Microvascular gas embolization clearance following perfluorocarbon administration

DAVID M. ECKMANN1 AND VLADIMIR N. LOMIVOROTOV2
1Department of Anesthesia and Institute for Medicine and Engineering, The University of Pennsylvania, Philadelphia, Pennsylvania 19104-4283; and 2Anesthesiology Department, Research Institute of Circulation Pathology, Novosibirsk 630055, Russia
Submitted 5 August 2002; accepted in final form 14 November 2002

Effective treatment of vascular gas embolism may be possible with emulsified fluorocarbon compounds. We tested the hypothesis that a fluorocarbon emulsion delivered before gas embolization would enhance bubble motion through the vasculature, favoring more rapid clearance. Air microbubbles were injected into the rat cremaster microcirculation in six groups of rats receiving Perftoran, an emulsified fluorocarbon, or saline immediately before, 2 h before, or after bubble injection. Embolism dimensions and dynamics were observed by using intravital microscopy. Surface area at lodging was equal between groups. Bubbles having smaller volume embolized smaller diameter vessels in the Perftoran pretreatment groups. A higher incidence of bubble dislodgement and larger distal displacement occurred in these two groups, with a 36% decrease in the time to bubble clearance and restoration of blood flow. Intravascular emulsified fluorocarbon administration before gas embolization affected initial bubble conformation, increased bubble dislodgement, and resulted in bubble displacement further into the periphery of the microcirculation. These dynamic events did not occur if embolization preceded fluorocarbon administration.

fluorocarbon; bubble; cremaster; microcirculation; reperfusion

A BRIEF REVIEW OF RECENT LITERATURE demonstrates that gas embolism is known to occur in cardiac surgery, both on-pump from bypass circuit sources (8) and off-pump (2). It happens in endoscopy (1), laparoscopic surgery (16), tissue biopsy (21), neurosurgery (30), liver transplantation (29), during central venous line insertion and removal (12), in orthopedic surgery (7), with laser surgery (20), during neuroangiography and cardiac catheterization procedures (26, 34), in cardiac ablation procedures (17), and in arthroscopy (14). It has been reported during cardiopulmonary resuscitation (15), with positive-pressure ventilation (19), during intravenous antibiotic delivery at home (24), with the use of ultrasound bubble contrast media (18), and as a result of iatrogenic embolization (31). Furthermore, it occurs in both recreational and commercial divers (27). Thus the exposure risk is large, the true incidence is unknown, and effective treatment remains to be established.

Intravascular administration of emulsified fluorocarbon compounds is one potential therapy. Fluorocarbon emulsions are stabilized with surfactants, which have been shown to alter adhesion force at gas-solid-liquid interfaces (9), promote bubble detachment from the wall in a flowing system (6), and decrease the time to reperfusion of embolized microvessels (4). Fluorocarbons are considered to be potential blood substitutes because they have a high oxygen-carrying capacity compared with plasma, and, if emulsified, they can be administered directly into the bloodstream (10, 11). It has been hypothesized that their presence in circulating blood may also increase the speed of bubble reabsorption by increasing the solubility of gases in blood (28).

We hypothesize that patterns of embolization (microbubble gas volume, diameter of vessel embolized, numbers of bubbles trapped) and subsequent dynamics (bubble dissolution, detachment and displacement further into the periphery, rate of gas reabsorption, time to reperfusion) can be modified by altering the mechanics at the air-blood interface with an emulsified fluorocarbon compound. We anticipate that use of an emul-
sified fluorocarbon will produce bubble conformations that favor bubble clearance and speed restoration of blood flow, minimizing embolism effects on endothelial-mediated responses. We further hypothesize that timing and order of the administration of the fluorocarbon relative to gas embolization are critical in generating effective therapy. We tested these hypotheses in the rat cremaster circulation pretreated and post-treated with either Perftoran, a perfluorocarbon emulsion, or saline as control. We used intravital microscopy to measure bubble dimensions and number after entrapment, bubble conformational changes postembolization, and effects of embolization and surfactant treatment on vessel reactivity.

MATERIALS AND METHODS

Surfactant characterization. Perftoran (OJSC SPC Perftoran, Moscow, Russia) is a proprietary 10% volume emulsion consisting of two perfluorocarbon compounds, Perfluorodecalin (C10F18, molecular mass = 462 Da) and Perfluoromethylecyclohexylpiperiden (C12F23N, molecular mass = 595 Da). The solution is stabilized by a surface-active block copolymer propylene oxide, Proxanol P-268, giving an average particle size in the range of 0.03–0.15 μm. The solution has a pH in the range of 7.2–7.8, osmolarity of 280–310 mosmol/l, and an oxygen-carrying capacity of 7.0% volume at 20°C. Perftoran is stored frozen (between 7 °C and refrozen up to −18°C) and can be thawed and refrozen up to five times. For this study, a 200-ml vial was thawed and partitioned into 3-ml sterile aliquots, which were immediately refrozen for later use.

A concentration of Perftoran was determined that would not significantly alter the surface tension at the bubble-blood interface in vivo from the value expected in whole blood. The air-liquid surface tension of serial dilutions of Perftoran in ultrapure water was measured repeatedly (n = 6) at 37°C using the Wilhelmy plate method with a KSV Sigma 703 surface tensiometer. (4, 6, 9). The surface tension of a 5% weight BSA solution was also measured repeatedly (n = 6) at 37°C over a range of Perftoran concentrations. BSA was used for these measurements because protein concentration is a major determinant of surface tension and eliminates interference from clot formation as occurs with blood.

Animal experiments. All experiments were performed by using adult male Wistar rats (250–325 g). Animals were handled according to National Institutes of Health guidelines and approved by the University of Pennsylvania Animal Care and Use Committee. The intact cremaster surgical protocol and muscle preparation have been described in Branger and Eckmann (3, 4). One important feature of this preparation is the insertion and positioning of a femoral artery microcatheter placed so that the ipsilateral cremaster microcirculatory bed branches with injection of saline. This preparation provides a reproducible method of gas embolizing the cremaster arteriolar microcirculation.

Experimental protocol. Anesthesia was induced (5%) and maintained (1.2%) with inhaled halothane delivered in an air-O2 mixture (inspired O2 fraction = 0.3). After induction, rats were laid supine on a Plexiglas tray and intubated through a tracheostomy. The animals were ventilated by using a positive-pressure, piston-driven small-animal ventilator. Blood pressure and heart rate were monitored with a right carotid artery catheter (PE-50). A PE-50 left jugular venous catheter was placed for intravenous fluid and drug administration. A PE-10 catheter was inserted into the left femoral artery for injection of air bubbles directly into the cremaster circulation. Body temperature was monitored with a rectal thermometer and maintained at 37°C with a heating pad.

The cremaster muscle was prepared as previously described (3, 4). Briefly, the cremaster muscle was exposed and separated from the surrounding tissue and organ through a midline scrotal incision and then through the muscle itself. Loose connective tissue was dissected away, and the muscle was spread over the transparent pedal portion of the Plexiglas tray. Several sutures were attached to keep the muscle flat on the platform. The cremaster was superfused at 2 ml/min with a warmed (34°C), gassed (95% N2-5% CO2) Krebs buffer containing 132 mmol/l NaCl, 25 mmol/l NaHCO3, 5 mmol/l KCl, 1.2 mmol/l MgCl2, and 2 mmol/l CaCl2. The cremaster muscle was allowed to equilibrate for 30 min before any experimentation was started. The cremaster temperature was monitored with an intramuscular thermocouple placed away from possible areas of interest. Cremaster temperature was maintained at 34–36°C by adjusting the temperature of the superfusate. After surgery and equilibration, a series of clearly visible consecutive branching arteriole vessels was selected, and other nearby arterial vessels were cauterized. This minimized a physiological environment while creating a more controlled vascular pathway in which the air embolism could be observed.

To demonstrate the preservation of robust vascular responses after cremaster muscle preparation, a 0.5-ml bolus of 10−4 mol/l ACh (Sigma Chemical, St. Louis, MO), diluted in Krebs buffer, was added topically to the muscle to test for endothelial-mediated vasodilation. A 0.5-ml bolus of 10−4 mol/l phenylephrine (PE; Sigma Chemicals), diluted in Krebs buffer and placed topically, was used to confirm smooth-muscle-mediated constriction. At least 10 min passed between the applications of each vasoactive agent. Tissue responses were considered intact if the PE elicited at least a 20% decrease in diameter and the ACh elicited at least a 50% increase in vessel diameter from baseline. The preparation was not further studied in one case because these criteria were not met. In the delayed embolization group defined below, topical application of PE and ACh 10 min after study compound delivery was used to demonstrate that vessels were reactive with no change from the prebolus responses elicited. Pancuronium bromide (1 mg/kg) was administered intravenously 10 min after vasoreactivity was demonstrated to be intact.

Six groups of animals (n = 6 per group) were studied for three treatment regimens with either study compound, Perftoran administration, or saline administration as a control. The three treatment regimens were immediate pretreatment, immediate posttreatment, and delayed embolization after pretreatment. For immediate pretreatment, animals received the treatment compound (Perftoran or saline), and cremaster embolization followed within 10 min of completion of treatment compound delivery. For immediate posttreatment, the cremaster muscle was embolized, and the treatment compound was delivered beginning 2 min later. For delayed embolization, animals received the treatment compound, and cremaster embolization followed 2 h after completion of treatment compound delivery.

For treatment compound administration, animals were given an intravenous bolus of either 0.9% NaCl or undiluted, freshly thawed (second thaw) Perftoran warmed to room temperature. The volume of saline or undiluted Perftoran delivered was calculated to be 10% of the individual animal’s estimated blood volume (64 ml/kg for rats). For Perftoran, this resulted in a 1% volume concentration in circulating blood. The bolus was delivered by syringe pump over 5 min. For gas embolization, single air bubbles were injected into
the femoral artery ipsilateral to the selected cremaster. Initially, a 3-μl bubble was injected, and this volume was increased in 1-μl increments, as necessary, in subsequent injections until a suitably sized embolism arrived in the cremaster circulation. The maximum bubble volume required for successful embolization was 5 μl. Once a bubble of sufficient size embolized the cremaster, no additional experiments were conducted in that animal. After complete bubble reabsorption or transarteriolar passage, the tests of vascular reactivity were conducted in the regions of the vessel in which bubbles had lodged.

Data analysis. Data analysis was performed by using the videotaped recording of each experiment with a calibrated video micrometer, as previously published (3, 4). Two or more bubbles lodging at a given location were considered to be a single embolism only if the bubbles touched each other. The total embolism volume was calculated as the sum of the individual bubble volumes in that case.

The length (L) and average diameter (D) of parent bubbles were measured at the time of initial entrapment. Initial embolism volume and aspect ratio (length/radius) were calculated by using the measured dimensions, assuming that the elongated bubble is approximated by a cylinder with hemispherical end caps, as was done previously (3, 4). Surface area at the moment of lodging was calculated as the sum of the end cap area (πD²) and the area of cylindrical central portion (πDL) of each bubble. Predicted absorption times (T_{predicted}) for parent bubbles were computed based on the initial volume and the aspect ratio measured using our mathematical model for bubble absorption as described by Branger and Eckmann (3) and subsequently used by the authors (4, 5). Actual elapsed time (T_{observed}) required for the last remaining observable remnant of parent bubbles to reabsorb from the embolized vessel was determined from the videomicroscopy recording. The percent change in observed (actual) absorption time from the time predicted (ΔT%) was calculated as

\[ \Delta T\% = \frac{T_{\text{predicted}} - T_{\text{observed}}}{T_{\text{predicted}}} \times 100\% \]  

Bubble entrapment was followed by “stick-and-slip” behavior. Stick-and-slip behavior generally refers to a lurching phenomenon occurring in the motion of large sliding interfaces, such as a creaky door hinge, a bowed violin string, or a moving geological fault. Stick-and-slip behavior is the result of the strong collective interfacial interactions of large numbers of surface molecules. An increase in stick and slip occurs as the surface interactions become weaker, indicating a transition between arrested motion and free motion of the surfaces relative to each other (25). The magnitude of stick and slip was quantified by measuring the number of episodes of bubble lodging and subsequent bubble motion during the first 2 min after initial entrapment and by measuring the distance a bubble traveled during a slip episode. Only bubble motion lasting >1 s, during which time the bubble moved at least one vessel diameter downstream, was considered to be stick and slip. The distance traveled was normalized to the local vessel diameter in each case for later statistical analysis and for comparison to the normalized bubble length, the bubble aspect ratio.

Statistical analysis. The results from the groups are presented as arithmetic means ± SD. Variances of the groups were examined by using the F-test. If the variances were equal, statistical significance between groups was established by using ANOVA, with P < 0.05 considered statistically significant by using the Bonferroni correction. Nine separate comparisons were applied: the six comparisons made between groups receiving the same compound at different times relative to the embolization; and the three comparisons made between groups for the same embolization timing but receiving different compounds. Changes within the same group at different time points were considered statistically significant for P < 0.05, calculated by using the paired Student’s t-test. The variances were not equal for analysis of the distance bubbles traveled in stick-and-slip behavior. In this case, the t-test for unequal variances was used to compare specific group means.

RESULTS

Surface tension. The surface tension of the 5% weight BSA solution was 50.6 ± 0.4 mN/m, compared with 50 mN/m for blood (33). The surface tension was 45.3 ± 0.5 mN/m for undiluted (10%) Perftoran, 50.4 ± 0.3 mN/m for 1% Perftoran diluted in ultrapure water, and 50.7 ± 0.8 mN/m for 1% Perftoran diluted in 5% weight BSA.

Hemodynamic stability and baseline vessel tone. Heart rate and blood pressure data before, midway through, and 5 min after infusion of the study compound or control injectate are presented in Table 1. There were no significant changes within or between groups (P > 0.65). The administration of either Perftoran or saline had no significant effect on arteriolar tone or reactivity. There was no appreciable change in second- or third-order arteriole diameter (<5.2% change in all cases, P > 0.79) 1 min after bolus delivery compared with the prebolus diameter.

Initial bubble lodging and bubble dynamics. Air emboli (range 4.8–9.9 nl) lodged in the cremaster microvasculature within 5 s of injection. The number of injections required to embolize successfully was the

Table 1. Heart rate and blood pressure before, during, and after bolus delivery

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Prebolus</th>
<th>Midbolus</th>
<th>5 Min Postbolus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart rate, beats/min</td>
<td>Blood pressure, mmHg</td>
<td>Heart rate, beats/min</td>
</tr>
<tr>
<td>Saline pretreatment</td>
<td>331 ± 32</td>
<td>85 ± 9/47 ± 8</td>
<td>325 ± 31</td>
</tr>
<tr>
<td>Saline posttreatment</td>
<td>324 ± 38</td>
<td>87 ± 8/44 ± 7</td>
<td>320 ± 29</td>
</tr>
<tr>
<td>Saline-delayed embolization</td>
<td>340 ± 35</td>
<td>83 ± 8/42 ± 6</td>
<td>335 ± 38</td>
</tr>
<tr>
<td>Perftoran pretreatment</td>
<td>328 ± 40</td>
<td>90 ± 6/46 ± 7</td>
<td>326 ± 44</td>
</tr>
<tr>
<td>Perftoran posttreatment</td>
<td>337 ± 36</td>
<td>88 ± 8/43 ± 6</td>
<td>332 ± 45</td>
</tr>
<tr>
<td>Perftoran-delayed embolization</td>
<td>330 ± 33</td>
<td>85 ± 7/46 ± 5</td>
<td>322 ± 39</td>
</tr>
</tbody>
</table>

Values are means ± SD. Blood pressures shown are systolic and diastolic.
same in all groups, ranging from one to three in all experiments. Either a single bubble or two adjacent bubbles (two cases) formed the embolism. There was a linear correlation between embolism volume and vessel diameter, as shown in Fig. 1. Data segregated into two clusters. The Perftoran pretreatment and delayed embolization data formed one cluster, and the remaining four groups formed the other. Mean values and bidirectional error bars for each cluster are also plotted. Bubbles in the two Perftoran pretreatment groups had smaller volumes (P < 0.001) and lodged in smaller vessels (P < 0.001) than in the other four experiments.

The means bubble volumes, embolized vessel diameters, initial bubble length, and initial aspect ratio are shown in Table 2 for each group. Values were not different among the three saline treatment groups. Bubble length and aspect ratio were not different among the Perftoran treatment groups. Volumes were smaller in both the Perftoran pretreatment and delayed embolization groups compared with their saline controls (P < 0.0056). Bubble volume was also smaller after Perftoran pretreatment compared with Perftoran delayed embolization (P < 0.0056). The initial bubble aspect ratio was larger for Perftoran pretreatment compared with saline pretreatment (P < 0.0056).

Bubble surface area after initial lodging is illustrated in Fig. 2. There were no differences between groups (P > 0.14 for all comparisons). End-cap surface area was smaller for both the Perftoran pretreatment and delayed embolization groups compared with their respective saline controls and compared with Perftoran posttreatment (P < 0.0056 for all comparisons) but not compared with each other (P > 0.37). Surface area of the cylindrical bubble portion was not different between groups (P > 0.18 in all cases).

A small decrease in vessel diameter occurred in the first 5–8 s after bubble entrapment in each experiment. Vessel constriction did not exceed 6.5% in any experiment, with group means ranging from 2.7 ± 1.9% (saline-delayed embolization) to 3.9 ± 2.2% (Perftoran pretreatment). A slight increase in bubble length, ranging from 6.7 ± 6.1% (saline-delayed embolization) to 7.5 ± 5.8% (Perftoran pretreatment) accompanied the decrease in bubble diameter. The aspect ratio increased ∼10–13% per group (range 9.9 ± 12.8% (saline-delayed embolization) to 13.1 ± 10.0% (Perftoran).

Table 2. Initial bubble geometric parameters after embolization

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Bubble Volume, nl</th>
<th>Bubble Diameter, mm</th>
<th>Bubble Length, mm</th>
<th>Bubble Aspect Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline pretreatment</td>
<td>8.4 ± 0.8</td>
<td>81.5 ± 5.8</td>
<td>1,568 ± 303</td>
<td>39.0 ± 10.0</td>
</tr>
<tr>
<td>Saline posttreatment</td>
<td>8.2 ± 0.8</td>
<td>78.7 ± 5.9</td>
<td>1,651 ± 332</td>
<td>42.6 ± 11.9</td>
</tr>
<tr>
<td>Saline-delayed embolization</td>
<td>8.0 ± 0.8</td>
<td>78.8 ± 6.3</td>
<td>1,625 ± 333</td>
<td>42.0 ± 12.1</td>
</tr>
<tr>
<td>Perftoran pretreatment</td>
<td>5.6 ± 0.6*</td>
<td>59.8 ± 6.1*</td>
<td>1,983 ± 313</td>
<td>67.7 ± 17.4*</td>
</tr>
<tr>
<td>Perftoran posttreatment</td>
<td>7.9 ± 1.4</td>
<td>74.8 ± 8.8</td>
<td>1,779 ± 428</td>
<td>49.0 ± 16.0</td>
</tr>
<tr>
<td>Perftoran-delayed embolization</td>
<td>6.1 ± 0.8†</td>
<td>57.7 ± 6.6‡</td>
<td>2,360 ± 605</td>
<td>84.5 ± 30.0</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.0056 compared with saline pretreatment; †P < 0.0056 compared with saline-delayed embolization; ‡P < 0.0056 compared with Perftoran posttreatment.
ran pretreatment). These changes in bubble diameter, length, and aspect ratio were not significant ($P > 0.81$ in all cases).

Minimal embolism breakup, or parent bubble dissolution into multiple smaller bubbles, followed bubble lodging. In five cases (one saline pretreatment, one saline-delayed embolization, two Perftoran pretreatment, one Perftoran-delayed embolization), breakup resulted in formation of two additional bubbles. Stick-and-slip events postlodging were accentuated in the two Perftoran pretreatment and delayed embolization groups. Bubbles in these groups moved appreciably further distally in the microcirculation, whereas bubbles in the other four groups essentially remained fixed once they lodged. Table 3 shows the average number of stick-and-slip events occurring in the first 2 min after embolism bubble lodging. Stick-and-slip events were rare in the saline-treated animals and in the Perftoran-delayed embolization group. Only two or three episodes occurred in these groups compared with 35 and 29 total episodes occurring after Perftoran pretreatment and delayed embolization, respectively ($P < 0.0056$). In the saline groups and the Perftoran posttreatment group, bubbles traveled approximately one bubble length (with a bubble aspect ratio of $\sim 40$) per slip event (Fig. 3). The normalized slip distance traveled was more than twice as far for both the Perftoran pretreatment and delayed embolization groups by comparison to their saline controls ($P < 0.0167$) and for Perftoran pretreatment compared with posttreatment ($P < 0.0046$). Coupled with the greater absolute number of slip events, bubbles in the Perftoran pretreatment and delayed embolization groups dislocated and moved 10 times further into the periphery of the microvasculature during the first 2 min. This corresponds to a displacement of $\sim 1.5$ cm compared with only 1.5 mm for the other four groups.

**Bubble reabsorption and reperfusion.** Predicted and observed reabsorption times for the six embolization experiments are presented in Fig. 4. The data fell into two distinct populations. One data cluster contains the three saline groups and the Perftoran posttreatment group. The other data cluster contains the Perftoran pretreatment and delayed embolization groups. Linear regression analysis was performed on each cluster. The regression line was forced through the origin because extremely small bubbles should reabsorb rapidly (3). The regression line slope of 0.994 for the data cluster from the four experimental groups is essentially the line of identity. The associated large correlation coefficient ($R = 0.933$) indicates that the observed reabsorption times measured collectively were closely predicted individually. This is further supported by the separate measure of the percent deviation between the two values calculated by Eq. 1 for each experiment. The values are presented in Table 3.

The slopes of the two regression lines in Fig. 4 were different ($P < 0.05$), with the slope of the regression line for the data cluster from the Perftoran pretreatment and delayed embolization groups being 1.557 and $R = 0.920$. This means that the observed reabsorption

---

**Table 3. Stick-and-slip events and differences in absorption time from predicted**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>No. of Stick-and-Slip Episodes</th>
<th>Mean $\Delta T%$</th>
<th>SD $\Delta T%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline pretreatment</td>
<td>0.5 ± 0.5</td>
<td>$-1.48$</td>
<td>4.1</td>
</tr>
<tr>
<td>Saline posttreatment</td>
<td>0.3 ± 0.5</td>
<td>$-0.65$</td>
<td>4.8</td>
</tr>
<tr>
<td>Saline-delayed embolization</td>
<td>0.3 ± 0.5</td>
<td>0.60</td>
<td>5.2</td>
</tr>
<tr>
<td>Perftoran pretreatment</td>
<td>5.5 ± 2.1†</td>
<td>38.3‡</td>
<td>3.9</td>
</tr>
<tr>
<td>Perftoran posttreatment</td>
<td>0.3 ± 0.5</td>
<td>0.70</td>
<td>7.3</td>
</tr>
<tr>
<td>Perftoran-delayed embolization</td>
<td>4.8 ± 1.5†‡</td>
<td>32.8‡</td>
<td>5.2</td>
</tr>
</tbody>
</table>

*No. of stick-and-slip episodes are means ± SD, $\Delta T\%$, percent change in observed absorption time from predicted time. $^*P < 0.0056$ compared with saline pretreatment; $^†P < 0.0056$ compared with saline-delayed embolization; $^{‡}P < 0.0056$ compared with Perftoran posttreatment.*

---

Fig. 3. Normalized bubble displacement distances traveled during stick-and-slip events (n). Statistical analysis showed $^*P < 0.167$ compared with saline pretreatment; $^{‡}P < 0.01$ compared with Perftoran posttreatment; and $^*P < 0.0046$ compared with saline-delayed embolization.

Fig. 4. Relationship between the actual elapsed time ($T_{observed}$) and the predicted time ($T_{predicted}$) required for the last remaining observable remnant of parent bubbles to clear from the embolized vessel. Linear regressions are forced through the origin. Symbols are as defined in Fig. 1 legend.

*J Appl Physiol • VOL 94 • MARCH 2003 • www.jap.org*
time in these two groups was 35.8% faster than predicted. This correlates well with the measures of the percent deviation between the two values calculated by Eq. 1 for these two groups (Table 3). For Perftoran pretreatment, reabsorption was significantly faster than was predicted, with the measured value deviating from the predicted value by 38.3 ± 3.9% (range 31.7–42.1%, P < 0.0056 compared with saline pretreatment and Perftoran posttreatment). In the case of Perftoran-delayed embolization, the measured reabsorption time was faster than predicted by 33.8 ± 5.2% (range 26.3–39.2%, P < 0.0056 compared with saline-delayed embolization and Perftoran posttreatment). An ensemble average for these two groups gives a measured reabsorption time that is 36.0 ± 4.9% faster than predicted, similar to the correlation analysis result.

The data in Fig. 4 have been recast in Fig. 5 to demonstrate the relationship between initial bubble surface area and the observed clearance time, as was done in Ref. 3. Linear regression analysis forced through the origin is included for the two resultant data clusters as were identified in Figs. 1 and 4. The two regression line slopes were different (P < 0.05). The slope from the three saline groups and the Perftoran posttreatment group was nearly double the slope derived from the Perftoran pretreatment and delayed embolization data. Thus for fixed bubble surface area, clearance took nearly twice as long if Perftoran was not given before embolization.

**DISCUSSION**

Gas embolization can obstruct blood flow, cause ischemia, initiate thromboinflammatory events, and injure or denude the endothelium, all of which can be extremely detrimental, particularly in the cerebral circulation (13, 22). Fluorocarbon emulsions have been thought to enhance the rate of bubble reabsorption by increasing the solubility of gas in the fluorocarbon-laden blood. The surfactants used to stabilize the emulsion have also been thought to lower surface tension and thereby promote bubble entrainment in more distal regions of the vasculature. Determining the effects of timing of delivery of such compounds in relation to the timing of embolization in the treatment of gas embolism is also important. Neither the duration of effect of any pretreatment nor the effectiveness of delivery of a treatment compound to the site of embolization after bubble deposition has occurred has been characterized. In these in vivo experiments, we have used intravital microscopy to quantify the effects of timing of delivery of a fluorocarbon emulsion, Perftoran, on air embolism bubble deposition into, and clearance from, the arterial microcirculation.

**Initial bubble lodging and bubble dynamics.** The use of Perftoran as a pretreatment was expected to promote bubble deposition, with smaller bubbles lodging in more distal vessels, and to accelerate reabsorption, whereas fluorocarbon emulsion installation after embolization was expected to have no effect (4, 23). Indeed, treatment delivered postembolization was not different between the Perftoran and saline control groups in any aspect of the study. This most likely indicates that the treatment compound did not reach the site of embolization. This would be expected, because the Perftoran would not be convected into those regions of tissue in which blood flow was obstructed. Neither did the emulsion appear to have diffused through stagnant blood to achieve any appreciable concentration at the gas-liquid interface or the sites of adhesion between the bubble and the vessel wall. Thus there would be no measurable effect of Perftoran if it were administered after a gas embolism that had already occurred. If, however, the emulsion were already circulating, it would have the following effects: decreased volume but equal surface area of the bubbles that lodged (Figs. 1 and 2, Table 2); decreased diameter of the vessel that was embolized (Fig. 1, Table 2); shorter length of time that they stayed lodged in one location (Fig. 3, Table 3); greater distance that they moved each time they dislodged (Fig. 3, Table 3); and shorter elapsed time until complete reperfusion had occurred (Figs. 4 and 5, Table 3).

When gas embolization was preceded by Perftoran administration immediately or 2 h before, the gas volume lodging was 25–35% less than if saline were administered or if Perftoran were given postembolization. Although this could in part be the result of increased gas solubility within the blood because of the nonzero fluorocrit, the very rapid transit time from bubble injection to intravessel lodging is not consistent with the time that would be required for gas transport out of the bubble and into the blood. Thus it is more likely a primary effect of “snap off” of small bubbles from the injected bubble, as was predicted by Tsai and Miksis (32) and seen in vivo by Branger and Eckmann (4). In the presence of the circulating fluorocarbon emulsion, which has some surfactant properties, it is possible that smaller bubbles split free from the injected embolism and subsequently enter the cremaster circulation.

The smaller bubbles in the Perftoran pretreatment and delayed embolization groups also lodged further...
out into the periphery of the microvasculature, resulting in bubble deformation, yielding a total surface area profile that was not different from that determined for the other groups. The fact that the surface area of the bubble end caps was smaller for these two treatment groups is indicative of the smaller vessel diameter, because the end-cap surface area depends only on diameter. The end-cap surface area contributes only \( \sim 5\% \) of the total surface area, leaving the central cylindrical portion of the bubble to contribute \( \sim 95\% \) of the total surface area (Fig. 2). With equal surface areas but smaller volumes, it is expected that bubbles in the Perftoran pretreatment and delayed embolization groups should reabsorb faster by diffusion of a smaller volume of gas across an equal surface area, as we have found (Fig. 5). But this finding does not account for the continued motion of bubbles further into the periphery of the vascular tree precipitated by preembolism administration of the study compound.

The finding that stick-and-slip events are enhanced in the present study suggests that a smaller adhesion force develops between the bubble surface and the vessel wall in the two groups receiving Perftoran before embolization. Although the exact nature of the adhesion force between the bubble surface and the vessel wall has not been identified, there is likely to be some adhesion interaction between elements of the endothelial surface (e.g., the glycocalyx or endothelial surface layer) and plasma-borne molecules that have adsorbed to the bubble surface (e.g., proteins). The circulating emulsion can also adsorb to the bubble surface. The surface-active components can compete with plasma-borne molecules to occupy the bubble interface, and this may lower the surface concentration of plasma-borne molecules, which could potentially decrease adhesion to the endothelial surface. Also, despite the fact that they are considered to be biologically and chemically inert (nonreactive), the molecular components of the emulsion may also have some interaction with the endothelial surface that reduces its adhesive interaction with adsorbed species, thus increasing intermolecular distances on the interface. This accentuates the effect of having a smaller initial surface area, due to smaller initial volume and spherical shape before lodging, available for adsorption. Furthermore, as the bubble progresses into the periphery, both the local blood pressure and the bubble cross-sectional area decrease, diminishing the net driving force for bubble movement until arrest of motion occurs. Such behavior could also result from effects on the bubble surface due to competition between blood-borne molecules and components of the emulsion for interfacial adsorption or from an interaction between the fluorocarbon emulsion and the vessel wall that lowers adhesiveness for the bubble. The net effect is to permit more distal gas embolization.

Perftoran has a lower oxygen-carrying capacity than does arterial blood. At a 10-fold dilution, as was used, there may still be a sufficient decrease in local oxygen tension so that hypoxemia-induced tissue-mediated events contribute to earlier reperfusion. One possible mechanism is hypoxia-mediated changes in endothelial surface layer structure, leading to a less adhesive surface. It is not possible to separate this effect from a molecular binding interaction, as described above, but the stick-and-slip phenomena (Fig. 3, Table 3) do reflect a decreased adhesiveness in the two groups receiving Perftoran before embolization. Bubbles detached and moved many more times in those groups. As the bubbles reabsorbed, the surface area in contact with the vessel wall shrank until a critical surface area was reached, at which point the bubble detached. This happened significantly more often in these two groups, suggesting that the strength of adhesion was reduced by the Perftoran. This stick-and-slip behavior induced by a circulating surface-active compound and influenced by the available interfacial surface area has also been observed in vitro by Cavanagh and Eckmann (6). The distance traveled with each dislodging episode was also higher in those same two groups, indicating that...
the bubble surface and the vessel wall did not form sufficient adhesion interactions until the bubble had displaced further downstream.

Bubble reabsorption and clearance. Bubbles in the two groups receiving Perftoran before gas embolism not only moved further out to the periphery, but they disappeared from the microcirculation sooner with movement further out to the periphery. As a result, blood flow was reestablished in less than two-thirds of the time predicted, whereas in the other four groups the actual restoration of blood flow occurred at the time predicted (Figs. 4 and 5, Table 3).

After embolization, there were no differences between treatment groups in the amount of vasoconstriction elicited or the degree of bubble elongation that occurred in the other groups (Figs. 4 and 5). The influence of the fluorocarbon on the rate of gas reabsorption was not assessed independently but is not believed to have been the major reason for this finding. The compound studied was selected in part because the oxygen-carrying capacity of Perftoran is actually less than that of whole blood. Thus it was not expected that a change in gas-carrying capacity would be the major mechanism responsible for more rapid reperfusion. Rather, it was by a combination of gas reabsorption and bubble movement out to the periphery. As bubbles moved further into the periphery, they elongated and became more slender, so that the surface area available for gas transport out of the bubble increased, as seen in Fig. 5. Bubbles in the Perftoran pretreatment and delayed embolization group did not cease to stick and slip after 2 min and thus continually displaced further into the periphery until small bubbles disappeared from view or appeared to pass transcapillary and enter directly into the venous circulation. As a result, blood flow was reestablished in less than two-thirds of the time predicted, whereas in the other four groups the actual restoration of blood flow occurred at the time predicted (Figs. 4 and 5, Table 3).

The net effect of the phenomena that we recorded was that bubbles traveled further into the periphery initially, continued to move further distal in quantum events, and cleared from the circulation faster than predicted only when Perftoran was given in advance of the gas embolism. Although no measurements of tissue ischemia, endothelial injury, inflammatory response, or thrombus formation precipitated by gas embolism were performed in this study, the 36% reduction in the duration of blood flow obstruction, coupled with the continued distal movement of bubbles, could limit the degree of injury developed. Targeting the mechanism of bubble adhesion to the vasculature provides a potential pharmacological approach to therapy for gas embolism.

The authors thank Lytal Kaufman-Grob and Stephen Armstead for experimental assistance and Dr. Dmitri Guvakov for help with procurement of the Perftoran. Dr. E. Andrew Ochroch provided statistical support.

This study was supported by National Heart, Lung, and Blood Institute Grant R01HL60230.

REFERENCES


