Journal of Clinical Toxicology

Hanton, J Clin Toxicol 2014, 4:3
DOI: 10.4172/2161-0495.1000200

Research Article Open Access

Echocardiography a Non-Invasive Method for Investigating Preclinical Drug Toxicity and Safety

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Received date: Arp 18, 2014; **Accepted date:** May 22, 2014 **Published date:** May 29, 2014

Abstract

Echocardiography (EC) is a method used for investigating cardiac morphology and function. Two-dimensional EC gives a visualization of the morphology of the heart. M-mode EC allows heart function to be monitored. Pulsed Doppler EC is the method of choice for measuring blood flows through valves and large vessels. EC is used in routine in clinic and veterinary practice but is infrequently applied to preclinical evaluation of drug toxicity and safety pharmacology despite a number of advantages. Since similar investigations can be done in laboratory animals and humans, preclinical and clinical findings can easily be transposed to each other. EC is totally non-invasive, it does not induce any suffering to the animals and has no impact on health and physiology. It allows repeated measurements and consequently monitoring of development and evolution of adverse effects. In this way, EC evaluates the functional adverse effects of drugs on the cardiovascular system and the consequences of induced lesions. Moreover, using the different modes of EC it is possible to determine the changes in heart contractility and hemodynamics that are involved in the development of cardiovascular lesions. This is illustrated by an experiment in dogs treated with minoxidil. The development of lesions in the right atrium and left ventricle were considered to be related to changes in the function of these cardiac structures as demonstrated by EC recordings. These findings confirm the usefulness of EC in assessing the pathogenesis of drug-related cardiac toxicity.

Keywords: Echocardiography; Cardiac lesions; Minoxidil; Cardiac function

Introduction

Echocardiography (EC) is a method used for the investigation of cardiac morphology and function.

A transducer is placed on the chest of the subject and emits ultrasounds that are reflected by the cardiac structures and surrounding tissues. The reflected ultrasounds are received by the transducer and then processed by the echographic device in order to form an image on a screen. The fraction of the ultrasounds that are reflected characterizes the echogenicity and depends on the physical properties of the tissues. Bones and air have a strong echogenicity and appear in white on the screen. Liquids such as blood have a weak echogenicity and appear as black areas corresponding to the cardiac cavities and lumen of large vessels. Fibrous tissues and muscles have an intermediate echogenicity and appear as grey structures corresponding to cardiac valves and the wall of heart and main vessels.

Echocardiography can be done in humans and in laboratory animals, in particular dogs and non-human primates.

The methodology of echocardiography and its usefulness in preclinical toxicology are reviewed in the current paper. Then an example of application of echocardiography for the investigation of the pathogenesis of cardiac lesions is given.

The Different Modes of Echocardiography

There are three modes of EC, which are usually performed successively for a complete examination of the cardiac structures, their movements over time and blood flows. Indeed the different modes give pieces of information that complement each other.

Two-dimensional echocardiography

Two-dimensional echocardiography (2-D EC) gives a view of the morphology of the heart on the screen of the echographic device. The transducer emits a planar beam of ultrasounds in which the cardiac structures are visualized.

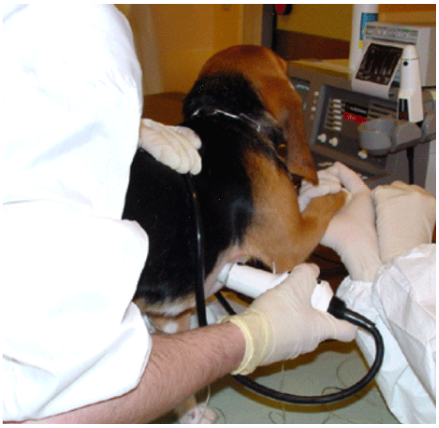

Figure 1: Echocardiography in dogs. Recording in right parasternal incidence.

Depending on the position of the transducer on the chest of the animal, different heart sections are obtained. By changing the

orientation of the transducer progressively, the operator can scan the heart in successive sectors from which the walls, the cavities, cardiac valves and main vessels are visualized.

The 2-D EC examination is usually performed in two different incidences.

In parasternal incidence, the transducer is placed on the right side (Figure 1) and the cardiac structures are visualized in two different sections.

A long axis (longitudinal) section is carried out across the left and right ventricles, left atrium and aorta (Figure 2).

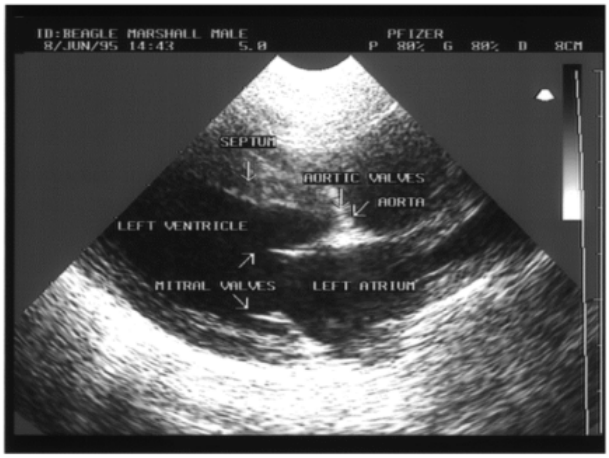

Figure 2: Two dimensional echocardiography in dogs. Long axis section in right parasternal incidence showing the different cardiac structures.

This section is used for the examination of the septum, free wall and cavity of left ventricle, wall and cavity of the left atrium, mitral valves and aortic root.

A short axis section allows visualization of the heart in successive transverse sections, from the apex to the upper part of the heart. The section at the upper level allows visualization of the aortic and pulmonary artery trunk and measurement of the pulmonary artery diameter (Figure 3).

For apical incidence, the transducer is placed on the left side of the animal at the level of the cardiac apex (Figure 4).

The four cardiac cavities are visualized simultaneously (Figure 5) and their areas can be measured in systole and diastole.

In 2-D EC views, guidance lines for M-mode EC or Doppler EC recording can be placed across cardiac structures.

Two-dimensional EC is thus the method of choice for visualizing the cardiac structures and assessing anatomical abnormalities. An ECG that is recorded simultaneously allows following the cardiac cycle. Images 2-D can then be frozen either in ventricular systole or diastole a number of measurements can be taken. For example the surface of the left ventricle can be measured in diastole and systole and the corresponding volumes of the ventricle can be estimated allowing a theoretical calculation of the stroke volume. However in 2-D EC changes in cardiac structures cannot be followed over the full cardiac cycle.

Time-motion EC

In time-motion (M-mode), the position and movements of the cardiac structures crossed by the guidance line are displayed for a few cycles. In this way, M-mode EC allows the observer to visualize changes in heart morphology over the full cardiac cycle and to monitor cardiac function. However the time-related changes can only be seen in a single dimension. M-mode EC is recorded from a 2-D view of the heart in either short axis or long-axis section.

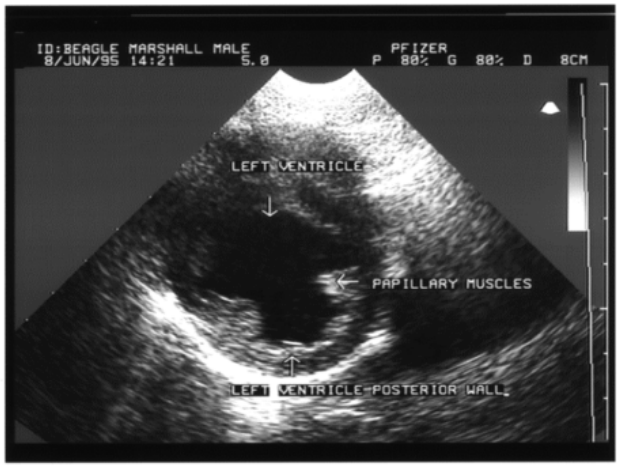

Figure 3: Two dimensional echocardiography in dogs. Short axis section in right parasternal incidence showing the different cardiac structures at the level of the papillary muscles.

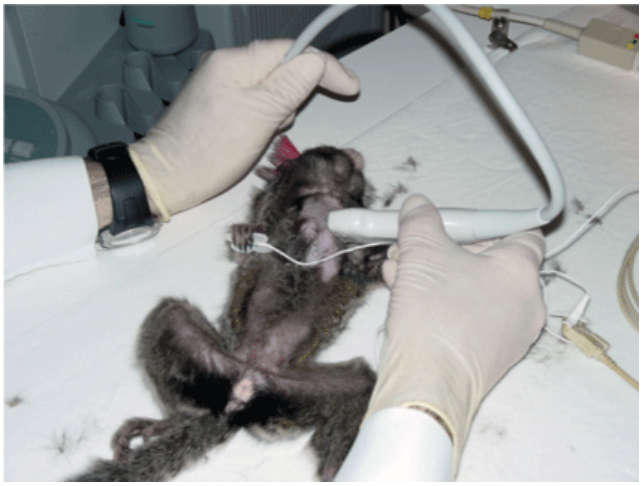

Figure 4: Echocardiography in marmosets. Recording in apical (left) incidence.

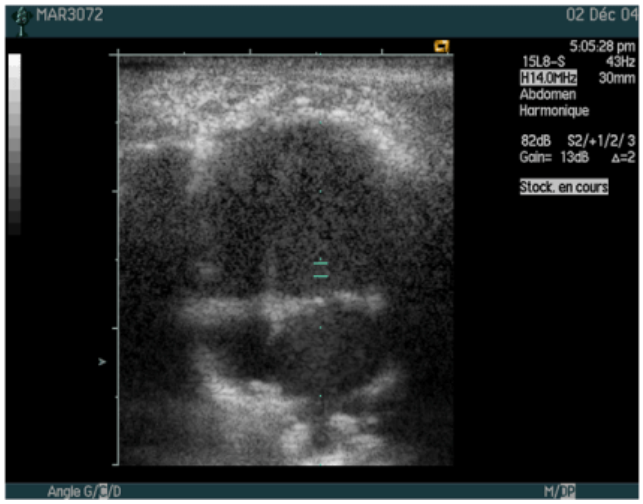

Figure 5: Two dimensional echocardiography in marmoset. Four cavity section in apical incidence showing the different cardiac structures and positioning of the Doppler guidance line for recording mitral flow. LV: Left Ventricle; RV: Right Ventricle; LA: Left Atrium; RA: Right Atrium.

In a short axis section, the upper part of the ventricle is visualized at the level of the papillary muscles close to the chordae tendinae. The guideline is positioned between the papillary muscles and the movements of the cardiac walls and septum are recorded (Figure 6A). Left ventricular end-diastolic (LVDd) and end-systolic (LVDs) diameters are measured at the time of maximum diastolic and minimum systolic dimensions. Thicknesses of the interventricular septum (IVSd and IVSs) and of the left ventricular posterior wall (PWTd and PWTs) are measured in diastole and systole (Figure 6B).

A number of parameters can be calculated

- End diastolic and end systolic volumes (EDV and ESV), calculated with the Teicholz formula: $V = 7D^3/(2.4 + D)$ with D=LVIDd or LVIDs respectively or EDV and ESV.
- Stroke volume: SV= EDV- ESV.
- Cardiac output and cardiac index: CO=SV*HR and CI=CO/body weight.
- Fractional shortening: FS=(LVIDd - LVIDs)/LVIDd.
- Ejection fraction: EF=SV/EDV (EF indicates the fraction of the ventricular diastolic volume that is ejected at each beat and is therefore considered as a key indicator of the ventricular contractile function).
- Percent of septum thickening: PST=(STd - STs)/STd.
- Percent of left ventricle posterior wall thickening: PWT=(LVPWd - LVPWs)/LVPWd.

The mean slope of the systolic wave of the free wall of ventricle is calculated between the onset and the peak of the wave, whereas the maximal slope is measured as a tangent of the wave at its onset. These slopes are further indices of left ventricle contractile function.

In long axis section, an area of the heart giving a clear longitudinal view of the cavities and walls, in particular these of the left heart is selected, and the guidance line is usually placed at two levels. For the evaluation of the ventricular function and morphology, the guidance line is positioned at the tips of the mitral valves and the movements of the cardiac walls and septum are recorded. The same parameters as those measured or calculated from M-mode recordings in short axis section can be obtained from this long axis section.

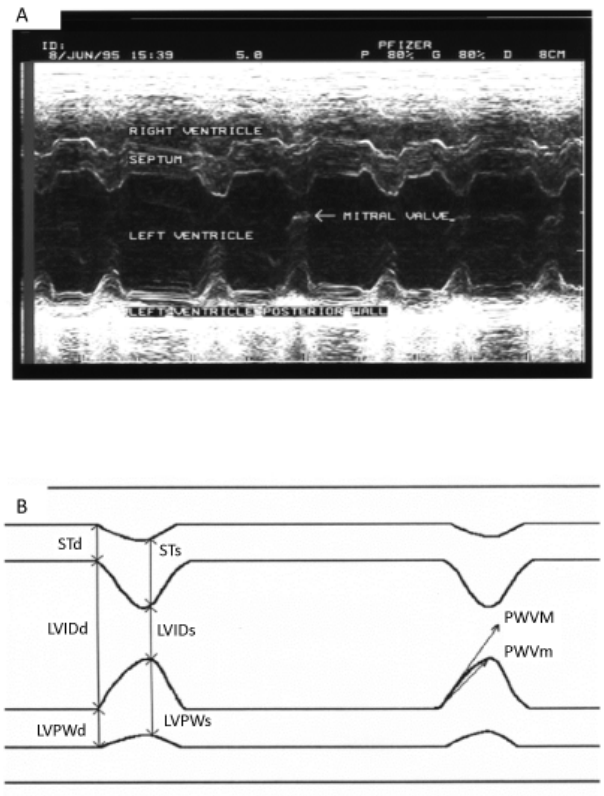

Figure 6: M mode recording in dogs. **(A)** example of tracing recorded from a long axis section in two-dimensional mode. The guidance line is positioned at the tip of the mitral valve. The movements of the septum and free wall of the left ventricle are followed over time. **(B)** Schematic representation of measured parameters.

In addition, the guidance line can be placed in long axis section in the upper part of the heart, across the aortic root and left atrium, for recording of their movements (Figure 7). Aortic diameter of the aorta and left atrium are measured in systole and diastole (ADs and ADd).

M-mode and 2-D EC give no information on intra-cardiac blood flows and only an indirect evaluation of stroke volume based on measurement of ventricle volumes in systole and diastole.

Doppler echocardiography

In pulsed Doppler EC, blood velocity is measured at the level of a window selected in a 2-D section. The spectrum of distribution of the velocities of the red blood cells and their variations over the cardiac cycle are recorded as successive waves produced by the pulsatile flows. The waves appear positive on the screen when the blood is flowing to the transducer and the waves are negative when the blood is flowing in the opposite direction. By measuring the speed of blood motion in vessels and cardiac cavities, pulsed Doppler EC allows the assessment

of flows patterns and, consequently, of the systolic and diastolic cardiac function. Physiological and pathological changes in pulmonary, aortic and atrio-ventricular flows can be investigated. Doppler EC is also a reliable method for measurement of stroke volume.

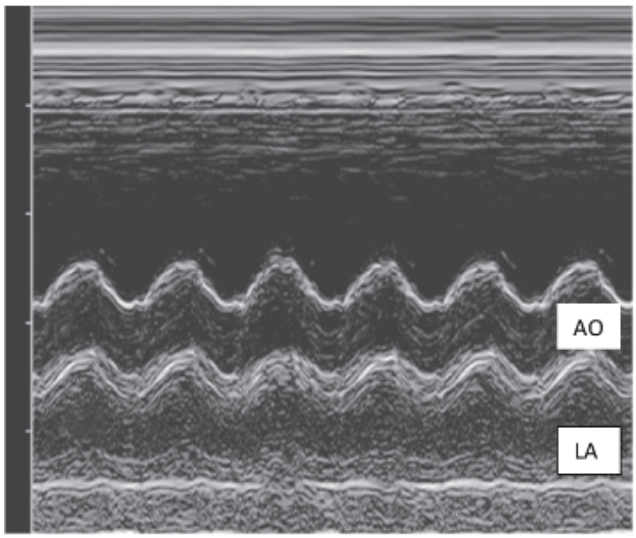

Figure 7: M mode recording at the level of upper part of the heart in marmosets from a long axis section in two-dimensional mode. The guidance line is positioned across the aorta and left atrium. The movements of aorta (AO) and left atrium (LA) are recorded over time.

The atrio-ventricular flows are assessed from a four-cavity section obtained in apical incidence. The Doppler windows are placed downstream of the flows, below the mitral or the tricuspid valves (Figure 5).

The flows are recorded and two positive waves occur at each cardiac beat (Figure 8). The rapid inflow E wave corresponds to the passive filling of the ventricle occurring during its diastole and is recorded during the isoelectric section of the ECG, between T and P waves. The A wave corresponds to the ventricular filling associated with atrial contraction and occurs at the time of the P wave. Peak velocities (V max) of E and A waves, their ratio (E/A), wave acceleration and the integral of velocity over time (VTI) of the two waves together are recorded and serve as indices of ventricle diastolic and/or atrial systolic functions. In particular, E/A gives an indication of the relative contribution of ventricular diastole and atrial systole to the ventricular filling.

Measurements

- Pre-ejection time from the Q wave of the ECG (a) to the onset of the Doppler velocity spectrum(b),
- Acceleration time from the onset to the peak of the velocity spectrum (c) and
- Ejection time from the onset to the end of the velocity spectrum (d).

The stroke volume (SV) is calculated for the pulmonary (SVPul) or aortic flows (SVAo) with VTI of the corresponding flow and the diameter (D) of the artery measured from a two-dimensional section.

$$SV_{Pul} = VTI_{Pul} * \pi * 1/4\ D_{Pul}^2$$

$$SV_{Ao} = VTI_{Ao} * \pi * 1/4\ D_{Ao}^2$$

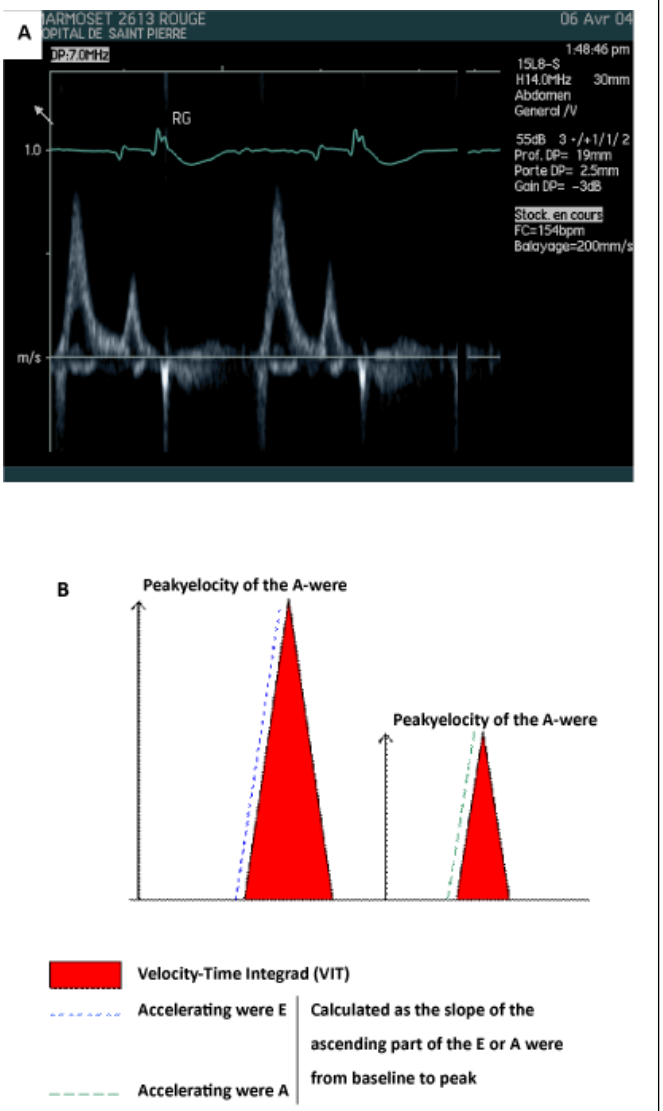

Figure 8: Doppler recording of mitral flow in marmosets. (A) example of tracing recorded from an apical section in two-dimensional mode. **(B)** Schematic representation of measured parameters.

In color Doppler EC, the flows in the cavities and large vessels are visualized in real time from a two-dimensional section, based on a color code. On a four-cavity apical view of the heart, the atrio-ventricular flows during ventricular diastole and atrial systole and the arterial flows during ventricular systole are observed at each cardiac beat. The flow appears in blue when the blood is flowing toward the transducer or in red when blood is flowing in the opposite direction. The brightness of blue or red color indicates the velocity of the blood.

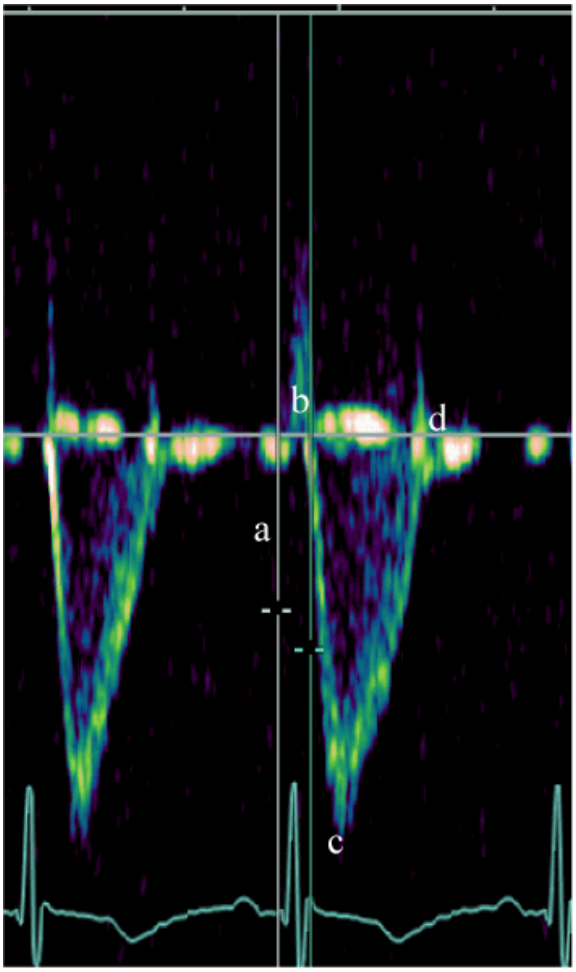

Figure 9: Pulsed Doppler recording of the aortic flow in a marmoset. Pulsed Doppler EC is also used to assess arterial flows. The Doppler window for aortic or pulmonary flows is placed just above (downstream of) the sigmoid valves. For either flow, a negative wave is recorded at each beat, from which V max , VTI, pre-ejection time, acceleration time (AT) and ejection time (ET) can be measured (Figure 9).

Color Doppler EC shows a number of qualitative blood flow changes, for example, laminar versus turbulent flows or abnormal timing and location of blood flows. Color Doppler is therefore a useful tool for the assessment of disturbed flow patterns associated with valve insufficiency or stenosis.

Doppler EC gives thus the information on intra-cardiac blood flows and the direct evaluation of stroke volume, which are missing when only 2-D and M-mode EC is recorded. Therefore Doppler is a key complement of the other EC modes but cannot be substituted to them, since it gives no information on cardiac morphology.

Usefullness of Echocardiography in Preclinical Toxicology

EC has been widely used in humans to investigate cardiac physiology and pathology and to evaluate the pharmacological effects of drugs.

EC has a number of applications in laboratory animals, in particular in dogs and nonhuman primates, but also in rodents and rabbit.

In dogs, the method of EC recording is well established and has been described in a number of papers, together with values in normal subjects [1-4]. In canine or feline veterinary practice, EC is routinely used and can assist in the diagnosis of cardiac morphological alterations or dysfunction.

EC is frequently used to assess toxicity of drugs in humans and in particular, Doppler EC is the gold method to assess the functional consequences of anthracycline's cardiotoxic effects [5-7]. In contrast, this technique is not routinely used for the preclinical toxicological evaluation of drugs despite its potential interest, mainly in dogs and non-human primates [8,9].

Using 2-D EC, it is possible to visualize and evaluate morphological changes induced by drug treatment, such as myocardium hypertrophy or cardiac chamber dilation.

Furthermore, the functional consequences of treatment induced arrhythmia or cardiac lesions in laboratory animals can be assessed by M-mode or Doppler EC. Changes in hemodynamic parameters (SV and flows patterns) and indicators of cardiac contraction (ejection fraction, fractional shortening and velocities of cardiac structures movements) would allow evaluation of the degree of cardiac function impairment.

EC is also of prime interest in assessing the cause of cardiac lesions. Toxic effects of cardiovascular drugs on the heart or blood vessels are often due to exaggerated pharmacological effects [10,11], resulting in marked changes in the cardiovascular function. M-mode and Doppler EC can quantify drug-induced changes in the patterns of cardiac contraction, flows, SV and cardiac output and in this way help to clarify the pathogenesis of cardiac or arterial lesions [12].

Value of echocardiography as a method of refinement

In contrast to most methods presently used in animal experimentation to investigate cardiac or vascular function, EC is non-invasive and does not necessitate surgery. It requires only a gentle restraint and, in some species, sedation or light anesthesia. EC does not induce any pain and no or minimal stress. It does not alter the cardiovascular function that it seeks to measure. Similarly, EC has no or little interference with the measurement of other parameters recorded in toxicity or pharmacology studies and has no effect on the health status of the animal. EC measurements are easily repeatable and therefore allow subsequent follow-up in the same animal.

Limitations of echocardiography

For obtaining accurate data from echocardiographic investigations, recordings should be done by highly trained people using a well standardized method. Even when it is the case, a full EC examination using the 3 modes is rather time consuming. Moreover measurements of changes in cardiac function are usually less accurate than for invasive methods. EC should therefore be limited to toxicological

investigations or in some occasions pharmacological investigations, when major changes in cardiac function are expected.

Example of application of echocardiography for assessment of the pathogenesis of cardiac lesions

EC is routinely used in the clinic and there are a number of publications describing the application of echocardiography for evaluating the functional consequences of drug-induced cardiac toxicity, in particular for monitoring patients treated with chemotherapeutic agents [13-16]. The consequences of cardiac lesions produced by doxorubicin in dogs have been assessed by EC [17]. Thus EC is occasionally used in preclinical toxicology but is not apply to the assessment of the cause of cardiac lesions [18]. Therefore we used echocardiography to assess the pathogenesis of cardiac lesions associated with minoxidil treatment in dogs. This compound is a potent vasodilator and produces necrotic lesions in the myocardium of the left ventricle when given at suprapharmacological doses. These adverse effects are considered to be due to marked changes in ventricular function and hemodynamics. The aim of the study was to confirm that these changes can be investigated by echocardiography. Details on these experiments can be found in previous publications [19,20].

Groups of three beagles received a single administration of minoxidil at doses of 0.5 or 2 mg/kg or the vehicle alone (controls). M-mode and Doppler echocardiography was performed under two-dimensional echocardiography guidance on three occasions the day before treatment, immediately before dosing and 1, 3 and 24 h after dosing. Lead I ECG was recorded by the echographic equipment and heart rate (HR) was calculated. From M-mode recording, the following parameters were measured or calculated: end diastolic, end systolic, and stroke volumes (EDV, ESV and SV), fractional shortening (FS), ejection fraction (EF), the percentage of thickening of the septum and of the left ventricle posterior wall (PST and PWT). Doppler was used for recording aortic flows and calculating the corresponding Vmax, VTI, ejection time (ET) and stroked volume. Cardiac output was calculated at SV*HR.

Minoxidil produced a marked tachycardia. The treatment was associated with a decrease in ESV and with marked increases in FS, EF, PST and PWT, in comparison with data from controls (Table 1), (Figures 10 and 11).

	PST	PWT	EDV	ESV	EF	HR
Control	-14	-17	-7	-10	2	2
0.5 mg/kg	72	25	-21	-62	28	59
2 mg/kg	51	25	-21	-74	34	111

PST: Percent of septum thickening; PWT: Percent of left ventricle posterior wall thickening; EDV, ESV: End diastolic, End systolic volumes; EF: Ejection fraction; HR: Heart rate

Table 1: Minoxidil effects on parameters of left ventricle function in dogs, measured by M-mode echocardiography. Change (%) in mean values recorded 1 hour after treatment (time of maximal amplitude of changes) compared to values recorded the day before treatment.

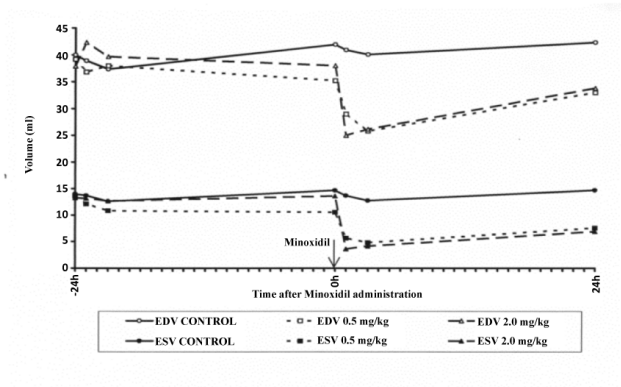

Figure 10: Effect of minoxidil on ventricular volumes in dogs. M-mode echocardiographic recording.

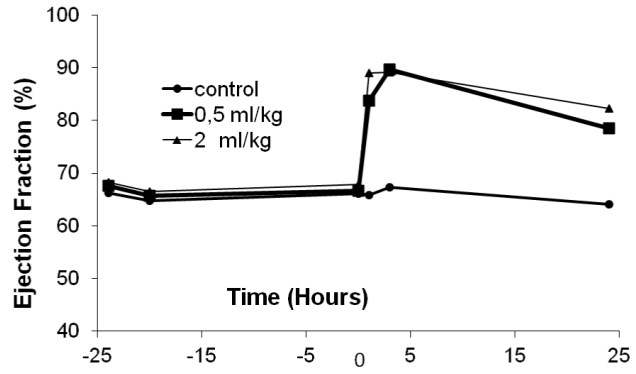

Figure 11: Effect of minoxidil on ejection fraction in dogs. M-mode echocardiographic recording.

	Vmax	VTI	ET	SV	CO
Control	16	14	-2	8	10
0.5 mg/kg	29	18	-17	22	93
2 mg/kg	53	25	-18	33	181

Table 2: Minoxidil effects on aortic flow in dogs, measured by Doppler echocardiography. Change (%) in mean values recorded 1 hour after treatment (time of maximal amplitude of changes) compared to values recorded the day before treatment.

Vmax: Maximum velocity of the wave; VTI: Velocity time integral; ET: Ejection time; SV: Stroke volume; CO: Cardiac output.

These changes are indicative of an increase in the amplitude of cardiac contraction. Minoxidil also produced a decrease in EDV, which indicates a decrease in left ventricle filling probably due to the tachycardia and consequent decrease in inter systolic time. Doppler measurements in table 2 showed an increase in the velocity of the aortic flow, which confirmed the increase in cardiac contractility. A decrease in ET is consistent with an increase in heart rate and a faster ventricular contraction. There was also a mild increase in stroke

volume, which together with the tachycardia resulted in a marked increase in cardiac output. Overall, the effects were dose-related.

The tachycardia and echocardiographic evidence of increased cardiac contractility are consistent with the vasodilatory properties of minoxidil [21,22]. The consequent hypotension provoked a reflex inotropic and chronotropic compensatory reaction on the heart [23]. Furthermore, by decreasing the afterdoad, the vasodilation also resulted in the increase in velocity and amplitude of ventricle contraction [24,25].

The increase in rate and force of contraction of the myocardium increases its energy expenditure and oxygen requirements [10]. Because of the tachycardia, the ventricular filling is reduced as indicated by a decrease in EDV. In addition, the increase in heart rate decreases the duration of the diastole when most of the coronary perfusion occurs. The compound-induced hypotension further decreases the coronary perfusion [26]. Therefore, the increase in energy requirement coupled with an impaired perfusion resulted in a condition of relative coronary insufficiency, which is the likely cause of the necrotic lesions in the left ventricular myocardium (Figure 12).

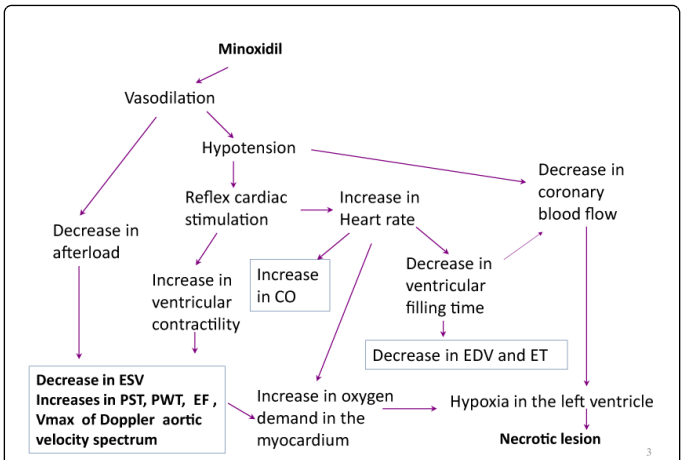

Figure 12: Relationship between changes produced by minoxidil on cardiac function and the development of left ventricular lesions in dogs.

Decrease in end systolic volume (ESV) and increases in percent thickening of the septum and left ventricle wall (PST and PWT), in ejection fraction (EF) and in maximum value of the Doppler aortic velocity spectrum (Vmax), which can be evaluated by echocardiography play a critical role and the development of the left ventricular necrosis. EDV: End diastolic volume; ET: ejection time; CO: cardiac output.

In conclusion, we have shown that echocardiography allows a non-invasive investigation of the changes in cardiac function that are considered to play a key role in the development of cardiac lesions produced by supra-pharmacological doses of a potent vasodilator in dogs.

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