Echocardiography a non invasive method for investigating preclinical drug toxicity and safety.

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What is echocardiography (EC)

- Ultrasound (US) are emitted by a transducer
- Reflection of US on tissues depends on their physical properties (echogenicity)
  - strong echogenicity: bones, air
  - weak echogenicity: liquids (blood, urine)
- Reception of reflected US by the transducer
- Processing of the information and image on the screen
Bidimensional echocardiography (2-D EC) in right parasternal incidence

Visualisation of the heart structures in the plane of the ultrasounds beam: longitudinal section
2-D EC: Longitudinal section
M-mode echocardiography

Positioning of a guidance line through the cardiac structures in 2-D

Visualisation of the movements of the cardiac structures
M-mode echocardiography
M-mode echocardiography
Schematic representation showing measured parameters
M-mode echocardiography calculated parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>End diastolic volume</td>
<td>( EDV = \frac{7 \cdot LVIDd^3}{2.4 + LVIDd} )</td>
</tr>
<tr>
<td>End systolic volume</td>
<td>( ESV = \frac{7 \cdot LVIDs^3}{2.4 + LVIDs} )</td>
</tr>
<tr>
<td>Stroke volume</td>
<td>( SV = EDV - ESV )</td>
</tr>
<tr>
<td>Cardiac output</td>
<td>( CO = SV \times \text{heart rate} )</td>
</tr>
<tr>
<td>Fractional shortening</td>
<td>( FS = \frac{LVIDd - LVIDs}{LVIDd} )</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>( EF = \frac{SV}{EDV} )</td>
</tr>
<tr>
<td>Percent of septum thickening</td>
<td>( PST = \frac{Std - Sts}{STd} )</td>
</tr>
<tr>
<td>Percent of posterior wall thickening</td>
<td>( PWT = \frac{LVPWd - LVPWs}{LVPWd} )</td>
</tr>
</tbody>
</table>
The different modes Doppler

Assessment of

- Quantitative parameters of cardiovascular function
  - Flows: Stroke volume, cardiac or extra cardiac shunt flow, left coronary blood flow,
  - Pressure changes across valves and orifices or in cardiac chamber and great vessels
- Qualitative blood flow changes:
  - Laminar vs disturbed flow patterns
Doppler recording of intra-cardiac flows

- Visualisation of the heart structures in a 2-D mode section using apical incidence
- Positioning of the Doppler Window at the level of the aorta, pulmonary artery, mitral or tricuspid valves
- Recording of the changes in blood velocity over a few beats
The different modes: Doppler

Four cavities view in apical incidence (marmosets)
Echocardiography in marmosets: mitral flow
Spectrum of distribution of erythrocytes velocities

E wave
Ventricle diastole
A wave
Atrial systole
Schematic representation of measurements on a Doppler velocity spectrum of the mitral flow

- Peak velocity of the E-wave
- Peak velocity of the A-wave
- Velocity-Time Integral (VTI)
- Acceleration wave E: calculated as the slope of the ascending part of the E or A wave from baseline to peak.
- Acceleration wave A

13
Aortic flow recording (marmoset)

Measurements
• Vmax, VTI,
• Ejection time (ET): from the onset (b) to the end (d) of the velocity spectrum
• 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 (d).
Doppler Echocardiography

Calculated parameters

- From tricuspid and mitral flow
  Ratio A/E waves for peak velocity or velocity-time integral:
    - Relative contribution of atrial systole vs ventricle diastole to ventricle filling

- From aortic flow
  Stroke volume = VTI x AA with
  - VTI: velocity time integral
  - AA: aortic diameter measured from M-mode trace
Application of echocardiography in preclinical safety assessment (1)

CONSEQUENCES of Cardiac toxicity

- Evaluation of morphological changes induced by test compounds (cardiac hypertrophy, dilation…)
- Measurement of functional consequences (changes in haemodynamic parameters and in contractility) of compound-induced cardiac lesions
- Measurement of haemodynamic changes associated with arrhythmias
Application of echocardiography in preclinical safety assessment (2)

CAUSE and MECHANISM of Cardiac toxicity

- Evaluation of pharmacological effects of cardiovascular drugs.
  - Measure of changes in cardiac contractility and in haemodynamic parameters
  - Clarification of the pathogenesis of cardiac lesions linked to exaggerated pharmacological effects: example of minoxidil
Value of echocardiography in toxicology as a method of refinement

- Non-invasive technique
  - No surgery
  - No pain or distress for the animal
  - Only a gentle restraint is needed

- No interference on cardiac function: measurement in normal situation

- No interference with the measurement of other parameters

- No influence on the results of the toxicity study
  - No medication
  - No effects of echography on the health status of the animal

- Measurements are easily repeatable and allow subsequent follow-up in the same animal
Minoxidil

- Potent vasodilator

- Cardiac toxicity of minoxidil in the dog
  - Produces necrosis of left ventricle at suprapharmacological doses (0.5-3 mg/kg)
  - Is due to the vasodilatory properties of the drug
Example of minoxidil
Experimental procedure

- Treatment with 0.5 or 2 mg/kg (single dose)
- 3 dogs/dose
- Measurement of echocardiographic parameters in M-mode and Doppler at different time points before and after dose
Minoxidil effects on parameters of left ventricle function evaluated by M-mode echocardiography

Change (%) in mean values recorded 1 hour after treatment compared to values recorded the day before treatment

<table>
<thead>
<tr>
<th></th>
<th>PST</th>
<th>PWT</th>
<th>EDV</th>
<th>ESV</th>
<th>EF</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-14</td>
<td>-17</td>
<td>-7</td>
<td>-10</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td>72</td>
<td>25</td>
<td>-21</td>
<td>-62</td>
<td>28</td>
<td>59</td>
</tr>
<tr>
<td>2 mg/kg</td>
<td>51</td>
<td>25</td>
<td>-21</td>
<td>-74</td>
<td>34</td>
<td>111</td>
</tr>
</tbody>
</table>

PST: Percent of septum thickening; PWT: Percent of left ventricle posterior wall thickening; HR: heart rate
EDV, ESV: end diastolic, end systolic volumes; EF: Ejection fraction
Effect of minoxidil on ventricular volumes
Effect of Minoxidil on Ejection Fraction
(measured in M-mode)

- Control
- 0.5 ml/kg
- 2 ml/kg

Ejection Fraction (%) vs Time (Hours)
Minoxidil effects on aortic flow measured by Doppler echocardiography

Change (%) in mean values recorded 1 hour after treatment compared to values recorded the day before treatment

<table>
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<tr>
<th></th>
<th>Vmax</th>
<th>VTI</th>
<th>ET</th>
<th>Stroke Volume</th>
<th>Cardiac Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>14</td>
<td>-2</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td>29</td>
<td>18</td>
<td>-17</td>
<td>22</td>
<td>93</td>
</tr>
<tr>
<td>2 mg/kg</td>
<td>53</td>
<td>25</td>
<td>-18</td>
<td>33</td>
<td>181</td>
</tr>
</tbody>
</table>

Vmax: maximum velocity of the wave  
VTI: velocity time integral  
ET: ejection time
Minoxidil effects on parameters of left ventricle function evaluated by echocardiography

- Increase in contractility
  - Increase in ejection fraction and percent thickening of the left ventricle wall and septum
  - Decrease in end systolic volume
  - Increase in Vmax of aortic flow
- Mild increase VTI and consequently in stroke volume
- Marked tachycardia leading to
  - Decrease in ejection time
  - Decrease in end diastolic volume indicating decreased filling of the ventricle (decrease in inter-systolic time)
- Marked increase in cardiac output
  - Due mainly to tachycardia and to a lesser extent to increase in SV
Relationship between changes produced by minoxidil on cardiac function and the development of cardiac lesions

Minoxidil

- Vasodilation
- Hypotension
  - Reflex cardiac stimulation
    - Decrease in afterload
    - Increase in ventricular contractility
    - Increase in CO
    - Decrease in ESV
    - Increases in PST, PWT, EF, Vmax of Doppler aortic velocity spectrum
  - Increase in Heart rate
    - Decrease in ventricular filling time
    - Increase in oxygen demand in the myocardium
    - Decrease in EDV and ET
    - Hypoxia in the left ventricle
    - Necrotic lesion
  - Decrease in coronary blood flow
Conclusion of minoxidil study

- Echocardiography allows the non-invasive investigation of changes in the cardiac function produced by a vasodilator known to play a critical role in the pathogenesis of cardiac lesions.
- In the past, these functional changes were assessed using highly invasive methods.
CONCLUSION

Echocardiography has potentially a great value as a method for investigation of cardiovascular effects of drugs in toxicology and safety pharmacology
Establishment echocardiography in dogs and marmosets
Drs Pierre Bonnet and Véronique Eder
Hopital Bretonneau / University of Tours, France

Minoxidil study, Scientific collaboration
M. Gautier, PhD student

Technical collaboration of
H. Petinay, N. Mauclair and O. Christin
Pfizer Research Center, Amboise, France
References

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- Serriere S., Tranquart F., Hanton G. Sonographic exploration of the mesenteric and renal arterial blood flows in adult rats. Toxicol Lett., 158, suppl 1, S237, 2005
THANK YOU
for your attention

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• Back Up slides
2-D echocardiography in right parasternal incidence

Scanning in transverse section
2-D EC: transverse section
M-mode echocardiography of the upper part of the heart in a marmoset. The guidance line is positioned across the aorta and left atrium. The movements of aorta (AO) and left atrium (LA) marmoset, are recorded over time.
Color Doppler of intra-cardiac flows. Ventricular diastole (marmoset).

The flow from left atrium to left ventricle through mitral valves appears in red.
Color Doppler of intra-cardiac flows: ventricular systole (marmoset)

The aortic flow appears in blue