Title: Impact of anesthesia on echocardiographic evaluation of systolic and diastolic function in rats

Running head: Anesthesia and echocardiography in rats

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Abstract:

Background: Echocardiography is used on rats but general anesthesia is usually necessary to be able to obtain a good quality echocardiogram. Each type of anesthetic agent has specific impacts on hemodynamics and therefore may affect differentially the echocardiographic measurements.

Objectives: To compare the echocardiograms of normal (NL) or rats with chronic aortic regurgitation (AR) under anesthesia using 1) ketamine-xylazine (KX) or 2) isoflurane (ISO)

Methods: Animals underwent an echocardiogram with both drugs sequentially. Echocardiographic measurements were compared.

Results: Mitral diastolic Doppler measurements (E and A wave velocities) were significantly affected by the type of anesthesia in the normal group but not left ventricular dimensions nor ejection fraction. Left ventricular dimensions were affected by the type of anesthesia in the AR group as well as diastolic Doppler flow.

Conclusion: The anesthetic agent has significant specific impacts on many echocardiographic measurements. Investigators working with rat models should be aware of those potential effects.

Key Words:

Introduction

Echocardiography has become widely used to evaluate cardiac function in a variety of animal models of cardiac diseases. Being non-invasive, echocardiography allows serial in vivo evaluation of various parameters such as cardiac dimensions, ejection fraction, filling parameters and evolution of valve disease. However, sedation is almost always used to perform an echocardiogram in small animals such as mice and rats. Anesthetic agents are known to affect cardiovascular hemodynamics, preload, afterload, as well as myocardial contractility ([1-17]). Therefore the choice of anesthetic agent has the potential to significantly alter echocardiographic parameters. Despite the reported differential effects of anesthetic agents on cardiac function and echocardiograms in mice ([18-25]) , much less is known about the impact of the choice of the anesthetic agent on the echocardiogram of sedated healthy or sick rats, especially if the animals suffer from a cardiac disease that is significantly affecting pre-load and afterload. Therefore the present study was performed to assess the differential impact of two types of anesthetic regimens (intraperitoneal ketamine-xylazine or inhaled isoflurane) on basic echocardiographic measurements 1) in healthy male adult rats and 2) in rats suffering from chronic aortic valve regurgitation (AR).

Methods

Animals:

Adult Wistar male rats (275-325g) were randomly divided as follows :

Normal animals (n=12-13/gr) : Group 1: anesthetized with intraperitoneal injection of 0,1 mg/kg ketamine and 0,75 mg/kg xylazine (NL-KX); Group 2: anesthetized with 1,5% inhaled isoflurane (NL-ISO).

Rats with aortic regurgitation (n=10/gr): Group 3: anesthetized with ketamine-xylazine (AR-KX) and group 4: anesthetized with isoflurane (AR-ISO).

Animals were considered sufficiently anesthetized with both anesthetic regimens when they became totally unresponsive to a moderate pain stimulus (moderate pinch of hind limb toe) while still normally breathing spontaneously (absence of respiratory depression, animals not intubated). A minimum of 48 hours was respected between both echocardiograms to avoid potential residual effects of the first anesthesia on the animal's heart.

This protocol was approved by the Université Laval's animal protection committee and respected the recommendations of the Canadian Council on Laboratory Animal Care.

In groups 3 and 4, aortic regurgitation was created as previously reported ([26-29]). Briefly, a rigid catheter was inserted in the right internal carotid artery and advanced in the ascending aorta towards the aortic valve under echocardiographic and hemodynamic guidance. Using the rigid catheter, the aortic valve leaflets were perforated to create severe aortic valve regurgitation.   Severity of aortic valve regurgitation was assessed semi-quantitatively by echocardiographic and hemodynamic criteria ([26-29]). Briefly, AR was considered severe when all the following criteria were met: AR jet width >60% of

LVOT diameter measured in the parasternal long axis view, presence of holodiastolic reversed flow with terminal velocity >18 cm/sec in descending aorta by pulsed Doppler, acute decrease of diastolic pressure >30% at AR surgery (measured invasively). AR severity was evaluated not only at initial surgery but also at each echocardiography. Only animals with severe AR remained included in the protocol. Animals were allowed to stabilize and were observed for 8 weeks before the echocardiograms under both anesthetic agents were performed in random order.

Echocardiography: A complete two-dimensional, M-mode and Doppler echocardiogram was performed under antesthesia. Left ventricular dimensions (end diastolic and end systolic diameters), wall thickness, ejection fraction (EF), heart rate (HR), aortic stroke volume (SV), cardiac output (SV x HR) were measured. Filling parameters were measured by pulsed Doppler analysis of mitral E wave and A waves. All studies were performed by an experienced sonographer using a 12 MHz probe coupled to a Sonos 5500 echographer (Philips Medical Imaging, Andover, MA) using harmonic imaging. Images were stored on videotapes and digitally for off-line analysis.

Statistical analysis:

Results are presented as mean ± SEM unless specified otherwise. Unpaired Student t tests were used for inter-group comparisons and paired t tests were used for intra-group comparisons.   Statistical significance was set at a p value of 0.05 or less. Data and statistical analysis were performed using GraphPad Prism version 4.02 for Windows, GraphPad Software, (San Diego California USA).

Results:

Feasibility:

Both types of anesthesia were well tolerated. All animals survived and we did not encounter any hemodynamic instability or arythmias during sedation.

Effects of anesthetic agents in normal animals (Table 1 and 2)

The hemodynamic effects in normal animals (groups 1 and 2) are summarized in table 1. As expected, both anesthetic regimens affected hemodynamics differentially. Heart rate remained higher and closer to a normal rat heart rate in group 2 (NL-ISO) compared to group 1 (NL-KX). Aortic stroke volume tended to be lower in group 2 (NL-ISO) vs group 1 (NL-KX) (p=0,08). The higher heart rate resulted in a significantly higher calculated cardiac output/index in the animals anesthetized with isoflurane (group 2).

Left ventricular dimensions and ejection fraction measured by echocardiography were not significantly affected by the type of anesthetic agent in normal animals (table 1). However, diastolic mitral filling parameters were significantly affected by the type of anesthesia as shown in table 2. Mitral E and A wave maximal velocities were significantly smaller in the NL-KX group whereas their ratio (E/A) remained unaffected. E wave downslope was significantly steeper in the NL-ISO group vs NL-KX group.

Effect of anesthetic agent in animals with chronic severe AR (groups 3-4)

Results for groups 3 (AR-KX) and 4 (AR-ISO) are summarized in figures 1-3.

As for normal animals (groups 1-2), KX and ISO had differential effects on hemodynamics in AR animals (fig.1). AR anesthetized with ISO had a higher heart rate

(closer to normal reported rat heart rate), higher aortic stroke volume as well as higher cardiac output than the same animals when anesthetized with KX.

Left ventricular dimensions were significantly affected by the type of anesthesia in AR in contrast with what we had found in NL (groups 1-2). When sedated with KX, AR had smaller end-diastolic and end-systolic dimensions (fig 2A and B). Calculated ejection fraction was not significantly affected (fig 2C).

Results of mitral Doppler filling parameters in AR are summarized in figure 3. AR sedated with KX had smaller E and A wave velocities. The effect was most pronounced for E wave velocities. There was also a trend towards a smaller E/A wave ratio in the AR-KX group (p=0,07). E wave slope was much steeper in the AR-ISO group when compared to the AR-KX group.

Discussion

For many years, small animal models have been used to study a wide variety of cardiac diseases as well as their response to treatment. While invasive hemodynamic intracardiac measurements and evaluation of the hearts of these animals have been mandatory in the past, many investigators have turned in recent years to echocardiography to obtain similar data non-invasively. Echocardiography is safe, quick and does not cause any damage to the animals heart, thereby allowing serial and repeated evaluation of cardiac diseases in vivo in live animals.   Except for rare exceptions, anesthesia is mandatory to perform a good quality complete echocardiogram in small animas such as rats and mice. Although anesthesia allows the investigator to obtain data of better quality and more quickly, it also has disadvantages.

Anesthetic agents routinely used to sedate rodents are known to have effects on myocardial contractility, heart rate as well as on preload and afterload. Therefore, the choice of anesthetic agent has the potential to affect echocardiographic data directly. Isoflurane acts as a direct vasodilator, reducing systemic vascular resistance and arterial blood pressure. As for other volatile anesthetic agents, it can result in a dose-dependent myocardial depression. Isoflurane administration can induce an endogenous catecholamine release and secondary increase in heart rate. Cardiac output is usually preserved under isoflurane anesthesia since the increase in heart rate and decreased afterload counterbalance the mild myocardial depression induced by the medication.

The combination of ketamine with xylazine results in mixed effects on the cardiovascular system. Ketamine administration by itself usually produces a catecholamine outburst resulting in increased systemic vascular resistance, increased blood pressure and heart rate. The overall effect of ketamine alone is therefore an increase in cardiac workload. Xylazine however is considered a clonidine analog, $\alpha2$ adreno-receptor agonist. It usually produces variable effects on arterial blood pressure (mostly a mild decrease) and mostly bradycardia (potentially severe), therefore counteracting the global cardiovascular effects of ketamine. The overall effects of the combination of the two drugs can therefore be variable depending on the subject and the dose administered.

Both ketamine and inhaled anesthetic agents have been previously shown in animal models to negatively affect diastolic function [30-32] probably by a direct effect on intra and/or extracellular calcium handling by cardiomyocytes. Unfortunately, inhaled

anesthetic gases and ketamine have never been compared head-to-head on that particular aspect and xylazine was not used in those animal models. Moreover, those experiments were conducted in the presence of pharmacologic blockade of the autonomic nervous system which was not the case in our rats. Although we did not explore the effects of both types of anesthetic agents at a cellular level in our study, it remains very probable that isoflurane and ketamine/xylazine had a specific influence on cellular calcium handling.

Under the light of the known cardiovascular effects of both isoflurane and the combination of ketamine-xylazine, it is interesting to evaluate their differential effects on a normal hearts and hearts suffering of a valve disease such as AR causing an increased preload and afterload. Our results show that the echocardiographic data of healthy rats are less affected by the type of anesthesia than those of rats with AR. In healthy rats as well as in AR rats, heart rate and cardiac output remain higher and closer to normal values when isoflurane was used for anesthesia. It seems that the bradycardic effects of xylazine overruled the potential increase of heart rate that would have been expected with the administration of ketamine alone. Left ventricular dimensions and ejection fraction are not significantly affected by the type of anesthesia in normal rats but this is not true for AR rats. Indeed, diastolic and systolic diameters were significantly smaller in AR rats sedated with KX whereas no effects were detected in normal animals. The smaller LV dimensions of AR rats with ketamine/xylazine anesthesia suggest a more optimal preload/afterload and/or less myocardial depression with this type of anesthesia in a ventricle that is strongly dependent on loading conditions due to the presence of severe AR.

Conclusion:

Echocardiographic measurements are significantly affected by the type of anesthesia. The effects of the anesthetic agents on the echocardiographic data are also different in healthy and in animal with a disease affecting both preload and afterload. Investigators using echocardiography to investigate rat models of cardiac diseases should be aware of those effects since they may significantly affect their data.

Figure legends:

Figure 1: Left ventricle diameters and ejection fraction in AR rats under ketamine/xylazine (ket/xyl) or isoflurane (iso) anesthesia. A: End-diastolic diameter (cm) B: end-systolic diameter (cm) and C: ejection fraction (%). Data are presented as mean ± SEM (n=20 for each group).

Figure 2: Hemodynamics of AR rats under ketamine/xylazine (ket/xyl) or isoflurane (iso) anesthesia. A: Heart rate B: stroke volume (µl) and C: cardiac output (ml/min). Data are presented as mean ± SEM (n=20 for each group).

Figure 3: Left ventricle filling parameters in AR rats under ketamine/xylazine (ket/xyl) or isoflurane (iso) anesthesia. A: E wave (cm/s) B: A wave (cm/s) and C: E/A ratio and D: E wave slope. Data are presented as mean ± SEM (n=20 for each group).

Table 1. Echocardiographic dimensions and hemodynamics in normal Wistar rats.

Parameters	Ket/Xyl (n=25)	Iso (n=25)	p value
EDD, mm	7.2 ± 0.11	7.2 ± 0.31	0.96
ESD, mm	3.9 ± 0.14	3.7 ± 0.11	0.25
ED Septal wall, mm	1.7 ± 0.02	1.7 ± 0.03	0.36
ED Posterior wall, mm	1.7 ± 0.03	1.7 ± 0.02	0.50
Ejection fraction, %	70.2 ± 1.46	73.1 ± 1.28	0.15
Relative wall thickness	0.47 ± 0.011	0.47 ± 0.011	0.90
Stroke volume, µl/beat	244 ± 8.5	223 ± 7.7	0.084
Heart rate, bpm	266 ± 5.4	363 ± 6.2	<0.0001
Cardiac output, ml/min	63 ± 2.7	99 ± 3.0	<0.0001

EDD: end-diastolic diameter; ESD: end-systolic diameter; ED: end-diastolic; Ket/Xyl:

Ketamine-xylazine; Iso: isoflurane. Values are means ± SEM.

Table 2 Diastolic function in normal Wistar rats.

| Parameters	Ket/Xyl (n=25)	Iso (n=25)	P value
Left atria diameter, mm	4.0 ± 0.11	4.1 ± 0.11	0.84
E wave, cm/s	79 ± 2.0	88 ± 2.5	0.0025
A wave, cm/s	40 ± 2.1	54 ± 4.9	0.014
E/A ratio	2.0 ± 0.09	1.9 ± 0.18	0.76
E wave slope (cm/s^2)	1581 ± 97.8	2479 ± 115.2	<0.0001

E wave: E wave maximal velocity by pulsed Doppler of mitral flow; A: wave: A wave

maximal velocity by pulsed Doppler of mitral flow. Values are means ± SEM.
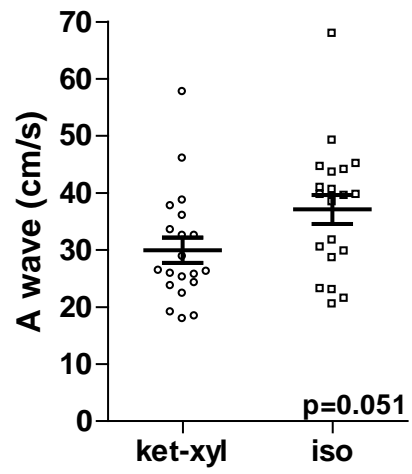
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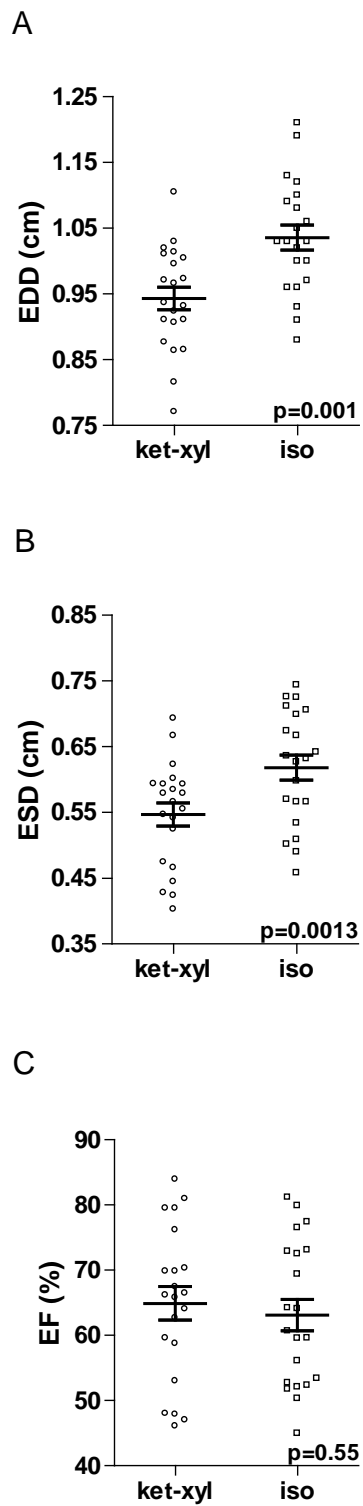
Plante et al. Figure 1

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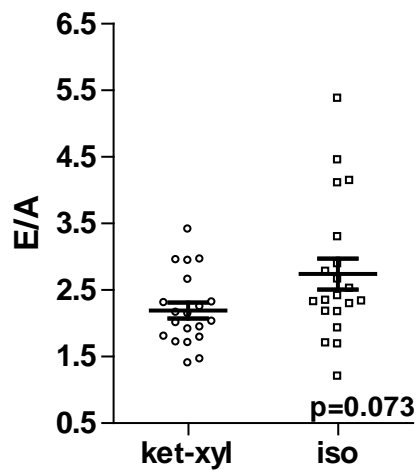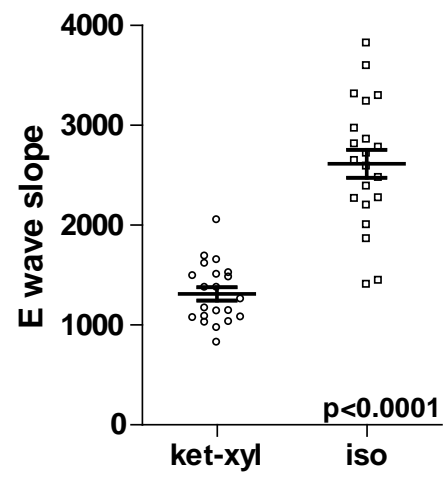Plante et al. Figure 2
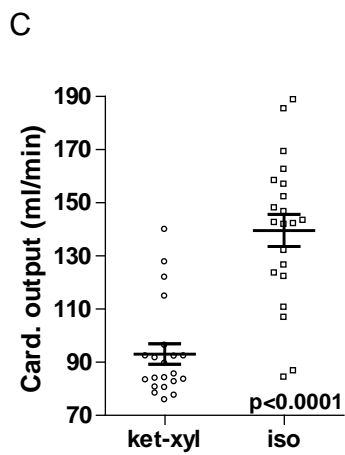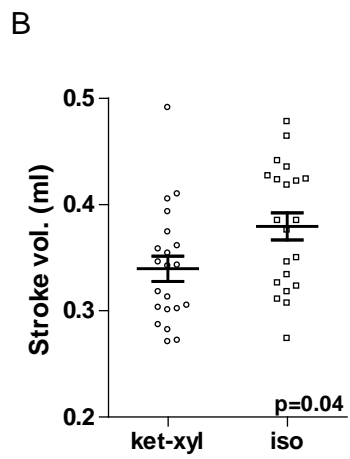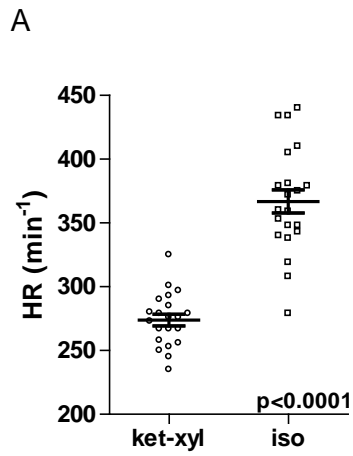
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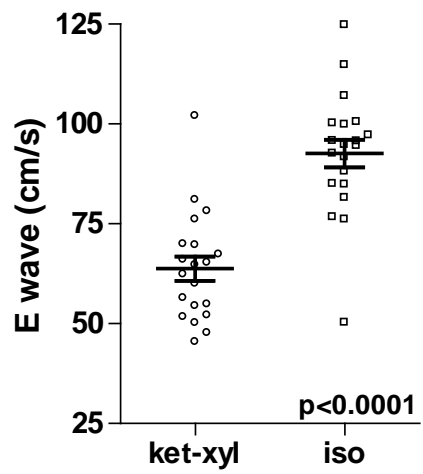
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Plante et al. Figure 3

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