Blood Flow Measurements by the Reference Sample Method with Microsphere Injection into the Aorta: An Accurate and Easy Approach (43444)

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Abstract. **The validity of hemodynamic measurements by the reference sample method with microspheres injection into the aorta, via a carotid artery catheter, was evaluated in rats and compared with the results obtained after left ventricle injection. In the aorta injection group, a good mix of microspheres was observed in 83% of the animals. Moreover, a symmetrical distribution of microspheres was observed in 10 out of 12 rats (83%). An excellent correlation between right and left kidney-testes blood flows was observed (r = 0.93 and 0.96, respectively; $P < 0.01$). Mean arterial pressure was not modified during microspheres injection into the aorta. Cardiac output (104 ± 26 vs 101 ± 23 ml/min, NS) and portal blood flow (14.2 ± 3.3 vs 13.5 ± 2.2 ml/min, NS) were similar after aorta and left ventricle injections series, respectively. Our results indicate that the injection of microspheres into the aorta is an adequate and easy approach to systemic and splanchnic hemodynamic measurements. This approach could be a good alternative to left ventricle injection of microspheres in experimental studies in rats.**

[P.S.E.B.M. 1992, Vol 200]

Simultaneous measurement of cardiac output and regional blood flow distribution can be made using the reference sample method with tracer microspheres (1, 2). The accuracy of this method depends mainly on the complete mixing of microspheres with the blood before they reach the target organ (2). Therefore, this requirement influences the choice of the microsphere injection site. Until now, all of the studies in portal hypertensive models have been performed by injection into the left ventricle (3–5). However, this is a difficult technique and may be associated with some methodological problems (left ventricle perforation or outflow obstruction). The aim of the present study was to evaluate blood flow measurements with microsphere injections into the aorta, comparing the results with those obtained after left ventricle injection.

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Received March 18, 1991. [P.S.E.B.M. 1992, Vol 200]
Accepted January 14, 1992.

0037-9727/92/2003-0375$3.00/0

Materials and Methods

Male Wistar rats were used in the study. The animals were housed in individual cages and had free access to normal rat chow and water until the end of the study.

Animals were anesthetized with ketamine (100 mg/kg body wt, im). The aorta and left femoral artery were catheterized with a PE-50 catheter to allow blood sampling and monitoring of arterial pressure. Radioactive microspheres (New England Nuclear) 15 μm in diameter labeled with ^{85}Sr and suspended in saline with one drop of Tween 80 were injected over a 15-sec period into the junction of the carotid artery with the aorta (n = 8) or the left ventricle (n = 8) (total vol, 0.4 ml). The injection catheters were flushed with 0.2 ml of normal saline. Entry into the aorta and left ventricle was evaluated under continuous recording of the pressure tracing. The exact position of the catheter was confirmed in the autopsy. Pressure measurements were performed using high sensitivity pressure transducers (P-23 Gould Staham); permanent tracings were obtained with a multichannel recorder (Dyne MCD). The microspheres were sonicated for 15 min in an ultrasonic bath and vigorously shaken in a vortex prior to injection. Ap-

proximately 60,000 microspheres were injected into either the aorta or the left ventricle. Starting 10 sec before the injection and continuing for 50 sec thereafter, arterial blood was drawn from the femoral artery at a constant rate (1 ml/min) using a high precision pump (Apema PC 10). This was used as reference sample for the calculation of regional blood flows and cardiac output (2).

After completion of the study, the animals were sacrificed with an intravenous injection of saturated KCl. The lungs, liver, spleen, small and large bowels, stomach, pancreas, mesentery, kidney, and testes were dissected, cleaned, weighed, and put into tubes. The larger organs were cut in pieces and placed in several vials to increase the geometric efficiency of counting. Samples were counted in a gamma counter for 1 min each and the average number of counts per minute were recorded.

Cardiac output was calculated by the following formula: injected cpm × reference sample blood flow/reference sample cpm. The arterial blood flow of each organ was calculated by the formula: organ cpm × reference sample blood flow/reference sample cpm. Portal venous inflow was the sum of arterial blood flow of the stomach, intestine, spleen, pancreas, and mesentery. In this study, the two latter tissues, which have a comparatively very low blood flow, were included with intestinal blood flow. The following requirements were obtained in each animal before data analysis: (i) >300 microspheres in the reference blood and organs; (ii) <10% difference in radioactivity in symmetrical organs (kidney and testes) in order to assess the correct mixing of the microsphere injection; (iii) the absence of large changes (±10 mm Hg) in mean arterial pressure during microsphere injection or blood sampling.

Results are reported as mean ± SD. Student's t test was used in the statistical analysis of the hemodynamic results.

Results

The body weight was similar in both groups (aorta injection 268 ± 34 g and left ventricle injection 262 ± 27 g, NS). We evaluated 24 animals. In the aorta injection group, a symmetrical distribution of microspheres was observed in 10 of the 12 animals (83%). An excellent correlation was found between right kidney-testes blood flow and left kidney-testes blood flow ($r = 0.93$ and 0.96, respectively; $P < 0.01$). In two rats, the number of microspheres obtained in the reference blood sample and organs was less than 300 (17%). Similar values of mean arterial pressure were observed before (126 ± 11 mm Hg) and after (127 ± 8 mm Hg, NS) microsphere injection into the aorta.

In the other group, left ventricle catheterization was successful in 10 out of 12 animals (83%). In two rats (17%), a high radioactivity in the lungs was observed, suggesting that right ventricular perforation had occurred; in two other animals (17%), an asymmetrical distribution of microspheres was obtained.

Tables I and II present systemic and splanchnic hemodynamic data in both groups. The mean values of arterial pressure and cardiac output were similar in both groups (Table I). Moreover, the hepatic hemodynamic measurements were not significantly different between aorta or left ventricle injection series (Table II).

Discussion

In recent years, the microsphere technique of blood flow measurement has been applied in different experimental models (3–7). The accuracy of this technique depends on several factors (2, 8). These include the number of microspheres trapped in the reference blood sample and organs, and the absence of systemic and local hemodynamic changes after microsphere injection and blood withdrawal (2, 8). Moreover, one of the main requirements for the validity of hemodynamic measurements with this method is a complete mixing of the

Table I. Systemic Hemodynamic Measurements[a]

	Aorta injection ($n = 8$)	Left ventricle injection ($n = 8$)
Mean arterial pressure (mm Hg)	126 ± 11	127 ± 8
Cardiac output (ml/min)	104 ± 27	101 ± 23
Cardiac index (ml/min/100 g body wt)	38.7 ± 8.5	38.5 ± 8.1

[a] Values are mean ± SD. Comparison between the two groups showed no significant differences.

Table II. Hepatic Hemodynamic Measurements[a]

	Aorta injection ($n = 8$)	Left ventricle injection ($n = 8$)
Portal venous inflow		
ml/min	14.2 ± 3.3	13.5 ± 2.2
ml/min/100 g body wt	5.6 ± 1.6	5.3 ± 1.3
% Cardiac output	14.6 ± 5.6	14.3 ± 5.7
Hepatic Artery Flow		
ml/min	1.6 ± 0.7	1.7 ± 0.7
ml/min/100 g body wt	0.6 ± 0.2	0.6 ± 0.3
% Cardiac output	1.6 ± 0.8	1.7 ± 0.7
Total hepatic flow		
ml/min	15.8 ± 3.0	15.1 ± 1.8
ml/min/100 g body wt	6.0 ± 1.7	5.9 ± 1.3
% Cardiac output	16.3 ± 5.8	15.9 ± 6.0

[a] Values are mean ± SD. Comparisons between the two groups showed no significant differences.

microspheres with the blood before they reach the target organ (2, 8). Therefore, the choice of microsphere injection site depends on that requirement. In this regard, recent hemodynamic studies have demonstrated that brain and coronary blood flow determinations may be inadequate with microsphere injection into the carotid artery (9, 10).

In the present study, we investigated the accuracy of blood flow measurements with microsphere injections performed into the aorta at the junction with the carotid artery. We assumed that if blood flow to splanchnic organs was to be measured, then the injection of microspheres into the aorta should be sufficient. We also compared these results with those obtained after left ventricle injection.

A poor mixing of microspheres, either in the heart or in the aorta, could result in a decrease in the number of microspheres in the reference sample. The present study shows that in 92% of the rats studied, the reference blood sample and organs contained >300 microspheres after the injection into the aorta. A symmetrical distribution of microspheres, evaluated by similar right and left renal and testes blood flow, was observed in 75% of the animals in this group. Similar results were reported by Laughlin *et al.* (11) and Sarin *et al.* (12). These authors demonstrated that the injection of microspheres into the aorta is a useful technique to evaluate hemodynamic changes in experimental animals (11, 12).

Laughlin *et al.* (11) also compared blood flow values obtained in both kidneys. However, in those studies, no systematic assessment of hemodynamic measurements between two different sites of microsphere injection was made. In this regard, in our study, similar values of systemic and splanchnic hemodynamic parameters were found between aorta and left ventricle injection series. Moreover, the radioactive microsphere technique was successful in an equal percentage in both groups (67%).

The results of the present study indicate that aorta injection is an adequate and easy approach to systemic and splanchnic hemodynamic measurements. This approach could be a good alternative to left ventricle injection of microspheres in experimental studies in rats.

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