Echocardiography in cardiovascular drug assessment

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SUMMARY The validity of using echocardiography to measure the effects of cardiovascular drugs on the heart has been investigated. A standardised method of measuring left ventricular diameters is described and used to measure variability and therefore reproducibility in 20 subjects. Immediate variability was measured in 10 normal subjects. In 5 of these subjects variability was measured every few minutes, hourly, and weekly during control periods of drug studies and also after an interval of 1 year. Estimates were made of the minimum significant change in heart size which could be defined under these circumstances. Reproducibility decreased with increasing time interval between studies but overall was very good (coefficient of variation in diameters from 1 to 8%). Even with a time interval of 1 year the individual absolute difference in the mean of diameters was never greater than 0.3 cm. Reproducibility was similar in small groups of patients with hypertension or ischaemic heart disease (without dyskinesia).

Estimated sensitivity could be increased for any time interval equally as much by increasing the number of measurements on each occasion and modifying statistical analysis accordingly as by doubling the number of subjects. There was a fourfold difference between minimum and maximum sensitivity.

After 0.5 mg sublingual glyceryl trinitrate, diastolic and systolic diameters decreased significantly in all subjects. Estimated ventricular and stroke volumes were reduced. Cardiac output was unchanged as heart rate increased from 67 to 80 beats per minute while blood pressure fell. The estimated ejection phase indices (ejection fraction, fractional shortening, and fibre shortening rate) all increased significantly.

With rigid standardisation echocardiography could be a potentially useful non-invasive method of measuring the effects of cardiovascular drugs on the heart.

Echocardiography is well established as a useful non-invasive method of cardiac diagnosis. Echocardiographic estimates of left ventricular internal diameter and derived haemodynamic indices have correlated well with standard angiographic measurements (Fortuin *et al.*, 1971; Feigenbaum *et al.*, 1972; Murray *et al.*, 1972) and have therefore been used to assess left ventricular function (Fortuin *et al.*, 1972; Belenkie *et al.*, 1973). These methods make assumptions about the echocardiographic internal diameter and its relation to a geometric model of the left ventricle which may be questionable (Linhardt *et al.*, 1975) especially at the extremes of cardiac size or where there is dyskinesia (Teichholz *et al.*, 1976). It has, however, been suggested that echocardiographic estimation of ventricular function is still suitable for the sequen-

tial study of individuals and has been proposed as a suitable method of measuring the effects of cardiovascular drug action on the heart (Gibson, 1973; Hirshleifer *et al.*, 1975). Though the ability of echocardiography to measure drug-induced changes in heart size in certain circumstances appears to have been shown (Redwood *et al.*, 1974), in most studies the drug-induced changes were small and where assessed have not necessarily correlated with other measures of cardiovascular drug action (Frishman *et al.*, 1975). Conversely, relatively large changes have not always been significant (Seabra-Gomes *et al.*, 1976).

The ability of echocardiography to measure drug-induced changes in heart size accurately depends in part on the sequential variation in echocardiographic diameters during repeated control measurements. Previous measurement of this variation has produced differing results (Pombo *et al.*, 1971; Gibson, 1973; Popp *et al.*, 1975; Stefadourous and Canedo, 1977). However, these studies have differed in technique and type of variation studied, and

¹Dr M. A. Martin was in receipt of a grant from May and Baker Ltd, and the work was supported by a grant from the University of Sheffield Medical Research Fund.

consequently it was decided to measure the variability and, therefore, reproducibility of echocardiographic measurements of left ventricular function specifically under the circumstances appropriate to the investigation of drug action.

Subjects and methods

Echocardiography was performed using an Ekoline 20A Ultrasonoscope with a 2.25 MHz, 1.3 cm diameter, 10 cm focus transducer in conjunction with an Ekoline 21 Strip Chart Recorder.

Recordings were taken from the standard inter-space (Popp *et al.*, 1975) where the mitral valve was seen clearly with the transducer perpendicular to the chest wall. Machine controls were adjusted to show the septal and posterior wall endocardium clearly throughout the cardiac cycle. An M mode scan was then made from the aorta to the apex and the position where the echo from the mitral posterior leaflet was first replaced by chordal echoes was used to define the position where tracings were then recorded with the transducer stationary (Fig. 1). A carotid artery pressure tracing was recorded simultaneously on the echocardiographic tracing using a Hewlett Packard pressure transducer (2105 1D). Only subjects in whom the whole procedure could be carried out unequivocally were used in the study (10 out of 15 normal subjects). Tracings were recorded at 100 mm/s paper speed and measurements were made on 3 out of 6 consecutive beats during quiet respiration (Gerstenblith *et al.*, 1977) and the mean results were taken.

End-diastolic diameter (EDD) was defined as the distance between the septal and posterior wall endocardial echo at the peak of the R wave on the simultaneously recorded electrocardiogram, and end-systolic diameter (ESD) was measured at the point of least perpendicular separation of the endocardial surfaces (Fortuin *et al.*, 1971). Distances were measured using the internally generated scale whose reproducibility was checked with a reference echo (coefficient of variation = 1%) and expressed to the nearest mm. Heart rate was measured as the mean of the 6 consecutive beats recorded on the electrocardiogram.

End-diastolic volume (EDV) in ml and end-systolic volume (ESV) in ml were estimated as the cube of the respective diameters (Pombo *et al.*, 1971). Other indices were estimated from standard derivations: diameter shortening (ΔD) = (EDD - ESD) cm; stroke volume (SV) = (EDV - ESV)

ml; ejection fraction (EF) = $\frac{SV}{EDV}$; cardiac output

(CO) = (SV × HR) ml; percentage diameter

shortening ($\% \Delta D$) = $\frac{EDD - ESD}{EDD} \times 100$ per

cent.

The mean normalised rate of circumferential fibre shortening, which has been suggested as a possible measure of contractility (Cooper *et al.*, 1972), was

calculated as $\left(\frac{EDD - ESD}{EDD \times ET} \right)$ circumferences/s

Fig. 1 M mode scan to define ventricular level for diameter measurements.

Fig. 2 Device for measuring the degree of the subject's lateral rotation.

where ET = ejection time in seconds. Ejection time was measured as the time interval from the upstroke to the dicrotic notch of the simultaneous carotid artery tracing using the internal time scale. The accuracy of this scale was confirmed by a standard time pulse (coefficient of variation = 2%) and the mean of the 3 beats used in the diameter measurements was taken. A previous comparison of ejection time measured in this way with that measured directly from aortic valve echoes showed close correlation ($r = 0.98$, $P < 0.001$, $n = 9$).

The initial tracings were evaluated using the wall volume method for reliability (Bennett and

Rowlands, 1976). Recordings were taken in the subject position where clear tracings could be obtained. The degree of each subject's cranial elevation was defined by the angle from the horizontal made by the adjustable back of the examination couch. The degree of lateral rotation, if any, was measured with a simple device designed for measuring the angle of movement at major joints (Fig. 2). This allowed subjects to be included in whom acceptable tracings were not obtained in the supine position, thus increasing the yield of suitable subjects.

On each occasion, the exact subject position was recorded along with the interspace used and the machine control settings. On all subsequent occasions these variables were reproduced, since controlling subject position and interspace had been shown in a preliminary study to reduce significantly the variance of sequential measurements.

In this study 2 diameter measurements were made in 5 subjects using different interspaces with subject position controlled and in different positions with interspace controlled. Two further measurements were then made with both variables controlled. In each case the variance was measured and compared. (Positional variance ratio for diameters = 7.1 $P < 0.01$; interspace variance ratio = 5.7 $P < 0.05$.)

The appropriate ventricular level for recordings was confirmed by matching the echocardiographic pattern from the mitral valve and/or apparatus in the tracings taken on each occasion in each individual (Fig. 3).

Using the above technique, short-term reproducibility was measured in 10 normal subjects

Fig. 3 Two echocardiograms from a normal subject recorded one week apart.

Fig. 4 Effect of sublingual glyceryl trinitrate on ventricular internal diameters.

(7 men, 3 women, age 21 to 38). Three measurements were made of the ventricular internal diameters at approximately 5-minute intervals. In between recordings the transducer was removed from the chest and then repositioned while the subjects maintained a constant posture.

The mean, range, standard deviation, and coefficient of variation of the ventricular diameters and all the derived indices were measured for each subject and the group means were calculated.

In a smaller group of these subjects ($n = 5$) sequential reproducibility was studied in greater detail during the course of drug studies. Each of the 5 subjects was given 0.5 mg glyceryl trinitrate sublingually after 3 control tracings at 5-minute intervals and echocardiographic recordings were made 5 minutes later, along with further measurement of pulse rate and blood pressure. During another study baseline measurements were made at the same time of day on 4 different days, one week apart. On one of these days the subjects received placebo after the baseline recording and measurements were made at hourly intervals for 6 hours.

Statistical analysis of the results was by Student's paired t test and an analysis of variance incorporating replicate control measurements.

The individual measurements from the 3 beats used from each tracing gave an estimate of beat-to-beat variation, and the control measurements of the trinitrate study ($n = 3$) were used to measure immediate variability. Similarly the placebo day

results were used as a measure of hourly variation ($n = 7$) and the baseline measurements on each study day ($n = 4$) as a measure of weekly variability. The effect of time interval between tracings on variability was assessed by comparing the first 3 measurements at each time interval. Short-term variability was remeasured after an interval of 1 year from the initial recordings. Short-term variability was also measured in a small group of patients with ischaemic heart disease without significant dyskinesia ($n = 5$), and hourly variation was measured in a group of hypertensives ($n = 5$). All the normal subject data were used to estimate the changes in echo diameters and derived indices which would have to occur, after pharmacological intervention, to give a high chance of being shown to be statistically significant.

These estimates were used as a measure of the sensitivity of the method under a variety of different experimental designs. The designs considered differed in the time interval between measurements, the number of tracings taken on each occasion, and the number of subjects examined. The influence of these factors on estimated sensitivity was examined in an attempt to define the optimum application of echocardiography to cardiovascular drug assessment.

Results

The immediate variation measured in the whole normal group was small, showing good short-

Table 1 Sequential variation in 3 measurements: group mean of individual mean results
Time interval = 5 min

	Mean			Range			Standard deviation			Coefficient of variation (%)		
	N = 10 T = 0	N = 5 T = 0	N = 5 T = +1 y	N = 10 T = 0	N = 5 T = 0	N = 5 T = +1 y	N = 10 T = 0	N = 5 T = 0	N = 5 T = +1 y	N = 10 T = 0	N = 5 T = 0	N = 5 T = +1 y
EDD (cm)	4.6	4.5	4.4	0.14	0.12	0.11	0.08	0.07	0.06	1.7	1.6	1.3
ESD (cm)	3.1	3.1	3.1	0.11	0.11	0.12	0.06	0.06	0.06	1.9	2.0	2.0
ΔD (cm)	1.5	1.4	1.3	0.11	0.10	0.11	0.06	0.06	0.06	4.0	4.1	4.4
EDV (ml)	94	93	90	10	8	6	5	4	3	5.6	4.9	3.9
ESV (ml)	27	30	30	3	3	3	2	2	2	6.3	6.6	5.5
SV (ml)	67	63	60	8	6	6	4	3	3	6.5	5.7	5.5
CO (l)	4.27	4.12	3.90	0.56	0.53	0.49	0.43	0.28	0.25	7.1	7.2	6.2
EF	0.71	0.68	0.68	0.03	0.03	0.03	0.02	0.01	0.02	2.3	2.0	2.4
% ΔD	34	32	32	2.3	2.2	2.2	1.2	1.2	1.2	3.6	3.6	3.9
VCF (circ/s)	1.14	1.11	1.10	0.10	0.07	0.09	0.05	0.04	0.05	4.6	3.3	4.3

T = Time of measurement.

term reproducibility using the above method. There was no difference between the overall group and the subset of 5 subjects who were studied in detail, suggesting that they were a representative sample (Table 1). In these 5 normal subjects the sequential variation in measurements increased with increasing time interval on almost every occasion (Table 2). Measurement of immediate variability remained unchanged even after a gap of 1 year (Table 1), and the difference in individual mean diameters 1 year apart was in no case greater than 0.3 cm and the changes were random. There was no significant difference in variability in either group of patients compared with the normal subjects for the appropriate time intervals (Table 2).

The estimates of the size of change in each measurement which would have to occur to be statistically significant varied with time interval, number of measurements made, and subject numbers. The size of the change under these different circumstances which would ensure a 95 per cent probability of an observed difference being declared significant at the 5 per cent level is shown in Table 3.

In all subjects both ventricular diameters decreased significantly after glyceryl trinitrate

(Fig. 4). Estimated ventricular and stroke volumes decreased but since heart rate increased cardiac output was unchanged. The ejection phase indices (EF, % ΔD , VCF) all increased significantly (Table 4).

Discussion

The size of sequential variation in echo diameters in this study was similar to some previous studies but substantially smaller than other reports. The more pessimistic of the previous estimates mainly come from an earlier phase in the development of the echocardiographic application to left ventricular measurement, where standardisation was not as rigid as now appears optimum. Initially the exact level through the mitral apparatus was not defined in detail (Pombo *et al.*, 1971) and the concept of standard interspace, which has been shown to reduce variability (Popp *et al.*, 1975) was not in use. The use of strip chart recorders rather than Polaroid photographs has improved resolution and the careful control of all variables which we have attempted to maintain during repeated measurements should have allowed us to keep random variability to a minimum.

Previous work has not compared variability at different time intervals. Sequential variation in

Table 2 Effect of time interval on reproducibility of echocardiographic diameters: group mean of individual mean results in cm

Interval	Range			Standard deviation			Coefficient of variation (%)		
	EDD	ESD	ΔD	EDD	ESD	ΔD	EDD	ESD	ΔD
Normals n = 5									
Beat to beat	0.08	0.07	0.10	0.04	0.04	0.05	1.0	1.3	3.8
Five minutes	0.12	0.11	0.10	0.07	0.06	0.06	1.6	2.0	4.1
One hour	0.13	0.20	0.14	0.08	0.08	0.08	1.8	3.3	5.8
One week	0.26	0.17	0.24	0.17	0.10	0.12	3.4	3.3	8.4
Ischaemic heart disease n = 5									
Five minutes	0.14	0.13		0.06	0.07		1.4	1.7	
Hypertension n = 5									
One hour	0.21	0.10		0.10	0.06		2.5	2.1	

Table 3 Magnitude of minimum change with 95 per cent probability of being true change ($P < 0.05$)

Time interval	Weeks	Weeks	Hours	Minutes	Minutes	Minutes	Minutes
No. of recordings							
1st occasion	1	1	1	1	1	3	3
2nd occasion	1	1	1	1	1	3	1
Subject no.	5	10	5	5	10	5	5
EDD (cm)	0.39	0.24	0.23	0.19	0.12	0.09	0.14
ESD (cm)	0.27	0.17	0.34	0.17	0.11	0.09	0.13
ΔD (cm)	0.36	0.22	0.32	0.17	0.11	0.08	0.12
EDV (ml)	22	14	14	11	7	6	8
ESV (ml)	7	4	9	5	3	2	3
SV (ml)	21	13	13	9	6	5	7
CO (l)	1.39	0.91	1.26	0.83	0.52	0.40	0.59
EF	0.08	0.05	0.10	0.04	0.03	0.02	0.03
% ΔD	6.2	3.9	7.3	3.4	2.1	1.7	2.4
VCF (circ/s)	0.22	0.14	0.29	0.12	0.07	0.06	0.08

echocardiographic measurements varies with the different time intervals, and the technique therefore will vary in sensitivity in different types of study.

Measurements every few minutes would be needed in an evaluation of short acting drugs such as glyceryl trinitrate. Variation here should represent measuring error, respiratory variation, and variability associated with transducer replacement. It was found to be small (coefficient of variation in diameter = 2%) and similar to a previous study using a carefully controlled technique (Popp *et al.*, 1975). Hourly measurements would be suitable for many single dose and infusion studies, and weekly or longer time intervals would be necessary for steady state evaluations.

These time intervals would also include variation from subject repositioning. They would also include any true change in heart size secondary to intrinsic changes in the determinants of cardiac performance that might occur. These sources of variation might be expected to differ between subjects.

The size of sequential variation with the longer time intervals (coefficient of variation in diameters—hourly 3.5%, weekly 5%) was significantly smaller

than some previous studies (Pombo *et al.*, 1971; Stefadouros and Canedo, 1977) but similar to long-term measurement of control subjects in a transplant study (Popp *et al.*, 1971).

The time interval between measurements used in a drug study will be dictated by the pharmacology and kinetics of the drug concerned. Repeated echocardiographic measurements have been shown to be feasible for all the appropriate time intervals and their reproducibility suggests that the method could be sensitive enough to measure clinically relevant drug-induced changes in heart size. Even after a period of 1 year the group mean of individual mean absolute differences in diameters was no more than 0.2 cm.

For optimal use of echocardiography it is necessary to increase sensitivity wherever possible as some drugs may produce only small changes in heart size. If sequential variability in a measurement is known for a given population, the size of the minimum induced change which can be identified can be estimated. This minimum difference was estimated for all the echocardiographic indices and used as a measure of sensitivity. The

Table 4 Effect of 0.5 mg glyceryl trinitrate sublingually ($N = 5$)

Control measurements $\times 3$		5 min after glyceryl trinitrate	
Group mean ± 1 standard deviation (1 SD)		Mean ± 1 SD	Significance
EDD (cm)	4.5 $\pm$ 0.53	4.1 $\pm$ 0.70	***
ESD (cm)	3.1 $\pm$ 0.40	2.8 $\pm$ 0.60	***
ΔD (cm)	1.4 $\pm$ 0.16	1.3 $\pm$ 0.20	
EDV (ml)	93 $\pm$ 29	75 $\pm$ 29	***
ESV (ml)	30 $\pm$ 10	23 $\pm$ 10	***
SV (ml)	63 $\pm$ 19	53 $\pm$ 20	***
HR (beats/min)	67 $\pm$ 8	80 $\pm$ 3	***
CO (l/min)	4.12 $\pm$ 1.0	3.96 $\pm$ 1.50	
EF	0.68 $\pm$ 0.03	0.72 $\pm$ 0.05	**
% ΔD	32 $\pm$ 2	34 $\pm$ 5	*
ET (s)	0.284 $\pm$ 0.02	0.258 $\pm$ 0.02	***
VCF (circ/s)	1.11 $\pm$ 0.10	1.34 $\pm$ 0.30	***
S (mmHg)	117 $\pm$ 4	104 $\pm$ 6	***
D	76 $\pm$ 3	69 $\pm$ 6	**

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$
S, systolic blood pressure; D, diastolic blood pressure.

minimum differences shown are all small in absolute terms (for diameters 0.1 to 0.4 cm). However, under conditions of minimum sensitivity some indices would have to change by more than one-third to be significant and there is up to a fourfold difference between the estimated minimum and maximum sensitivity.

The estimated sensitivity of the method increased not only when the number of subjects was increased but also when more than one recording was made at intervals of a few minutes on each occasion. In the latter case the increase in sensitivity arises because the variation in the repeated recordings made on each occasion can be used to assess experimental error, thus separating the components of variation attributable to subject repositioning and any associated random changes which differ between subjects. The sensitivity of this design remains constant at different time intervals. The form of statistical analysis used in this situation is to analyse the data as arising from a two-way classification model with an interaction term and replicate observations in each cell. It can be shown that a similar increase in sensitivity may be obtained either by doubling the subject numbers or by making replicate recordings on each occasion (Table 3). This may be important logistically as it is usually far easier in studies to perform multiple measurements on each subject on each occasion than to use more subjects.

These estimates, however, make a number of assumptions. It is assumed that this study group, though small, was representative. This appears valid compared with the whole group of 10. However, it is possible that there might be differences in subjects with cardiovascular disease. Though this is not certain for all types of cardiac disease, variability in repeated hourly measurements in hypertensives and of 5-minute measurements in patients with coronary artery disease without dyskinesia was the same as the normal group. The estimates also presume that all subjects would react to interventions similarly both qualitatively and quantitatively and therefore probably represent idealised estimates. These estimates should, therefore, not be considered highly accurate in themselves. However, they should accurately reflect the way that changing subject numbers or measurement frequency will affect sensitivity.

If relatively large consistent changes occur in echocardiographic indices in a drug study, such as those shown with glyceryl trinitrate (Table 4), some will appear significant with single echocardiographic measurements in a small group. However, in circumstances where the changes are small or variable the increased sensitivity of

multiple measurements and more efficient statistical analysis may be necessary to demonstrate significance. For example, when the trinitrate data were assessed by a Student's paired *t* test using only one control measurement the changes in some indices (EF, % Δ D, V_{CF}) were not shown to be significant. This was also true when, as is common practice, the 3 control measurements were used only to calculate mean control results. In both cases the other indices were shown to be significant but at a lower level of significance. However, by employing an analysis of variance which took into account the multiple measurements, individual variability could be determined, allowing a more sensitive statistical assessment (Table 4).

When the effects of glyceryl trinitrate were assessed in this study all the derived echocardiographic haemodynamic indices were estimated. The accuracy of these derived indices is limited by the assumptions involved in using the cube formula and may have variable validity in different subjects. They appear to be reasonably sound when applied to normal sized hearts (Fortuin *et al.*, 1971) but can produce substantial errors in subjects with cardiac disease (Popp *et al.*, 1973) especially where dyskinesia is present (Teichholz *et al.*, 1976). However, alternative methods of estimation such as using regression equations (Fortuin *et al.*, 1971) or correction formulae (Teichholz *et al.*, 1976) have not been shown to produce greater accuracy on general application (Cooper *et al.*, 1972; Martin, 1978). In consequence some workers have suggested avoiding derivations (Fortuin and Pawsey, 1977). However, if the cube formula is applied to subjects without dyskinesia before and after drug interventions then it is likely that whatever errors are present will remain reasonably constant throughout, allowing valid measurement of any drug-induced changes.

The changes in estimated haemodynamics after glyceryl trinitrate in this study group are compatible with the effects of a reduction in preload and afterload with compensatory adjustments. These changes are similar to those seen in angiographic studies (McAnulty *et al.*, 1975) and suggest that derived echocardiographic data can give insight into the haemodynamic effects of the drug's action, as has previously been suggested (Burggraf and Parker, 1974).

Echocardiography therefore appears to be a useful method of assessing the effects of cardiovascular drugs on the heart where there is no dyskinesia and when using individuals as their own controls. If the method is standardised rigidly it appears to have good reproducibility. In studies with rapidly changing drug effects it is

recommended that a number of baseline measurements be taken every few minutes followed by single subsequent measurements. In single dose studies it would be prudent to take a number of baseline measurements on each day for comparison as well as including a placebo day, modifying the statistical analysis accordingly to take full advantage of the replication of measurements. Similarly in steady state evaluations multiple measurements will allow optimum application of this potentially useful non-invasive method of cardiovascular drug assessment.

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