion loop was released, and blood flow was re-established.

Four weeks later, rats were re-anesthetized for systemic and left ventricular hemodynamic recordings (chronic AR) acquired from the intact, patent, right common carotid artery. Systemic and left ventricular hemodynamics were recorded and analysed with a computerized system processor (Windaq AT/Codas; Dataq Instruments, Akron, OH, USA). The following parameters were analyzed: systemic systolic blood pressure (SBP, mmHg), systemic diastolic blood pressure (DBP, mmHg), systemic arterial pulse pressure (pulse pressure, mmHg), left ventricular end-diastolic pressure (LVEDP, mmHg), maximum rate of increase in left ventricular pressure ($+dP/dt$, mmHg/s), and maximum rate of decrease in left ventricular pressure ($-dP/dt$, mmHg/s). The latter two parameters were used to evaluate left ventricular systolic and diastolic function, respectively. Coronary driving pressure (mmHg) was estimated as the difference between DBP and LVEDP.

Cardiac remodelling

Immediately after chronic AR hemodynamic measurements, rats were sacrificed with an overdose of anesthesia. The heart was arrested in diastole with administration of 100 mM cadmium chloride via the common carotid artery. After removing and rinsing the heart in saline, the cardiac weight index was calculated as the ratio of heart to body weight (g/Kg).

The injury to the aortic valve was evaluated by examining the damaged leaflet(s) with a surgical microscope (M900, DF Vasconcellos, São Paulo, Brazil) at $\times$10 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 (mm^2); hypertrophy, based on left ventricular wall area (mm^2); 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 $\times$1000 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 $\pm$ 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.

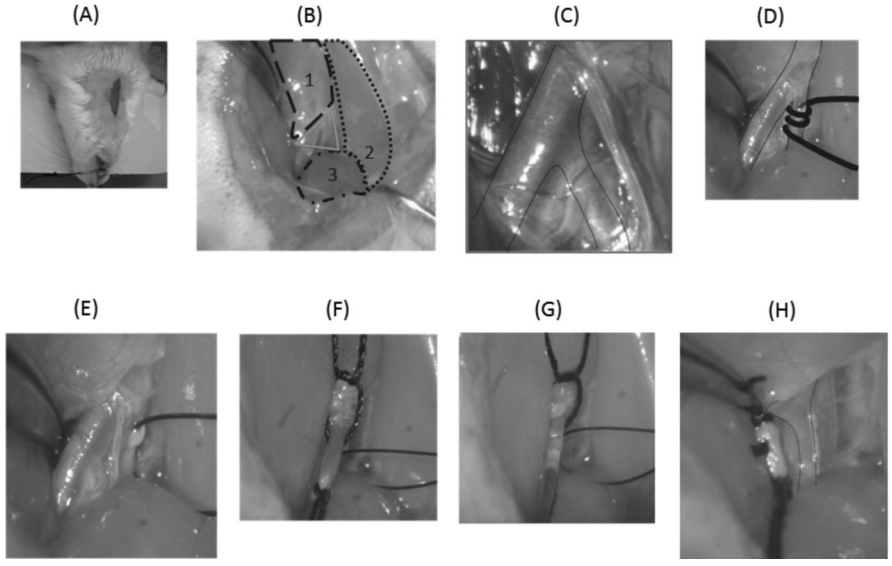

Figure 1 - Temporary occlusion of the right carotid artery and catheterization of the external right carotid artery. (Panel A) A midline skin incision on the ventral side of the neck was performed. (Panel B) This allowed access to the carotid triangle (blue line), bounded by the omohyoid (1), sternocleidomastoid (2), and digastric (3) muscles. (Panel C) The same surgical field is shown at 13-fold magnification to display the right carotid artery bifurcation (outlined). (Panel D) The internal carotid artery was occluded by tightening a double loop 6.0 nylon thread around the vessel, shown schematically. (Panel E) The black thread indicates the position of the occluded internal carotid artery. (Panel F) The external carotid artery is isolated and occluded. (Panel G) A polyvinyl catheter (OD 0.8 mm) is introduced into the external carotid artery to measure left ventricular hemodynamics and to guide the introduction of the metal rod used to damage the aortic leaflets. (Panel H) Finally, after removing the catheter, the external carotid artery is permanently ligated, and the internal carotid artery occlusion is released (opened vessel is outlined) to re-establish blood flow.

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 $-dP/dt$ and $+dP/dt$ 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).

	Baseline	Acute AR	Chronic AR
SBP (mmHg)	119 (116-123)	91 (88-92)	181 (174-186)$^{\#}$
DBP (mmHg)	76 ± 8.4	48 ± 5.2*	86 ± 7.9*$^{\#}$
Pulse pressure (mmHg)	43 ± 5.8	42 ± 7.2	91 ± 7.2*$^{\#}$
LVEDP (mmHg)	6.9 ± 1.6	11.1 ± 5.0*	9.2 ± 0.2
+dP/dt (mmHg/s)	8901 (7758-10264)	5559 (4759-6210)*	8755 (8522-8970)$^{\#}$
−dP/dt (mmHg/s)	6959 (5616-7398)	3756 (2968-4141)*	4617 (4398-4981)$^{\#}$
CDP (mmHg)	71 ± 12.5	37 ± 9.4*	77 ± 7.8$^{\#}$

SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEDP, left ventricular end diastolic pressure; + dP/dt, maximum positive first derivative of left ventricular pressure; − 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.

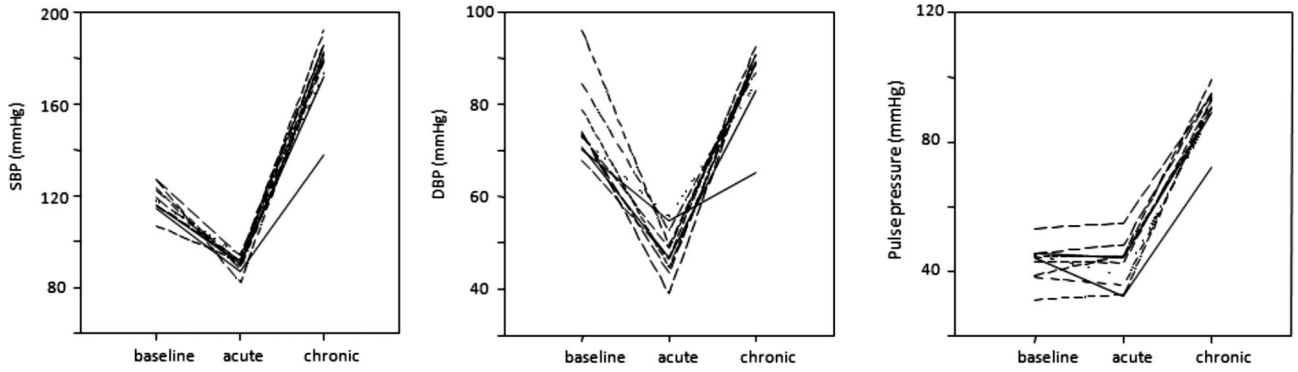

Figure 2 - Changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure at baseline and following acute and chronic aortic valve insufficiency.

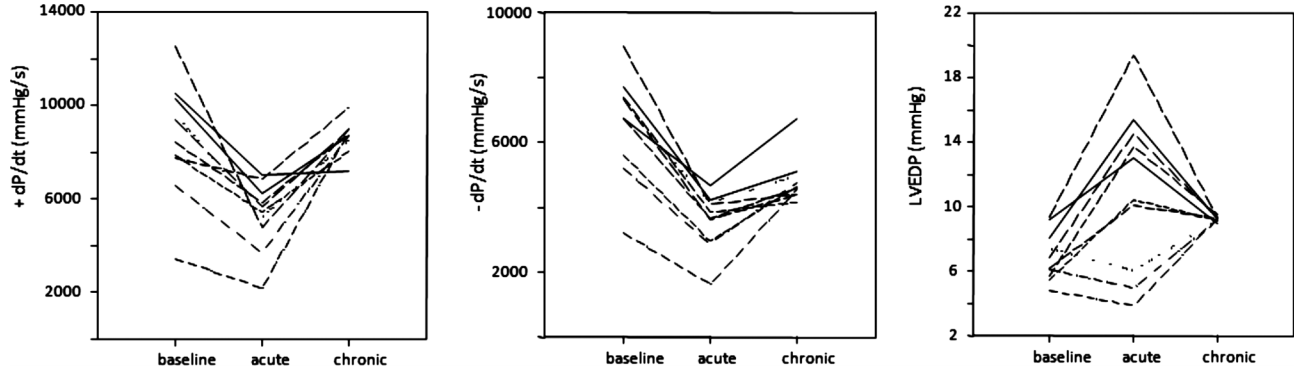

Figure 3 - Changes in left ventricular systolic and diastolic function (+ dP/dt and dP/dt, respectively) and end-diastolic pressure (LVEDP) at baseline and following acute and chronic aortic valve insufficiency.

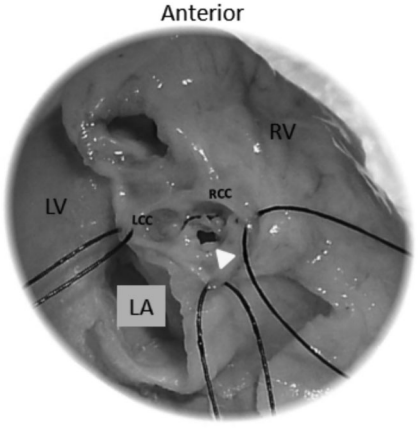

Figure 4 - Photomacrograph shows the base of a rat heart with a large perforation in the posterior cusp of the aortic valve (yellow arrowhead). LV, left ventricle; RV, right ventricle; LA, left atrium; LCC, left coronary cusp; RCC, right coronary cusp.

Recent investigations have shown that echocardiography appeared to be effective for studying experimental aortic insufficiency[6]. 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.

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

	Control	AR	p-value
Cardiac weight index (g/kg)	3.01(2.93-3.11)	3.49(3.06-4.00)	0.0232
Myocyte diameter (μm)	11.29 ± 1.30	13.99 ± 0.94	<0.0001
LV lumen area (mm^2)	0.06 ± 0.02	0.08 ± 0.01	0.0045
LV wall area (mm^2)	0.30 ± 0.05	0.58 ± 0.08	<0.0001
Relative wall thickness	4.90(3.58-6.45)	6.60(6.48-7.10)	0.0155

Mean ± SD or Median(25%-75%); LV: left ventricular

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

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[8-10]. 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[11-12]. 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 aórtica, 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 insuficiencia aortic 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 ocluído. O ramo externo foi cateterizado em sua porção patente proximal. Parâmetros hemodinâmicas ventriculares foram medidos antes e depois dos danos aos folhetos valvares via guia metalico. O cateter foi retirado e o ramo direito externo foi ocluido permanentemente. A carótida interna direita foi liberada e o fluxo sanguíneo restabelecido. Após quatro semanas, a hemodinâmica do ventrículo esquerdo foi realizada a partir da artéria carótida comum direita.

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 cateterização da artéria carótida permite duas medidas hemodinâmicas seqüenciais distintas e caracteriza bem o modelo de insuficiencia aortica em ratos.

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