A RADIONUCLIDE METHOD FOR THE SIMULTANEOUS STUDY OF THE EFFECTS OF DRUGS ON CENTRAL AND PERIPHERAL HAEMODYNAMICS

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1 The central and peripheral cardiovascular effects of hydralazine and glyceryl trinitrate (GTN) have been contrasted using radionuclide techniques.

2 Following intravenous injection of technetium-99m labelled human serum albumin, radionuclide ventriculography was performed by the equilibrium blood pool method using a mobile gamma camera. Simultaneous measurements of 'peripheral venous volume' were made using a collimated scintillation probe positioned above the patient's calf.

3 Ten patients with angina pectoris were studied at rest, after sublingual administration of 0.5 mg GTN and after intravenous administration of 10 mg hydralazine.

4 GTN caused a mean reduction in the end diastolic volume of the left ventricle of $14.6\% \pm 4.5\%$ ($P < 0.005$) but ejection fraction increased by 0.034 ± 0.007 ($P < 0.005$) so that stroke volume was only reduced by $4.9\% \pm 5.0\%$ (NS). There was a mean increase in heart rate of 10.8 ± 2.3 beats/min ($P < 0.001$) but no significant change in cardiac output. The calculated systemic vascular resistance fell by $10.0\% \pm 5.4\%$ ($P < 0.05$). Associated with these changes there was a mean increase of $9.6\% \pm 1.5\%$ ($P < 0.05$) in the counts from the calf.

5 Hydralazine caused a significant reduction in blood pressure and increase in heart rate. End-diastolic volume was reduced by $6.0\% \pm 2.7\%$ but there was a mean increase in ejection fraction of 0.058 ± 0.010 ($P < 0.001$) so that in this instance stroke volume increased by $9.0\% \pm 3.7\%$ ($P < 0.05$) and cardiac output increased by $16.4\% \pm 4.4\%$ ($P < 0.005$). The calculated systemic vascular resistance fell by $18.9\% \pm 3.8\%$ ($P < 0.001$). Despite these haemodynamic changes there was no significant change in counts from the calf.

6 The results confirm that GTN has a predominant venodilator effect while hydralazine acts largely on the arterial bed. These relatively simple radionuclide methods will allow a more detailed assessment of the cardiovascular effects of drugs.

Keywords hydralazine glyceryl trinitrate radionuclide assessment haemodynamics

Introduction

Many drugs produce cardiovascular effects by altering the peripheral circulation. Changes in arterial tone can be assessed by relating changes in pressure to changes in flow to give a measure of systemic vascular resistance, but changes in venous tone are more difficult to assess. Strain gauge plethysmography (Whitney, 1953) has been the traditional method but this technique can be inaccurate and is cumbersome and difficult to perform (Clements *et al.*, 1981). Radionuclides are being used increasingly to examine drug effects on the heart (Muir *et al.*, 1981) and two recent reports suggest that changes in peripheral or 'venous' vascular volume may also be determined by a modified equilibrium blood pool method using a

gamma camera (Clements *et al.*, 1981; Rutlen *et al.*, 1981). However this technique precludes the simultaneous study of central haemodynamics unless two gamma cameras are available and can be suitably positioned in close proximity to each other. We have used a collimated scintillation probe with a simple nucleonics system as the peripheral counting device to assess 'venous volume', while simultaneously measuring changes in ejection fraction, ventricular volume and cardiac output by a gamma camera. To test the sensitivity of this method to changes in 'arteriolar' and 'venous' tone we studied the effects of the administration of hydralazine and glyceryl trinitrate (GTN) respectively.

Methods

Patients

Ten patients with angina pectoris who were undergoing routine investigation by radionuclide ventriculography were studied. All gave informed consent to the study which had the approval of our hospital's Ethical Committee. None was on therapy with adrenoceptor antagonists or calcium channel blockers. No patient received glyceryl trinitrate (GTN) or other nitrate preparations for a minimum of 6 h prior to the study. The patients were divided randomly into two equal groups and after control measurements the first group received GTN and then after a restabilisation period of approximately 20 min were given hydralazine. The second group received the hydralazine and then, after a similar restabilisation period of 20 min, GTN.

Drugs

GTN (Evans Medical) was given sublingually, crushed, in a dose of 0.5 mg and the hydralazine (Ciba Laboratories) as 10 mg slow intravenous injection. The second drug was administered only after blood pressure, heart rate, and calf counts (*vide infra*) had returned to steady base line values.

Measurements

Patients were studied while resting supine in a room at 22°C and measurements commenced after an initial 15 min period of rest. The heart rate was monitored from a continuous ECG recording and blood pressure measured by an independent observer using conventional sphygmomanometry (diastolic Phase V). Mean blood pressure was calculated as diastolic blood pressure plus one third of the pulse pressure.

Studies were performed by the ECG-gated equilibrium blood pool method after the intravenous injection of 750 MBq technetium-99m electrolytically labelled human serum albumin (Millar *et al.*, 1979). Imaging was performed in the 30° left anterior oblique projection with a 10° caudal tilt using a Searle mobile gamma camera interfaced to a Cromemco (System 3) microcomputer. Images were acquired in 20–40 ms intervals throughout the cardiac cycle and were updated over 500 heart beats. At the end of each study the images were displayed as a movie sequence on a television monitor and the left ventricular region identified by an independent observer. The volume-time curves from individual pixels around the border of this region were displayed and the original region altered, where necessary, to ensure that the entire left ventricle was included and the left atrium excluded from the final region of interest. From this final region a ventricular volume curve was obtained and

left ventricular ejection fraction (EF) calculated from: $EF = (EDC - ESC)/EDC$ where EDC and ESC represent the background corrected counts from the left ventricle at end-diastole and end-systole respectively. Details of the data acquisition and analysis methods have been published (Muir *et al.*, 1980).

End-diastolic counts were corrected for physical decay and biological clearance by sampling peripheral blood and the percentage changes in end-diastolic volumes measured (Hannan *et al.*, 1980). In a recent study to assess the reproducibility of these methods 20 patients referred for routine radionuclide ventriculography were studied on two occasions 1 h apart. The mean difference in ejection fraction between the two studies was only 0.017 ± 0.012 (s.d.) and the mean difference in end diastolic volume was $2.7\% \pm 2.2\%$ (s.d.).

As stroke volume is the product of the end-diastolic volume and ejection fraction, relative values for stroke volume, and hence also cardiac output, could be determined. 'Systemic vascular resistance' was expressed as:

$$\frac{\text{mean arterial pressure}}{\text{relative cardiac output}}$$

Changes in peripheral vascular volume were measured with a simple scintillation detector. This consisted of a 4 cm diameter by 2 cm thick sodium iodide crystal coupled to a 5 cm diameter photomultiplier and positioned within a 6 cm internal diameter brass collimator. The detector was connected to a portable nucleonics system (Alrad Instruments Ltd, Model MS310E) and printer (Datac Ltd, Model 412 L). Since a relatively high activity of technetium-99m was required for the simultaneous gamma camera measurements the sensitivity of the scintillation probe has to be reduced to avoid pulse pile-up effects. This was achieved by positioning the probe 15 cm above the patient's calf. Positioning the probe in this manner gave a uniform response across the patient's calf and reduced any changes in detector response caused by patient movement. In addition the patient's ankle was held steady using bean bags. Radioactivity from the patient's other leg was shielded using a lead screen. In three additional patients the changes in the count rate detected by the scintillation probe were compared with those from the opposite leg when measured by the gamma camera.

Study periods

Heart rate and peripheral or calf blood pool counts were measured continuously. Blood pressure was recorded every 2 min. Ventriculograms were constructed in each control period and over the period of the peak effect of the drugs (approximately 3 min

after giving the GTN and 10 min after hydralazine). Peak effects were assumed from maximum changes in heart rate and blood pressure. The collection period for each ventriculogram was approximately 6 min.

Statistical methods

No difference was observed whether the hydralazine or GTN was administered first and therefore both group data were pooled. The results for the 10 values in each treatment period are expressed as the mean change $\pm$ s.e. mean. The changes from baseline values were analysed using Student's *t*-test for paired comparison. The group data are shown in Table 1.

Results

Preliminary observations

When no pharmacological agent was administered the decay-corrected counts from the calf varied by less than $\pm 2\%$ once initial equilibrium had been secured. There must have been biological clearance and correction for this using venous sampling suggested that there was indeed an increase of 8% per hour. This may represent the technetium leakage into the calf with time.

In three patients where the peripheral counts were measured by both the single probe and the gamma camera the relative changes in count rate were identical.

Pharmacological studies

In each case the administration of GTN caused similar changes (Figure 1). The mean changes for the group are summarised in Figures 2 and 3. There was a rise in heart rate of 10.8 ± 2.3 beats/min ($P < 0.001$) after GTN. The changes in blood pressure were small with the systolic blood pressure decreasing by a mean of 5.5 ± 2.5 mm Hg ($P < 0.05$) with no significant change in diastolic pressure. Ejection fraction increased by a mean of 0.034 ± 0.007 ($P < 0.001$) after GTN and end-diastolic volume fell by $14.6\% \pm 4.5\%$ ($P < 0.005$). Although there was a significant increase

in heart rate there was a mean reduction in stroke volume of $4.9\% \pm 5.0\%$ (NS) so that cardiac output was only increased by $7.8\% \pm 5.3\%$ (NS). The calculated systemic vascular resistance fell by $10.0\% \pm 5.4\%$ ($P < 0.05$). Associated with these relatively small changes there was a mean increase of $9.6\% \pm 1.5\%$ ($P < 0.005$) in the counts from the calf.

Hydralazine increased heart rate by 5.2 ± 1.3 beats/min ($P < 0.005$) but produced greater falls in blood pressure, the systolic falling by a mean of 12.5 ± 1.5 mm Hg ($P < 0.001$) and the diastolic by a mean of 8.0 ± 1.3 mm Hg ($P < 0.01$). There was a mean reduction in end-diastolic volume of $6.0\% \pm 2.7\%$ ($P < 0.05$) but ejection fraction increased by 0.058 ± 0.010 ($P < 0.001$) so that in this instance stroke volume was increased by $9.0\% \pm 3.7\%$ ($P < 0.05$) and cardiac output increased by $16.4\% \pm 4.4\%$ ($P < 0.005$). The calculated systemic vascular resistance fell by $18.9\% \pm 3.8\%$ ($P < 0.001$). Despite these greater haemodynamic changes the calf count rates were little changed ($-2.0\% \pm 2.6\%$, NS).

Discussion

Approximately 70% of the blood volume is contained in the venous side of the circulation (Shepherd & Vanhoutte, 1979) and there is no reason to suspect that this situation does not pertain to the calf. Thus the measurement of radioactivity from this region using a blood pool marker must to a large extent be a measurement of venous capacitance. Rutlen and colleagues (1981) contrasted changes in forearm intra-vascular volume as determined by equilibrium blood pool imaging using a gamma camera with standard plethysmographic technique and showed changes in volume produced either by pressure occlusion or by the administration of nitroglycerin correlated closely by both techniques. Indeed they showed that the administration of 0.4 mg GTN produced similar changes to the inflation of the occluding cuff to a pressure of 15 mm Hg, suggesting that they were indeed measuring changes in venous capacitance produced by the nitrate.

To be certain that this method was not influenced by changes in arteriolar tone we chose to contrast the

Table 1 Mean group data $\pm$ s.e. mean. The significance levels for paired comparison are given in the text

	Control	GTN	Control	Hydralazine
Heart rate (beats/min)	74.9 $\pm$ 6.4	85.2 $\pm$ 5.3	71.6 $\pm$ 5.5	77.1 $\pm$ 6.3
Diastolic blood pressure (mm Hg)	76 $\pm$ 4	77 $\pm$ 3	80 $\pm$ 3	72 $\pm$ 3
Systolic blood pressure (mm Hg)	133 $\pm$ 8	128 $\pm$ 7	138 $\pm$ 7	126 $\pm$ 8
Ejection fraction	0.470 $\pm$ 0.046	0.503 $\pm$ 0.048	0.447 $\pm$ 0.041	0.504 $\pm$ 0.040
Relative systemic vascular resistance	100.0	88.9 $\pm$ 5.1	100.0	81.1 $\pm$ 3.6
Relative calf counts	100.0	109.6 $\pm$ 1.5	100.0	98.0 $\pm$ 2.6

Figure 1 Counts detected by the scintillation probe positioned above a patient's calf. The counts detected in one minute intervals are shown for the control period, after intravenous administration of 10 mg hydralazine (H) and after oral administration of 0.5 mg of glyceryl trinitrate (G). Each data point has been corrected for physical decay.

findings with GTN with those produced by hydralazine. Although the mechanism and mode of action of hydralazine is unclear it seems to suppress contraction in the artery. A variety of mechanisms, including chelation of certain ion elements required for excitation contraction coupling (Koch-Weser, 1976) or the inhibition of conversion of dopamine to noradrenaline have been suggested (Songkittiguna *et al.*, 1980). Although it is regarded as having its main site of action on the arterioles data concerning its effect on veins is sparse. Worcel (1978) has demonstrated failure of hydralazine, even at high concentrations, to significantly inhibit portal vein tone. Hydralazine's effect on resistance and capacitance of the forearm vessels was contrasted with sodium nitrate by Ablad and co-workers (1963). Using intra arterial injections and assessing changes with plethysmography hydralazine caused a greater increase in forearm blood flow than forearm volume while the reverse applied for sodium nitrate. In clinical studies left ventricular end diastolic pressures show a modest fall after hydralazine but this could result from a reduction in afterload allowing more complete left ventricular emptying. Massie and colleagues (1977) noted that arterial pressure did not fall after hydralazine in patients with heart failure. Thus it is likely that hydralazine does not have any marked veno-dilator effect and our own results showing systemic vascular resistance was greatly reduced by hydralazine while there was no significant change in calf counts are in keeping with this view. GTN in contrast produced smaller changes in vascular resistance but a significant increase in counts from the calf and decrease in end-diastolic

volume suggesting its main effect is on the venous capacitance bed. Thus the method allows distinction between changes in 'arteriolar' and 'venous' tone and should allow a greater understanding of drugs acting on the cardiovascular system. The changes in central haemodynamics produced by both drugs emphasise the role of the peripheral vascular bed in regulating cardiac performance. This is of particular importance as recent therapy in heart failure has been directed to manipulating the vascular beds to secondarily enhance the heart function (Miller *et al.*, 1982).

To detect changes in venous capacitance alone this method only requires an inexpensive scintillation probe and the total dose of radionuclide can be very low indeed (10 MBq technetium-99m). However, if the cardiac effects are to be studied simultaneously the standard dose of technetium-99m will be required. It is essential to provide a blood pool label that stays within the vascular compartment. We have shown previously that electrolytically labelled albumin still remains within the vascular compartment at 1 h (Millar *et al.*, 1979) and the small amount of leakage we could identify is compatible with this. Other workers (Clements *et al.*, 1981; Rutlen *et al.*, 1981) have used an *in vivo* red cell labelling technique and although this provides a good blood pool label the initial uptake of technetium-99m by the pretreated red cells can be variable. Recently Callahan *et al.* (1982) have described a modified *in vivo* red blood cell labelled technique which would have major advantages. The method might be extended further to measure absolute peripheral intravascular volume (Rutlen *et al.*, 1981) or to determine the relative

Figure 2 Mean values $\pm$ s.e. mean for the absolute changes in heart rate, systolic blood pressure (SBP) and diastolic blood pressure (DBP) and the percentage changes in venous calf counts for glyceryl trinitrate (●) and hydralazine (■) with respect to control values. Significance levels were obtained from Student's *t*-test for paired comparison. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ and **** $P < 0.001$.

or absolute compliance of the vascular bed both in health and disease. In conclusion, peripheral counting by a scintillation probe of an equilibrium blood pool label is a sensitive means of detecting changes in vascular compliance and is not altered by changes

Figure 3 Mean values $\pm$ s.e. mean for the absolute changes in left ventricular ejection fraction (LVEF) and the percentage changes in end diastolic volume (EDV), cardiac output (CO) and systemic vascular resistance (SVR) for glyceryl trinitrate (●) and hydralazine (■) with respect to control values. Significance levels were obtained from Student's *t*-test for paired comparison. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ and **** $P < 0.001$.

in vascular resistance. Simultaneous measurements of central and peripheral haemodynamics may be carried out in any department having conventional nuclear medicine facilities and provides a sensitive means of assessing the cardiovascular effects of drugs.

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