New method of cardiac output measurement using ultrasound velocity dilution in rats

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Numerous techniques are available to measure cardiac output (CO) in humans or animals. Thermodilution, dye dilution (indocyanine green), and radiolabeled microspheres are commonly used techniques. Other methods for measuring CO include direct Fick oximetry, electromagnetic flowmetry, impedance plethysmography, and Doppler echocardiography. However, these methods have several drawbacks, and an easy, inexpensive, accurate, and minimally invasive technique for measuring CO is still needed, especially in laboratories for small animals such as the rat.

Transonic transit time ultrasound (TTU) perivascular flow probes have been developed in the last 10 years for easy and accurate measurement of volume flows. These probes have been validated and successfully used to measure blood flows in several vessels including the ascending or abdominal aorta (9, 42), carotid artery (26), renal artery (41), portal vein (8), mesenteric artery (13), and pancreatic vessels (17).

TTU technology is based on the fact that ultrasound travels faster when the propagation liquid is moving in the same direction (downstream transit time) and slower when the propagation liquid is moving in the opposite direction (upstream transit time). The difference between the upstream and downstream transit times is proportional to the blood flow in a given vessel. One advantage of TTU flowmetry for vascular blood flow measurements is that vessel diameter does not need to be measured by the operator (30). Detailed descriptions of TTU have been provided elsewhere (3, 5, 23, 31).

CO by ultrasound-dilution (COUD) is a new method using classic dilution principles (10a, 34) and Transonic ultrasound technology in which CO is calculated from an ultrasonic speed dilution curve generated on an intravenous injection of saline. CO by COUD has been assessed in patients with an extracorporeal device (12, 28), but it has never been applied to small animals.

Portal hypertension is a disease in which CO is increased as a result of systemic vasodilatation (hypokinetic syndrome). Therapy for portal hypertension may include systemic vasoconstrictors such as vasopressin analogs. The effects of these drugs on CO have so far been evaluated in portal hypertensive rats by using the radiolabeled microsphere method. However, serial measurements in the same animal were limited because each CO measurement required injection of
microspheres that potentially induced hemodynamic instability in the animal. By contrast, CO measurement by the COUD method requires only injection of physiological saline and may be an interesting alternative to the microsphere method in pharmacological studies.

The primary aim of the present work was to describe the measurement of COUD in rats. The secondary aims were to evaluate: 1) the accuracy of COUD compared with the radiolabeled microsphere method, and 2) the ability of COUD to detect pharmacological or pathological alterations in CO, and 3) the reproducibility of COUD between observers and over time.

MATERIALS AND METHODS

Animals

The accuracy of COUD was evaluated by comparisons with the microsphere method (group 1) and with direct aortic TTU flowmetry (group 2). COUD measurements were performed in both groups under two conditions: 1) at baseline and 2) after pharmacological manipulation. The pharmacological variations in CO were induced by a bolus of terlipressin because this vasopressin analog is a vasoactive drug known to induce a decrease in CO (25, 27). In addition, we evaluated in a third group of rats the ability of COUD to detect CO increase due to different dosages of losartan, which is a vasodilatory drug (15). The intra- and interobserver agreement studies of COUD were performed in a fourth group of rats in which two consecutive CO measurements were determined. The reproducibility as a function of time of COUD (also called repeatability) was evaluated in a fifth group of rats.

The COUD method was assessed in normal and portal hypertensive rats (Sprague-Dawley male rats, Etablissements Depré, Saint Doulchard, France). Portal hypertension was induced either by bile duct ligation as previously described (24) or with a 1% N-nitrosodimethylamine saline solution (DMNA; Sigma Aldrich, Saint-Quentin Fallavier, France). DMNA was administered intraperitoneally at a dose of 0.1 ml/100 g body wt (i.e., 10 mg DMNA/kg rat) for 3 consecutive days per week during 4 wk. Sham rats were similarly treated for 4 wk except that saline was injected instead of DMNA. All rats weighed between 270 and 400 g at the time of hemodynamic measurements, which were performed in rats anesthetized with pentobarbital sodium (Nesdonal; 5 mg in 0.1 ml/100 g body wt). Protocols were approved by the French Agriculture Office in conformity with European legislation on research involving animals. All rats had free access to food and water until 14–16 h before the study. During hemodynamic measurements, body temperature was maintained at 37°C with a homeothermic blanket system (Harvard Apparatus, Edenbridge, Kent, UK).

General Measurements

Mean arterial pressure (MAP) and heart rate (HR) were measured via an indwelling femoral arterial polyethylene catheter (PE10, 0.28 mm id, Clay Adams). The abdomen was opened by abdominal midline incision, and a second catheter was inserted into a small ileal vein and gently advanced to the bifurcation of the superior mesenteric and splenic veins to measure portal vein pressure (PP). PP, mean arterial pressure (MAP), and heart rate (HR) were monitored by using a multichannel recorder (Nihon Kohden, Tokyo, Japan).

COUD Technique

Principle and devices. Ultrasound velocity (1,560–1,590 m/s) in the blood is known to be mainly dependent on blood protein (32) and ion concentrations (10) and, to a lesser extent, on temperature. Ultrasound velocity in saline at 37°C is lower than in the blood: 1,533 m/s. Thus the injection of a saline bolus (0.2 ml) in the rat bloodstream will generate an ultrasound velocity dilution curve due to the transient dilution of global protein levels in the bloodstream (21, 22).

The ultrasound velocity dilution curve was recorded on a personal computer (PC) screen using the following modifications on the original flowmeter. First, an ultrasound velocity signal was derived from a T206 flowmeter main electronic board (Transonic Systems, Ithaca, NY) using a connection that was drawn between the board and a data acquisition system (model MP 100A, Biopac Systems, Santa Barbara, CA). The Biopac system was then connected to a PC for analysis and for display of the ultrasound velocity curves over time using the Acqknowledge 3.01 software (Biopac Systems). When a TTU probe (Transonic Systems) (Fig. 1) was placed around a vessel, the intravascular ultrasound velocity as well as the average and instantaneous blood flows of the given vessel were then reviewed on the PC screen on-line using this software (Fig. 2).

CO determination by COUD. To measure CO by COUD, we used the following protocol in three steps. First, a 1-mm TTU probe from the R series with a back cable exit (1RB) was placed around a carotid artery, using an ultrasound transmission gel for acoustic coupling (HR lubricating jelly, Carter Products, New York, NY). R series probes are designed for acute measurements in relatively large vessels in the rat (0.7- to 1.8-mm diameter). The device described above (Fig. 2) provided a display on the PC screen of three curves corresponding to the carotid ultrasound velocity and the average and instantaneous carotid blood flows. When stable ultrasound baselines had been registered for the three curves for at least 5 min, 0.2 ml of 0.9% saline at body temperature was injected into the rat venous bloodstream (through a femoral vein catheter). The saline injection lasted ~1–2 s (CO values remained unchanged whatever the injection speed). The sa-

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The saline bolus underwent mixing with the blood in the heart, and the diluted blood was then delivered to the carotid artery. The Transonic TTU system recorded and displayed on the PC screen a transient decrease in ultrasound velocity (sound velocity dilution curve) from the probe placed around the carotid artery after the saline bolus injection (Fig. 3A). The decrease in ultrasound velocity was followed by a slow return to baseline. This transient variation of ultrasound velocity described an area under the curve (AUC).

In the second step of COUD, we calculated the AUC off-line. As with any dilution curve, the return to baseline after saline bolus did not always occur rapidly (Fig. 3A). This delay can be attributed to a recirculation of the saline bolus. To make sure the AUC was accurate, we first smoothed the dilution curve (Fig. 3B) with Acqknowledge software. We then used Mathlab software (Mathlab 4.2c, MathWorks, Natick, MA) to extrapolate the end of the dilution curve (Fig. 3C), thus preventing an AUC underestimation bias (14).

In the third step, we calculated CO using the value of the AUC previously evaluated. It has previously been shown (12, 21, 22, 28) that the dilution curve has an AUC inversely proportional to CO and directly proportional to the volume of the injectate (like any classic dye dilution curve) according to the following formula

$$CO = \frac{V}{(K \times AUC)}$$

where $V$ is volume of saline injected into the vein (0.2 ml), AUC is the area under the sound velocity dilution curve (V·min), and $K$ is the calibration factor (V$^{-1}$).

**Determination of $K$.** It is necessary to determine $K$, the calibration factor which is, by construction, a characteristic of a given probe. In theory, $K$ is a constant that needs to be calculated only once (i.e., in only one rat). In practice, $K$ has to be determined regularly, approximately once a month, because the characteristics of the probe can change over time as a result of protein deposition and probe epoxy aging.

$K$ represents the relationship between changes in sound velocity and the number of concentration units (ml [saline]·ml [blood]$^{-1}$·V$^{-1}$, with square brackets indicating concentration). The speed of sound in saline and blood do not need to be measured by the operator. $K$ can be considered as a factor that represents the way a probe can sense the difference between saline and blood (and diluted blood), and $K$ is related to the area under the sound velocity curve ($AUC_b$) generated by the injection of a bolus of saline upstream from the site of the probe. The $K$ value was determined, using a protocol similar to COUD, in three steps similar to the steps described for COUD.

First, a catheter was placed in an ileal vein. The same 1RB probe used in COUD was placed around the superior mesenteric vein, 1–2 cm above the tip of the ileal catheter (ileal veins are drained in the superior mesenteric vein). Recording with the PC of both superior mesenteric vein flow and ultrasound velocity was then begun. When stable baseline was achieved, 0.05 ml of 0.9% saline at body temperature was injected through a femoral catheter generating a dilution curve which is detected by the TTU T206 Transonic flowmeter. A computer running a Biopac system was used for display and analysis of the dilution curve. AUC, area under the curve; $k$, calibration factor.

![Fig. 2. Cardiac output (CO) by ultrasound dilution (COUD) method. Apparatus and protocol used in COUD are shown. A saline bolus is injected through a femoral catheter generating a dilution curve which is detected by the TTU T206 Transonic flowmeter. A computer running a Biopac system was used for display and analysis of the dilution curve.](http://jap.physiology.org/)

![Fig. 3. Carotid artery blood flow and ultrasound velocity dilution curves. A: carotid artery blood flows (top curves: average and instantaneous blood flows, respectively) and an ultrasound velocity dilution curve that was generated after a bolus of 0.2 ml of physiological saline (lower curve). To have an accurate area under the curve, the ultrasound velocity dilution curve has to be smoothed (B). Then the curve was extrapolated to have a return to the baseline (C).](http://jap.physiology.org/)
injected in ~2 s through the ileal catheter, resulting in transient dilution of blood protein and ions and then a change in the velocity signal as registered by the superior mesenteric vein probe. As this was previously observed in COUD, a dilution curve was generated.

In the second step, the AUC was measured as described previously in COUD.

In the third step, \( K \) was calculated using the following formula

\[
K = \frac{V_s}{Q \times AUC_s}
\]

where \( Q \) is the blood flow detected by the TTU probe in a given vessel (ml/min), e.g., the superior mesenteric vein; \( V_s \) is the volume of isotonic saline injection (0.05 ml); and \( AUC_s \) (V·min) was measured as described above. The above-mentioned formulas 1 and 2 are derived from the general Stewart-Hamilton (10a, 34) formula

\[
Q = \frac{V}{(K \times AUC)}
\]

Choice of the superior mesenteric vein for the determination of \( K \). In theory, determination of \( K \) can be achieved with any vessel as long as its diameter fits to the probe used for CO determination (i.e., 1RB probe). In practice, one should remember that when a catheter is inserted in a small vessel, it obstructs the vessel and blood flow is therefore stopped. This does not happen when the catheter is inserted in an ileal vein while the probe is placed on the superior mesenteric vein because this latter drains several ileal veins. The superior mesenteric vein was also chosen for the determination of \( K \) because it was long enough to let a minimal distance between the tip of the catheter and the body of the TTU probe. If a minimal distance (~1–2 cm) was not observed, the bolus of saline injected upstream from the catheter would disturb dramatically the blood flow and would saturate the signals measured by the TTU probe.

Radiolabeled Microsphere Method

For CO measurements via the microsphere technique, a 0.7-mm-diameter polyethylene catheter with a Silastic medical-grade tube tip (0.012 in. ID, 0.025 in. OD, Dow Corning Medical Products, Midland, MI) was inserted into the left cardiac ventricle via the right carotid artery. This catheter was used for microsphere injections. CO measurement was performed as previously described (19). Briefly, 0.1 ml of a suspension of radiolabeled microspheres (~60,000 microspheres, 15.5 ± 0.1 μm in diameter, mean specific activity 10 mCi/g, range 2–47 mCi/g, New England Nuclear, Boston, MA) was suspended in Ficoll 70 (10%, Pharmacia Fine Chemicals, Uppsala, Sweden) and Tween 80 (0.01%). After sonication, the microsphere solution was injected and flushed with 0.9 ml of saline for 30 s into the left ventricle. A reference blood sample was withdrawn with a femoral artery indwelling catheter by using a motor-driven syringe (Harvard Apparatus pump 22) at a rate of 1 ml/min for 1 min during microsphere injection. CO (ml/min) was calculated as: radioactivity injected (cpm)/reference blood sample radioactivity (cpm). This was expressed per 100 g body wt [cardiac index (CI)]. Systemic vascular resistance (SVR) was calculated by the following formula: SVR [(dyn·s·cm⁻²·100 g)] × 10³ = MAP (mmHg) × 80/CI (ml·min⁻¹·100 g⁻¹).

To assess terlipressin-induced CO variations in those rats that were so treated, ¹⁴¹Ce-labeled microspheres were used for the first CO measurements (i.e., baseline CO just before terlipressin bolus) and ⁵¹Cr-labeled microspheres were used for the second CO measurements. Errors in measuring the radioactivity induced by spillover of ⁵¹Cr into the ¹⁴¹Ce channel were corrected by use of ⁵¹Cr and ¹⁴¹Ce standards.

Direct Aortic Flowmetry

The surgical approach for ascending aorta blood flow measurement was performed according to Wen et al. (42) and to the manufacturers’ recommendations. Briefly, anesthetized rats were placed in dorsal recumbency on a heating pad and mechanically ventilated by a small animal ventilator (Harvard rodent ventilator) through a tracheal cannula. A median sternotomy was performed. After the pericardium was opened, the ascending aorta was isolated by blunt dissection. A 3-mm TTU probe (3 SB probe, Transonic Systems) was then positioned around the ascending aorta, and an ultrasound transmission gel (HR lubricating jelly, Carter Products) was used to replace all air space through the probe’s acoustic window adjacent to the aorta. After at least 15 min stabilization, aortic blood flow was recorded.

Protocol Design

This design is summarized in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Model</th>
<th>n</th>
<th>Methods</th>
<th>Main aims</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sham</td>
<td>14</td>
<td>COUD</td>
<td>1) Comparison of both methods</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>and</td>
<td>2) Comparison of pharmacological decreases due to terlipressin</td>
</tr>
<tr>
<td></td>
<td>DMNA</td>
<td>32</td>
<td>microsphere</td>
<td>((n = 9))</td>
</tr>
<tr>
<td>2</td>
<td>Sham</td>
<td>8</td>
<td>COUD</td>
<td>1) Comparison of both methods</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>and aortic TTU</td>
<td>2) Comparison of pharmacological decreases due to terlipressin</td>
</tr>
<tr>
<td>3</td>
<td>BDL</td>
<td>35</td>
<td>COUD</td>
<td>Detection of dose-related increases due to losartan</td>
</tr>
<tr>
<td>4</td>
<td>Sham</td>
<td>13</td>
<td>COUD</td>
<td>Reproducibility (observer agreement)</td>
</tr>
<tr>
<td>5</td>
<td>DMNA</td>
<td>12</td>
<td>COUD</td>
<td>Repeatability (over time)</td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>20</td>
<td>COUD</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Summary of protocol design

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induced CO variations. Terlipressin-induced CO variations were studied in nine DMNA rats from group 1 and in all rats from group 2 (n = 8). In these rats, after the baseline CO measurements by both techniques (either COUD and microsphere method or COUD and direct aortic flowmetry), terlipressin (bolus of 50 μg/kg body wt) was injected through the femoral vein indwelling catheter. Ten minutes later, both CO techniques were performed in the same rats. Acute changes in CO after terlipressin were compared with baseline values for each method (paired comparisons), and these variations (Δ) between the two methods (paired comparisons) were compared.

Group 3: ability of COUD to detect changes in CO due to different dosages of losartan. Group 3 rats with bile duct ligation were prepared with one catheter in the femoral artery (for MAP measurements) and one in the femoral vein. Then a TTU 1RB probe was placed around the carotid artery (for COUD).

These rats had been allocated into three groups: 16, 11, and 8 rats were treated with saline and 10 and 5 mg·kg−1·day−1 of losartan, respectively, for 28 days. Treatment (losartan and placebo) was orally given by gavage 6 days/wk. Rats were weighed twice a week for dosage adjustment. For the same weight, whatever the model, the volume administered was the same. Hemodynamic measurements were performed at day 28. The chronic effects were compared between the three groups.

Group 4: Reproducibility of COUD between observers. Group 4 rats were prepared with three catheters: one in the femoral artery (for MAP measurements), another in the femoral vein (for the COUD technique), and the last in the portal vein (for PP measurement). The carotid artery was then isolated, and a TTU 1RB probe was placed around this vessel (for COUD).

An evaluation of the reproducibility of COUD was based on intraobserver and interobserver agreement. In the intraobserver study, CO was measured twice at a 1-min interval by the same investigator who was blinded to the previous results. In the interobserver study, CO was measured at a 1-min interval in random order by two investigators who were blinded to the results of the other investigator. For each measurement by the same or the second investigator, the probe was detached from the carotid, then replaced, and a CO determination was performed as previously described, starting with an injection of a saline bolus (0.2 ml). Then, the dilution curve was analyzed off-line for AUC measurement (smoothing of the curve and extrapolation of the end of the dilution curve with Matlab software). The operator calculated CO from the AUC value he had found using the formula 

\[ CO = V(K \times AUC) \]

with the K value determined monthly by one of the investigators.

Group 5: Repeatability of COUD measurements. Group 5 rats were prepared with two catheters: one in the femoral artery (for MAP measurements) and one in the femoral vein (for COUD). The carotid artery was then isolated, and a TTU 1RB probe was placed around this vessel (for COUD). Because the total blood volume of the rat may be around 20 ml, volume of saline injected each time (0.2 ml) is ~1% of total volume. To evaluate the limit of the repeatability of COUD, which may be affected by several injections of saline, CO measurements were performed either every 2 min (group 5A, n = 11 rats) or every 10 min, for 20 min (group 5B, considered as the control group, n = 9 rats).

Statistics

Quantitative variables were expressed as means ± SD and compared with a t-test (paired or unpaired) unless otherwise specified. The correlation between two variables was evaluated according to the Pearson’s coefficient. To test the influence of potentially confusing factors (e.g., the influence of rat group on the correlation between the CO methods), the correlation was calculated and adjusted for a qualitative variable (e.g., rat group) using the adjusted Pearson’s coefficient (r_a), with the Snedecor (33) method, including an interaction test. A significant interaction means that the correlation is significantly different as a function of the confusing factor (e.g., the rat group) and thus precludes an adjustment. Intra- and interobserver agreement of quantitative variables were evaluated by the intraclass correlation coefficient (r_i), which measures in a single test similarity in addition to conventional correlation (2).

RESULTS

Ability of the COUD Method to Detect Pathological CI Changes (Group 1)

Histological examination showed extensive liver fibrosis in the centrolobular area in DMNA rats. Portal hypertension was shown by a significant increase in PP in DMNA rats compared with sham rats (12.0 ± 2.4 vs. 7.7 ± 1.1 mmHg, P < 10^-4).

CI (which is CO calculated for 100 g of body wt) measured by COUD were significantly increased in DMNA rats (n = 32) compared with sham rats (n = 14) (34 ± 11 vs. 24 ± 12 ml·min⁻¹·100 g⁻¹, P < 0.01) (Table 2).

Comparison of COUD vs. Microsphere Method: Baseline and Acute Pharmacological-Induced CI Decrease (Group 1)

At baseline, the correlation between CO by COUD and microsphere method was good (r_i = 0.76, P < 10^-4, n = 32 DMNA + 14 sham rats) (Fig. 4).

As expected, the following significant changes were observed in the DMNA rats treated with terlipressin (n = 9, randomly chosen from group 1): MAP increased by 15 ± 13%, whereas HR decreased by 10 ± 7%, and CI decreased by 33 ± 19% (Table 3). The decreases in CO were similar when the microsphere method (−33.5 ± 20%) and COUD [−33 ± 19%, not significant (NS)] were used and were well correlated (r = 0.83, P < 0.01) (Fig. 5).

Comparison of COUD vs. Direct Aortic Flowmetry: Baseline and Acute Pharmacological-Induced CI Decrease (Group 2)

At baseline, CO performed by COUD and by direct aortic TTU flowmetry were well correlated (r = 0.79, P < 0.05, n = 8 sham rats) (data not shown).

Table 2. Comparison of baseline cardiac index as measured by COUD or microsphere methods in sham and DMNA rats (group 1)

<table>
<thead>
<tr>
<th>Data Set</th>
<th>Sham Rats</th>
<th>DMNA Rats</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Microspheres</td>
<td>21 ± 6</td>
<td>30 ± 14</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>COUD</td>
<td>24 ± 12</td>
<td>34 ± 11</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SD (in ml·min⁻¹·100 g⁻¹).
Under terlipressin, the decreases in CO were similar for COUD and direct aortic TTU flowmetry ($-37.9 \pm 12$ vs. $-39.4 \pm 12\%$, NS) and were well correlated ($r = 0.86$, $P < 0.01$, $n = 8$ sham rats) (data not shown).

**Ability of COUD to Detect Chronic Pharmacological-Induced CI Increase (Group 3)**

Losartan induced a significant decrease in MAP and SVR, whereas CI was significantly increased compared with the placebo group (Table 4). These changes were proportional to losartan dosages.

**Repeatability (Group 5)**

Within each group, the blind measurement of CI by COUD did not vary significantly between baseline value and the ten measurements (group 5A, $n = 11$ sham rats) or two measurements (group 5B, $n = 9$ sham rats) over 20 min (Wilcoxon test for each two paired series, or Friedman test for all the paired series together) (Fig. 8). The three common measurements of CI (at 0, 10, and 20 min) were not statistically different between the two groups at 10 min ($11 \pm 21$ vs. $9 \pm 27\%$, NS, unpaired test, respectively, group 5A vs. 5B) or at 20 min ($9 \pm 20$ vs. $9 \pm 22\%$, NS, unpaired test, respectively, group 5A vs. 5B).

**Table 3. Effects of terlipressin administration on systemic hemodynamics in 9 DMNA rats (group 1)**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After Terlipressin</th>
<th>P</th>
<th>δ, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>111 ± 17</td>
<td>127 ± 18</td>
<td>&lt;0.01</td>
<td>15 ± 13</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>407 ± 53</td>
<td>368 ± 67</td>
<td>&lt;0.01</td>
<td>-10 ± 7</td>
</tr>
<tr>
<td>Cardiac index, ml·min⁻¹·100 g⁻¹, with microspheres</td>
<td>25 ± 5</td>
<td>17 ± 7</td>
<td>&lt;0.01</td>
<td>-33.5 ± 20</td>
</tr>
<tr>
<td>Cardiac index, ml·min⁻¹·100 g⁻¹, with COUD</td>
<td>29 ± 5</td>
<td>19 ± 6</td>
<td>&lt;0.01</td>
<td>-33 ± 19</td>
</tr>
<tr>
<td>SVR, dyn·s·cm⁻⁶, 100 g⁻¹·10³</td>
<td>363 ± 65</td>
<td>712 ± 324</td>
<td>&lt;0.01</td>
<td>92 ± 73</td>
</tr>
</tbody>
</table>

Values are means ± SD. MAP, mean arterial pressure. Systemic vascular resistance (SVR) values were based on cardiac output data by the microsphere method.

**Table 4. Effects of losartan administration on systemic hemodynamics in BDL rats (group 3)**

<table>
<thead>
<tr>
<th></th>
<th>Placebo ($n = 16$)</th>
<th>5 mg·kg⁻¹·day⁻¹ ($n = 11$)</th>
<th>10 mg·kg⁻¹·day⁻¹ ($n = 8$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>95 ± 18</td>
<td>72 ± 21†</td>
<td>66 ± 25†</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>409 ± 55</td>
<td>385 ± 68</td>
<td>385 ± 52</td>
</tr>
<tr>
<td>Cardiac index, ml·min⁻¹·100 g⁻¹</td>
<td>64 ± 20</td>
<td>71 ± 24</td>
<td>85 ± 23†</td>
</tr>
<tr>
<td>SVR, dyn·s·cm⁻⁶, 100 g⁻¹·10³</td>
<td>159 ± 20</td>
<td>92 ± 15‡</td>
<td>83 ± 20‡</td>
</tr>
</tbody>
</table>

Values are means ± SD. SVR values were based on cardiac output data by COUD. All the intergroup differences were significant by ANOVA, so post hoc comparisons were made: †$P < 0.01$ vs. placebo; ‡$P < 0.05$ vs. placebo; ‡‡$P < 0.0001$ vs. placebo.
DISCUSSION

The present study evaluated the use of a TTU system combined with the classical CO dilution method (COUD) for easy measurement of CO in normal and portal hypertensive rats.

Comparison Between the Aortic Blood Flowmetry and COUD

TTU probes were used directly in the ascending aorta by Wen et al. (42) to measure CO. We tried to evaluate COUD accuracy by comparisons with the ascending aorta blood flows measured with TTU probe. We found that baseline CO and terlipressin-induced CO variations were well correlated between both methods. However, aortic blood flowmetry was quite invasive, as it required open-chest surgery. Moreover, it should be noted that the commercially available probes were so bulky and so large compared with the arch of the ascending aorta in the rat that the probes themselves could alter blood flow. For these reasons, we focused our comparative studies on the microsphere technique to evaluate the accuracy of COUD method.

Comparison Between the Radiolabeled Microsphere Technique and COUD

The microsphere technique is extensively used to measure CO in laboratories involved in portal hypertension studies that use rat animal models (6). It is classically considered a reproducible and reliable technique (19). Moreover, it requires steady-state conditions only for a short time (19) (1 min), and there is less surgical preparation than that required for direct

Table 5. Comparison of practical conditions between microsphere and COUD techniques for the measurement of cardiac output

<table>
<thead>
<tr>
<th></th>
<th>Microspheres</th>
<th>COUD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery</td>
<td>1 catheter in the femoral artery, 1 catheter in the cardiac ventricle</td>
<td>1 catheter in the femoral vein, 1 TTU probe around the carotid</td>
</tr>
<tr>
<td>Operator training</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Success rate</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Regional blood flows</td>
<td>All</td>
<td>Some</td>
</tr>
<tr>
<td>Animal death</td>
<td>Yes (for regional blood flows)</td>
<td>No</td>
</tr>
<tr>
<td>Repeatability</td>
<td>Twice</td>
<td>At least 10 times within 20 minutes</td>
</tr>
<tr>
<td>Direct costs</td>
<td>One study</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>Repeated studies</td>
<td>High</td>
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<tr>
<td></td>
<td></td>
<td>Low</td>
</tr>
</tbody>
</table>

vs. 2 ± 26%, NS, unpaired test, respectively, group 5A vs. 5B).

Fig. 6. Intraobserver agreement for cardiac index (ml·min⁻¹·100 g⁻¹) (○, sham rats; ●, portal hypertensive rats); \( r_{bc} \), intraclass correlation coefficient.

Fig. 7. Interobserver agreement for cardiac index (ml·min⁻¹·100 g⁻¹) (○, sham rats; ●, portal hypertensive rats).

Fig. 8. Repeatability of cardiac index (ml·min⁻¹·100 g⁻¹) measured by COUD.
measurements with an electromagnetic or TTU flow probe on the ascending aorta or pulmonary artery.

In the present study, we evaluated the validity of the COUD method as an alternative to the microsphere technique in the rat. We found that the COUD and microsphere methods provided similar CO values and were well correlated. In addition, COUD detected expected and significant changes: first, an increase in CI in portal hypertensive rats that is due to the well-known hyperkinetic syndrome in portal hypertension (37), and, second, a decrease in CO after terlipressin administration, a vasoconstrictor, which is also well known (25, 27). In addition, the changes in CO observed by both methods (COUD and microspheres) after terlipressin administration were similar and well correlated. Finally, losartan, a vasodilator, induced a dose-related increase in CO in response to decrease in SVR. Intra- and interobserver agreements for COUD were excellent.

COUD has, however, several advantages over the microsphere method (Table 5). The radiolabeled microsphere technique requires skillful and careful handling to avoid errors in CO calculations. Multiple potential errors of the microsphere method have been described (4). Most of these errors are related to the number of microspheres injected or trapped in the reference blood sample (4, 19, 35, 36). Moreover, CO measurements with the microsphere method are limited in the same rat because only one evaluation of CO can be performed with each type of radiolabeled microsphere. If CO must be measured twice, as in the present pharmacological study, then two types of radiomarkers must be used. Two injections are classically a maximum because excessive injected microspheres (>10^5) alter systemic hemodynamics in the conscious rat (35). In contrast, COUD can be evaluated several times in the same rat without any significant alterations in CO as shown in our study.

Finally, we calculated that a 1-yr use of microspheres would cost US$10,400 [price of 141Ce or 51Cr microspheres (50 ml, 500 µCi) is ~US$1,300, their maximum period of validity being 3 mo] without taking into account indirect costs of microsphere method (gamma counter, etc.), vs. US$10,800 for the COUD method (price of a flowmeter with one 1RB probe). So the COUD technique is less expensive than microspheres without any additional indirect costs, especially when several studies are planned over the time.

Is There a Gold-Standard Technique for CO Measurement in the Rat?

In this study, COUD accuracy could be questionable because the correlation coefficients r were not very high (r_s = 0.76 between COUD and microsphere methods, and r = 0.79 between COUD and direct aortic TTU flowmetry). However, similar r values were found for CO measured in rats when comparing electromagnetic flowmeter to the Fick method (r = 0.79) (38) or to thermodilution (r = 0.66) (39). More importantly, exact accuracy of any CO technique designed for rats is difficult to determine because of the lack of a gold-standard technique for the rat. Several CO techniques are frequently used in humans, but they are not suitable for the rat: the thermodilution technique, for example, is not recommended in the rat because of the rapid loss of heat in small animals compared with bigger animals (18, 20); the dye dilution technique requires a blood withdrawal, which is not compatible with the small total blood volume of the rat; and direct aortic flowmetry such as electromagnetic or TTU flowmeters are traumatic because of the thoracotomy.

With the microsphere method used as the reference technique, COUD validation was limited. Indeed, validation against the microsphere method mainly involved comparisons across different groups of animals rather than across a large range of values within animals. Within-animal comparisons were limited by the amount of microspheres that can be administered. Finally, because accuracy of COUD cannot be firmly proved in this setting, the choice of a technique should be based also on sensitivity, reproducibility (agreements and repeatability), and feasibility.

TTU Flowmetry as an Alternative to the Microsphere Technique for CO (COUD) and Regional Blood Flows?

One advantage of the microsphere method is its ability to measure regional blood flows. However, several studies have confirmed the validity of TTU probes for the measurement of several regional blood flows (1, 13, 17, 40–42). In recent studies, we measured the main collateral blood flow in portal hypertensive rats with TTU probes placed around the splenorenal shunt (7, 29). We demonstrated excellent reproducibility and accuracy of TTU for measuring blood flow inside a vessel (7). Thus TTU probes could be an alternative to microspheres for many volumetric flow measurements (1, 7, 42).

In conclusion, TTU appears to be a valid alternative to the microsphere method for COUD in addition to blood flow measurements in isolated vessels. The microsphere method is, however, convenient when numerous organ blood flows have to be measured simultaneously. COUD is especially suitable for pharmacological studies requiring repeated CO measurements without blood removal and without open chest surgery.

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REFERENCES
