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Accelerated Arteriolar Gas Embolism Reabsorption by an Exogenous Surfactant

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Background: **Cerebrovascular gas embolism can cause profound neurologic dysfunction, and there are few treatments. The authors tested the hypothesis that an exogenous surfactant can be delivered into the bloodstream to alter the air–blood interfacial mechanics of an intravascular gas embolism and produce bubble conformations, which favor more rapid bubble absorption.**

Methods: **Microbubbles of air were injected into the rat cremaster microcirculation after intravascular administration of either saline (control, n = 5) or Dow Corning Antifoam 1510US (surfactant, n = 5). Embolism dimensions and dynamics were directly observed after entrapment using intravital microscopy.**

Results: **To achieve embolization, the surfactant group required twice as many injections as did controls (3.2 ± 1.3 *vs.* 1.6 ± 0.9; $P < 0.05$). There was no difference in the initial lodging configuration between groups. After bubble entrapment, there was significantly more local vasoconstriction in the surfactant group (24.2% average decrease in diameter) than in controls (3.4%; $P < 0.05$). This was accompanied by a 92.7% bubble elongation in the surfactant group *versus* 8.2% in controls ($P < 0.05$). Embolism shape change was coupled with surfactant-enhanced breakup into multiple smaller bubbles, which reabsorbed nearly 30% more rapidly than did parent bubbles in the control group ($P < 0.05$).**

Conclusions: **Intravascular exogenous surfactant did not affect initial bubble conformation but dramatically increased bubble breakup and rate of reabsorption. This was evidenced by both the large shape change after entrapment and enhancement of bubble breakup in the surfactant group. These dynamic surfactant-induced changes increase the total embolism surface area and markedly accelerate bubble reabsorption.**

THE entry or formation of bubbles within the circulation may precipitate cerebrovascular gas embolism in surgery or during decompression sickness. Clinical manifestations of cerebrovascular gas embolism range from extreme neurologic impairment (*e.g.*, convulsions, stroke, or death) to lesser problems characterized by memory loss, speech impairment, or attention deficit.[1] The specific role of cerebrovascular gas embolism in surgical morbidity is unknown, but it likely contributes to the large percentage (approximately 50–70%) of patients demonstrating some neurologic dysfunction after bypass surgery.[1]

The introduction of gas emboli is often unpreventable, and treatment options are limited. Hyperbaric therapy is used in severe cases of decompression sickness but is uncommonly used to treat the surgical patient. Development of new clinically applicable methods of prevention and treatment for gas embolism would therefore be extremely valuable for reducing morbidity, mortality, and the cost of care. A potential therapy is the intravascular use of exogenous surface-active agents. Surfactants were originally shown to decrease the mortality rate in dogs receiving a large bolus of intravascular air by as much as 50%.[2] Injection of surfactants along with air into the coronary arteries of pigs has also been shown to reduce the duration and degree of myocardial depression caused by air alone.[3]

The protective mechanism of action involved with the use of intravascular surfactants has been speculated but never directly visualized. Surfactants lower surface tension at the bubble–blood interface. This allows greater bubble deformability so that spherical bubbles can undergo shape change and become long and slender.[4] Surfactants also alter surface wettability at gas–solid–liquid interfaces, or contact lines, thus changing the adhesion characteristics between the interfaces.[5] Some of the benefits theorized to result from these effects include entrapment of bubbles in smaller diameter vessels and breakup of large bubbles into multiple smaller bubbles at vessel bifurcations,[3] facilitation of bubble dispersion,[6] and reduction in resistance to blood flow caused by the gas embolism.[2]

Our study investigates the hypothesis that bubble deposition patterns and subsequent dynamics can be modified by altering the mechanics at the air–blood interface with an exogenous surfactant to produce bubble conformations that favor more rapid reabsorption. We tested our hypothesis in the rat cremaster circulation pretreated with either a silicone-based surfactant (Dow Corning Antifoam 1510US; Dow Corning, Midland, MI) or saline as control. We used intravital microscopy to measure bubble diameter, length, and number after entrapment, as well as the ensuing conformational changes.

Materials and Methods

Surface Tension Measurements

A concentration of the nonionic, silicone emulsion-type surfactant (Dow Corning Antifoam1510US) was determined that would suitably lower the surface tension at the bubble–blood interface *in vivo*. The air–liquid surface tension of a 5 weight % bovine serum albumin

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(BSA) solution was measured repeatedly (n = 5) at 37°C over a range of surfactant concentrations using the Wilhelmy plate method with a KSV Sigma 7^{03} surface tensiometer (KSV Instruments, Helsinki, Finland). Because protein concentration is the major determinant of surface tension, BSA was used as a blood surrogate for these measurements. This also helps to avoid measurement interference from clot formation in whole blood, as well as effects of anticoagulants, which have unknown effects on blood surface tension.

Animal Experiments

All experiments were performed using adult male Wistar rats (weight, 250–350 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 was previously described by Branger and Eckmann.[7] One important feature of this setup is the use of a microcatheter inserted *via* a femoral artery and positioned so that the ipsilateral cremaster microcirculatory bed blanched on injection of saline. With the catheter tip positioned as such, this provides a reliable means of introducing embolism bubbles into the microvasculature. Some modifications were made to the method described previously by Branger and Eckmann,[7] as mentioned in the Experimental Protocol section, below.

Experimental Protocol

Anesthesia was performed with halothane (5% induction, 1.2% maintenance) in an air–oxygen mixture (fraction of inspired oxygen = 0.3). The cremaster temperature was monitored with a thermocouple inserted into the muscle itself (away from possible areas of interest) and maintained at 34–36°C by controlling the temperature of the water bath through which the superfusate was circulating. After surgery and equilibration, a series of clearly visible consecutive branching arteriolar vessels were selected; other nearby arterial vessels were cauterized. This created a controlled vascular pathway in which the air embolism dynamics could be observed while still maintaining a physiologic environment.

To demonstrate the preservation of robust vascular responses in the prepared cremaster muscle, a 0.5-ml bolus of 10^{-4} M acetylcholine (Sigma Chemicals, St. Louis, MO), diluted in Krebs buffer, was added topically to the muscle to test for endothelial-mediated vasodilation. In addition, a 0.5-ml bolus of 10^{-4} M phenylephrine (Sigma Chemicals), also diluted in Krebs buffer and given topically, was used to confirm smooth muscle–mediated constriction. A minimum of 10 min passed between the applications of each vasoactive agent. Responses of the tissue preparation were considered intact if the phenylephrine elicited at least a 20% decrease in diameter and the acetylcholine elicited at least a 50% increase in vessel diameter from baseline. If these criteria were not met, the preparation was not further studied. Pancuronium bromide (1 mg/kg) was administered intravenously 10 min after vasoreactivity was demonstrated to be intact.

In preliminary experiments, animals developed severe hypotension with injection of pure, but not diluted, surfactant, as well as with intravenous, but not intraarterial, injection of the surfactant. Therefore, pretreatment injections were delivered over 10 min *via* the femoral artery contralateral to the cremaster under observation. For pretreatment administration, animals were divided into two groups and given either a 2.0-ml bolus of 0.9% NaCl (control group, n = 5) or a 2.0-ml diluted surfactant dose (study group, n = 5). The volume of undiluted surfactant in the dose was calculated to be 1.5% of the individual animal's estimated blood volume (65 ml/kg for rats). This was then diluted with sufficient 0.9% NaCl to bring the total volume to 2.0 ml for constant volume dosing between groups. Beyond mitigating the hypotensive response elicited by delivery of undiluted surfactant, the additional crystalloid also replaced the surgical blood losses of approximately 0.5 ml per animal.

After delivery of the pretreatment dose, single air bubbles were injected into the femoral artery ipsilateral to the selected cremaster. Initially, 3 μl bubbles were injected, and this volume was incrementally increased by 1 μl, as necessary, in subsequent injections until a suitably sized embolism arrived in the cremaster circulation. The maximum bubble volume required for successful embolization was 6 μl. Once a bubble of sufficient size embolized the cremaster, no additional experiments were conducted in that animal.

Data Analysis

Data analysis was conducted on the videotaped recording of each experiment with a calibrated video micrometer.[7] From previous experience[7] and in these experiments, bubbles having a volume less than 4.0 nl typically lodge in fourth-order (or smaller) vessels, and bubbles having a volume greater than 10.0 nl tend to lodge in the vessels larger than second-order arterioles. Thus, only emboli with volumes between 4.0 and 10.0 nl, which lodged in vessels of second or third order, were considered for data analysis. If two or more bubbles lodged at a given location, this was regarded as a single embolism only if bubbles were touching. The total embolism volume was calculated as the sum of the individual bubble volumes. All experiments were conducted so that emboli lodged not less than 5 min and not more than 20 min after pretreatment.

The number of femoral air injections and bubbles lodging together in each case was noted. Dimensions (average diameter and length) of parent bubbles were measured just before or during the initial entrapment. Initial embolism volume and aspect ratio (length/radius) were calculated

Table 1. Formation of New Bubbles

Subject	Pretreatment	No. of Bubbles at Initial Lodging	No. of Bubbles Postelongation	No. of Bubbles Newly Created
Animal 1	Saline	1	3	2
Animal 2	Saline	1	2	1
Animal 3	Saline	1	2	1
Animal 4	Saline	2	2	0
Animal 5	Saline	1	1	0
Animal 6	Surfactant	2	8	6
Animal 7	Surfactant	1	14	13
Animal 8	Surfactant	2	2	0
Animal 9	Surfactant	1	12	11
Animal 10	Surfactant	3	12	9

using the dimensions measured, assuming that bubble shape approximates a cylinder with hemispherical end caps.[7] Predicted absorption times ($T_{predicted}$) for parent bubbles were computed based on the initial embolism volume and the initial aspect ratio dimensions measured using our mathematical model for bubble absorption as described Branger and Eckmann[7] and subsequently used by the authors.[8] Actual elapsed time ($T_{observed}$) required for parent bubbles or their last remaining observable post-breakup remnant to reabsorb from the embolized vessel was determined from the video microscopy recording. The percent change in actual (observed) absorption time from the time predicted, ΔT%, was calculated as follows:

$$\Delta T\% = \frac{T_{predicated} - T_{observed}}{T_{predicted}} \times 100\% \quad (1)$$

Bubble entrapment was followed almost immediately by vasoconstriction, with maximal bubble elongation occurring within 5–30 s. The corresponding average constricted embolism diameter was calculated using the initial embolism volume and the maximum measured embolism length, ignoring the 1–5% of total gas volume predicted to have been absorbed.[7] Any splitting or coalescence of bubbles was noted.

Statistical Analysis

The results from the two groups, control and surfactant, are presented as arithmetical mean ± SD. Statistical significance between groups at a given time point was established using the unpaired Student *t* test with equal variances at $P < 0.05$. Changes within the same group at different points were considered statistically significant at $P < 0.05$, calculated using the paired Student *t* test.

Results

Surface Tension

The surface tension of the 5 weight% BSA solution was 50 mN/m (compared with blood at 50 mN/m).[3] The neat (undiluted) surfactant had a surface tension of 19 mN/m. The surface tension of the protein solution decreased as the concentration of Antifoam increased. An Antifoam concentration of 1.5% by volume gives an intermediate surface tension of 34 mN/m, a value midway between the maximum (whole blood) and minimum (pure Antifoam) surface tensions measured.

General Hemodynamic Stability

The only observable change in overall blood pressure after the injection of either saline or Antifoam was a transient small increase (always < 10 mmHg systolic) accompanied by a slight widening of the pulse pressure (always < 7 mmHg). This was attributed to an acute increase in the intravascular fluid volume of the animal.

The administration of either pretreatment solution in the femoral artery did not significantly change vessel tone in the cremaster circulation. The Antifoam caused a maximal average dilation (11 ± 45%; $P = 0.61$) in the two vessels under observation for each experiment. The large SD for the change of vessel tone in the Antifoam experiments demonstrates that, despite an average dilation, several vessels showed constriction or no change in response to the surfactant. If dilation did occur, the changes were transient, lasting less than 2 min after completion of the surfactant injection and being fully resolved at the time of gas embolization (< 2% change in diameter at 2 min compared with preinjection baseline; $P = 0.29$). A pretreatment injection of saline caused no significant change in vessel diameter (< 1%; $P = 0.45$). There was no evidence of change in the global hemodynamics (heart rate or blood pressure) of either group when air was injected into the cremaster circulation.

Initial Bubble Entrapment

Air emboli, in the range of 4.2–10.0 nl, lodged in vessels within 6 s of their appearance in the cremaster circulation for both groups. An average of 1.6 ± 0.9 air injections were given in the five control experiments before an embolism greater than 4.0 nl lodged in the circulation, significantly fewer than the 3.2 ± 1.3 injections required in the surfactant group ($P < 0.05$). In only one control case did more than one bubble become entrapped to form the embolism; however, the majority of surfactant experiments had two or more bubbles

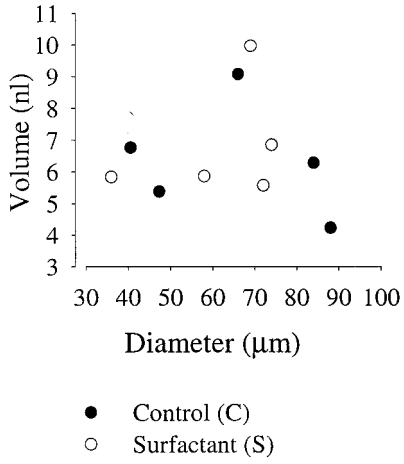

Fig. 1. Gas embolism volume in relation to the entrapping vessel diameter. Symbols signify the emboli in control (filled circles) and surfactant-pretreated (open circles) animals.

lodged together (table 1). There was no correlation between the embolism volume and the diameter of the vessel in which the bubble lodged, for either experimental condition, demonstrated in figure 1.

The average diameter of the lodged emboli in the control group was 65.2 ± 21.2 μm, no different than after Antifoam pretreatment (61.8 ± 15.7 μm; $P = 0.78$). The difference between groups in initial bubble lengths at lodging was also not significantly different: 2,523 ± 1,777 μm in controls *versus* 2,669 ± 1,768 μm for the surfactant group ($P = 0.90$). Dividing the bubble length by the radius gave a normalized value for the bubble configuration, or the aspect ratio, independent of bubble volume.[7] The control group had an average initial aspect ratio of 100.5 ± 97.6 compared with 108.4 ± 118.3 ($P = 0.91$) for the surfactant group.

Bubble Dynamics

Emboli in the control group underwent little change in diameter after entrapment. The average embolism diameter went from 65.2 ± 21.2 to 63.2 ± 21.3 μm, an average vessel constriction of 3.4 ± 2.4%. In contrast, the bubbles in the surfactant group underwent large conformational changes 6–30 s after entrapment. Arteriolar constriction reduced the average bubble diameter from 61.8 ± 15.7 to 45.5 ± 9.6 μm, an average decrease of 24.1 ± 14.0%, significantly different from controls ($P < 0.01$; fig. 2).

The decrease in embolism diameter was accompanied by a bubble elongation of 8.2 ± 7.1% in controls, compared with 92.7 ± 80.9% in the Antifoam group ($P = 0.048$; fig. 2). These length and diameter changes also appeared in the embolism aspect ratio. The difference, $\Delta X = X_m - X_i$, between the aspect ratio before lodging, X_i, and at maximal elongation, X_m, gives a representative value of an absolute shape transformation for each embolism. The average ΔX in the control group was significantly smaller than in the surfactant group (12.2 ± 13.3 *vs.* 103.9 ± 61.2; $P < 0.01$; fig. 2). The average value of X_m was significantly different than the average X_i for the surfactant group (212.3 ± 118.3 *vs.* 108.4 ± 118.3; $P < 0.02$) but not in controls (112.3 ± 103.7 *vs.* 100.1 ± 96.6; $P = 0.13$).

The considerable degree of embolism shape change, which occurred in the surfactant group, was often associated with the formation of multiple bubbles from the original, or parent, embolism, a phenomenon described as embolism breakup. There was only one control case in which breakup resulted in more than two bubbles from the parent bubble (table 1). In all but one of the five surfactant experiments, breakup led to the formation of six or more additional bubbles from the parent bubble. An example is shown in figure 3.

Vessel constriction in the segment of vessel containing the embolism bubble typically appeared to be transient. An example of this time-dependent phenomenon, taken from a single surfactant and control experiment, is shown in figure 4. The vessel originally constricted within 10 s, accompanied by bubble elongation (location 2 on the graph) and remained constricted for approximately 10 s, coinciding with bubble breakup (location 3 on the graph). Within 90 s the vessel returned to within 20% of its original diameter, separating the smaller, broken-up bubbles (location 4 in the graph). The dynamic steps in figure 4, illustrating the change in vessel diameter, correspond to the photographs in figure 3, demonstrating the resultant bubble behavior. Ensemble data for this behavior are also presented in figure 5. Data are normalized to the baseline vessel diameter, and error bars represent the SD from the mean at each time point.

Bubble Reabsorption

Predicted and observed reabsorption times for each embolization experiment are presented along with initial bubble volumes in table 2. The predicted absorption times for parent bubbles in the control animals (range, 14.7–21.8 min) were similar to the times predicted for reabsorption of parent bubbles in the surfactant-treated group (range, 15.0–23.1 min). The observed reabsorption times measured in the control group ranged from 14.5 to 23.1 min and were closely predicted individually. The percent deviation between the two values, as calculated by equation 1, ranged from −6.2 to 7.7%, with a mean (± SD) of −0.7 ± 5.5% for the group.

The observed reabsorption time for the last remaining remnant of parent bubbles after breakup in the surfactant group ranged from 10.2 to 14.8 min. This was much faster than was predicted, deviating from the value predicted for the parent bubble in the absence of surfactant by 29.3 ± 5.5% (range, 23.8–36.0%; $P < 0.00003$ compared with control).

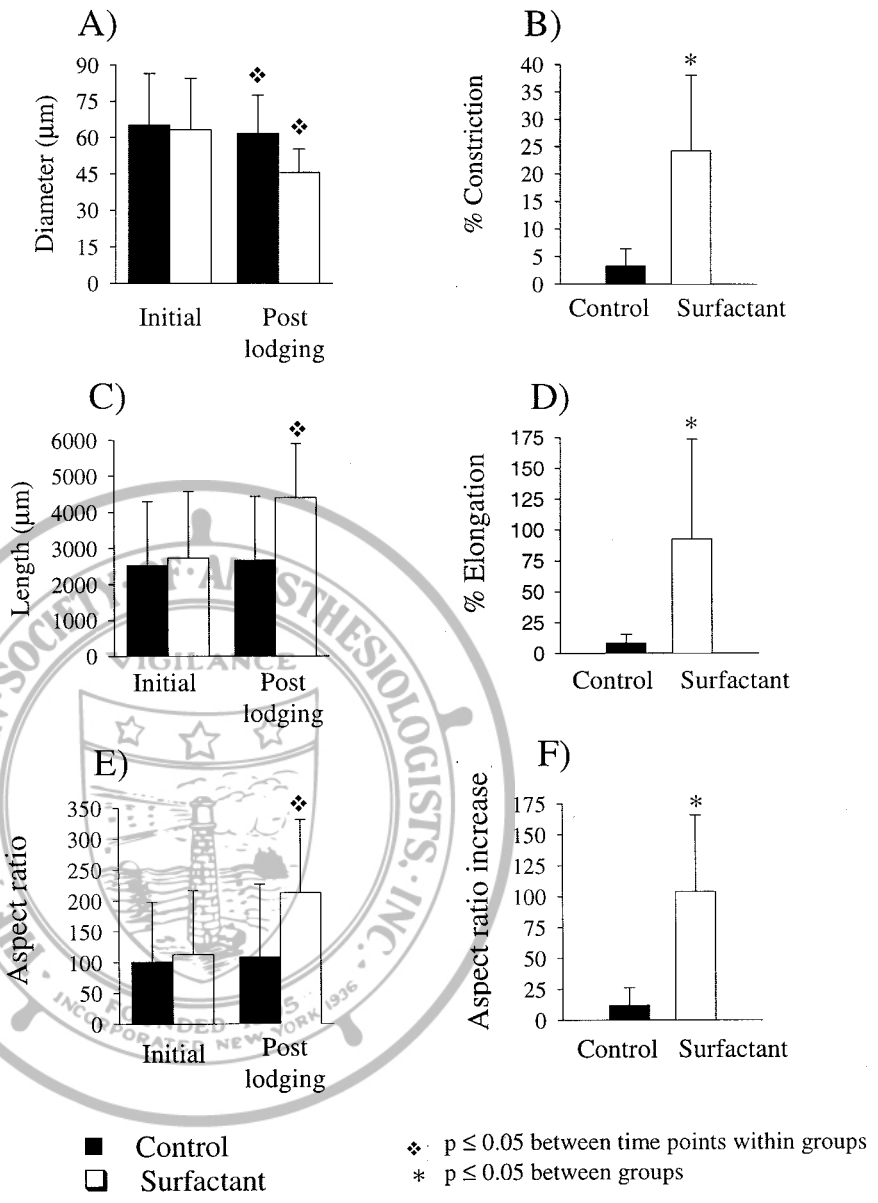

Fig. 2. Air embolism conformational changes during entrapment. Embolism (*A*) diameter, (*C*) length, and (*E*) aspect ratio (bubble length/radius) at: initial lodging (Initial) and maximal deformation (Post-lodging). Parameters of (*B*) percent bubble constriction, (*D*) percent bubble elongation, and (*F*) nondimensional embolism deformation, presented as increase in aspect ratio. Animal groups are control (filled squares) and surfactant pretreatment (open squares). Statistical significance noted at $P < 0.05$ between groups (asterisk) and between time points within groups (diamonds).

Discussion

Air bubbles in the circulation block blood flow, cause tissue ischemia, damage endothelial cells, and initiate other thromboinflammatory effects that can be extremely serious in the cerebral circulation.[9,10] It has been hypothesized that lowering surface tension at the air–blood–vessel interface mitigates embolism-induced injury by enhancing bubble breakup and more rapid reabsorption, along with promoting entrapment in more distal vessels. Our *in vivo* experiments use intravital microscopy directly to examine the modulation of air bubble behavior by a surfactant, Dow Corning Antifoam 1510US.

Surface Tension

As expected, increasing the surfactant concentration reduced surface tension of a 5 weight% BSA solution in concentration-dependent fashion *in vitro*. We selected an *in vivo* surfactant concentration of 1.5% of the estimated rat blood volume as the target dose. This concentration was assumed to decrease the air–blood interfacial tension significantly *in vivo*, based on the BSA result, while being well tolerated hemondynamically by the animals.

Initial Bubble Entrapment

The addition of surfactant was expected to enhance bubble splitting, increase bubble deformability, and speed reabsorption.[3,6,7] Our results support these hypotheses and, as explained in the succeeding two paragraphs, the interfacial fluid mechanics literature provides the physical and chemical rationale for these findings. First, twice as many air injections were required to embolize successfully the surfactant group. In

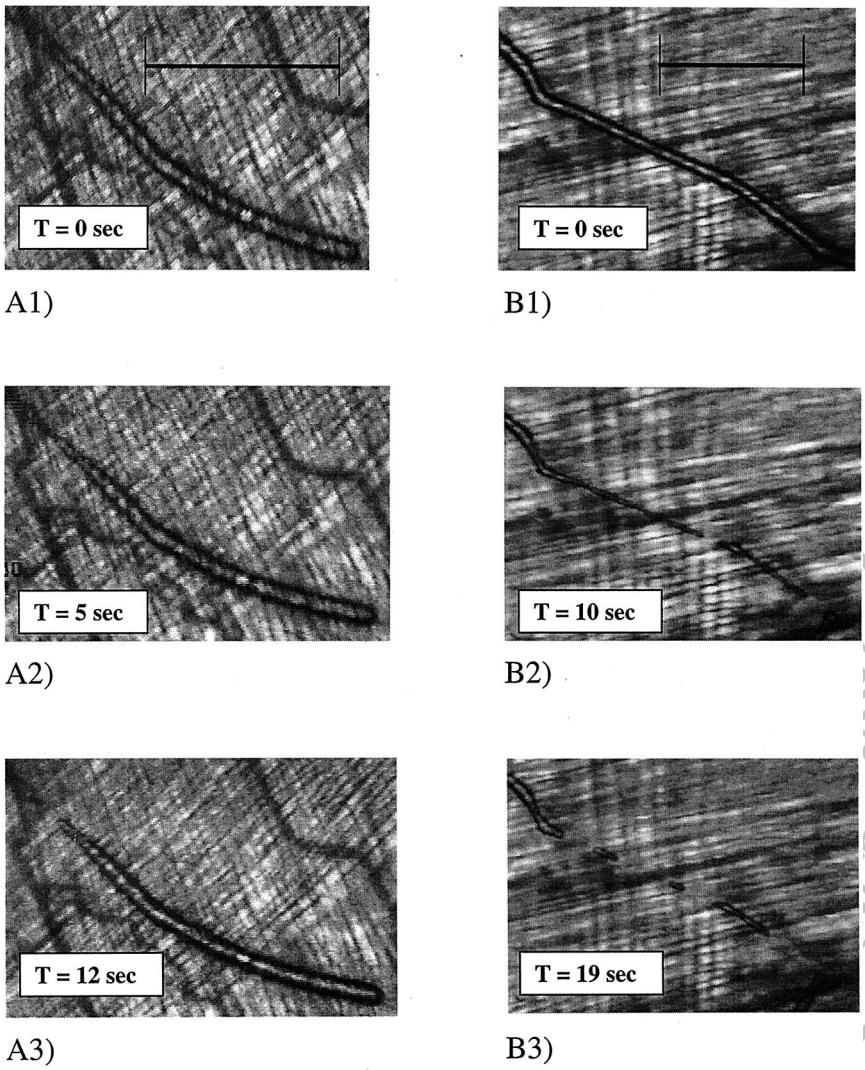

Fig. 3. Dynamic sequence of events of *in vivo* gas embolism in (*A*) control and (*B*) surfactant-pretreated animals. In both cases, numbers represent (1) initial air entrapment, (2) embolism constriction, and (3) breakup into smaller bubbles. Control case (average initial embolism diameter = 66 μm; scale bar = 700 μm) (*A*) shows only very local constriction, whereas surfactant case (average initial embolism diameter = 58 μm; scale bar = 450 μm) (*B*) demonstrates overall constriction and bubble breakup.

addition, in the majority of experiments in the surfactant group, the embolism initially consisted of two or more bubbles lodged together. This occurred only once in the saline-pretreated animals (table 1). The rate of gas reabsorption was accelerated in the surfactant-treated group (table 2). This is evidence that surfactant pretreatment resulted in smaller bubble volumes and more rapid disappearance from the circulation.

Tsai and Miksis[11] and Manga[12] predicted the first two of these behaviors in computational works. Tsai and Miksis[11] calculated that a bubble flowing through a constricted capillary tube demonstrates enhanced splitting, or "snap-off," of smaller bubbles, if surfactant were present. In the mechanism identified, surfactant alters the interfacial tension gradients at the bubble boundary, causing inhomogeneous stress fields to develop and inducing a radially inward motion of the interface. As the bubble surface area changes, the surfactant is redistributed along the interface. This time-dependent process results in a nonuniform surfactant concentration on the interface. The surfactant gradients that arise accelerate local motion of the deforming interface until the snap-off process is completed. The mechanism favors parent bubble breakup into multiple smaller bubbles as the bubble travels through the constriction.[11]

Bubbles are predicted to be more prone to enter the low-flow-rate branch of a bifurcation as the bubble size decreases.[12] Larger bubbles were predicted to deform and migrate preferentially into the high-flow-rate branch. Smaller bubbles, in contrast, were unable to deform as rapidly and, although they could still enter high-flow-rate branches, were more likely than large bubbles to enter low-flow-rate branches.

Combining these two findings yields a sound explanation for experimental results. Assuming that bubbles traveling from the injection site (femoral artery) to the cremaster vasculature encounter multiple areas of local constriction, bubbles injected in the surfactant group show more pronounced splitting (snap-off) than do controls. Although the distribution of the surfactant on the bubble interface is unknown, the observable difference in parent bubble breakup between the groups strongly

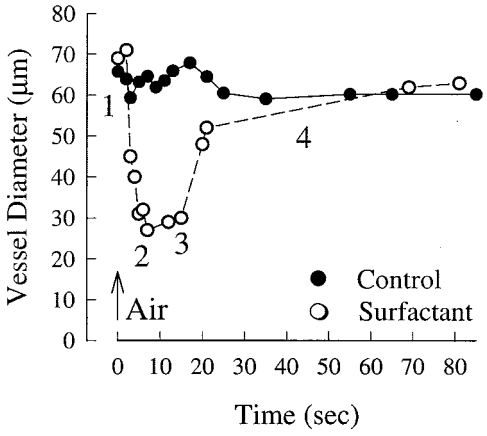

Fig. 4. Transient behavior of vessel diameter after exposure to air embolism in control (filled circles) and surfactant-pretreated (open circles) animals. Numbers define the specific events: (1) air becoming entrapped, with no change in vessel diameter; (2) rapid, massive vessel constriction over air; (3) continual vessel constriction causing embolism breakup into multiple smaller bubbles; (4) gradual return to original vessel diameter, separating individual bubbles from one another.

suggests that the surfactant plays a dominant role in eliciting this behavior.

The smaller bubbles resulting from breakup are more likely to enter vessels having lower flow rates. Such vessels do branch off the inferior epigastric artery and lead to end organs other than the cremaster muscle. The position of the injection catheter tip within the femoral artery is assumed to be very near the origin of the vessel supplying the cremaster, yet certainly the majority of the gas injected is directed to other tissues and does not end up in the cremaster. In the presence of the surfactant, this could be exacerbated. Blood flow is expected to decrease in embolized vascular beds. More gas from smaller bubbles (as a result of the surfactant) should follow into such regions, leaving less gas volume to enter the cremaster microcirculation. This mechanism is consistent with the result that, after surfactant pretreatment, more air injections were required for successful embolization of the cremaster circulation.

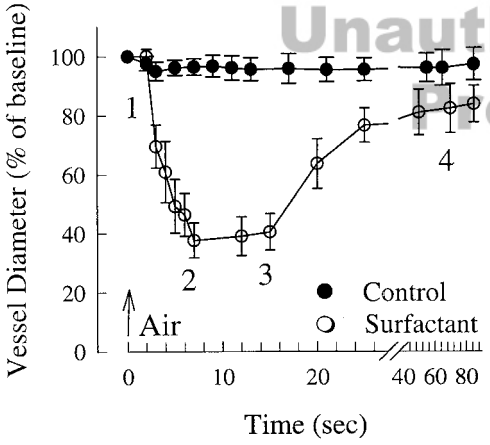

Fig. 5. Normalized ensemble data for transient behavior of vessel diameter after exposure to air embolism in control (filled circles) and surfactant-pretreated (open circles) animals. Numbers represent the same events described in fig. 4.

Another possible explanation is that, along with the transient effects on cremaster vessel tone caused by the surfactant, it may also have caused a redistribution of blood flow. The complete resolution of the surfactant effect on vascular tone observed in one tissue bed does not signify that the global distribution of blood flow is the same before and after administration of the Antifoam. This may have reduced flow to other tissue beds so that gas is preferentially diverted away from the cremaster. Only a narrow range of embolism volumes was examined after entrance into the cremaster circulation; therefore, the number and size or distribution of gas emboli in other tissues is speculative.

Our initial observations demonstrate no correlation between bubble volume and embolized vessel diameter in either group (fig. 1). These data do not support the hypothesis that a lower surface tension enables bubble entrapment in smaller diameter vessels[3]: in our experiments bubbles of the same average volume, length, and aspect ratio lodged in vessels of the same average diameter irrespective of treatment. This may result, in part, from limited transport time for the surfactant to diffuse out of the blood and onto the interface.[13] The data do not suggest that surfactant has no effect on bubble deformability, but merely demonstrate surfactant pretreatment did not change the initial conformation of the embolism. The data do indicate that surfactant increased bubble deformability, as described in the Bubble Dynamics section, below.

Bubble Dynamics

The presence of the surfactant did not appear to affect vascular reactivity directly. In a separate experiment, average vessel constriction and dilation to phenylephrine and acetylcholine, in the absence of air, did not differ by more than 5%, regardless of whether Antifoam was present in the bloodstream. However, bubble dynamics were significantly different with surfactant pretreatment. Within 30 s of lodging, emboli in the surfactant group had a larger decrease in average diameter (24.2 *vs.* 3.4% in controls; figs. 4 and 5) and consequently elongated more (92.7 *vs.* 8.2%; fig. 2).

The explanation for how the exogenous surfactant influenced these *in vivo* findings follows from the early observations documenting that muscular arteries in rabbit duodenal mesenteric loops constricted temporarily in the presence of air[14]; however, no constriction was noted in the arterioles. This is likely to have occurred because, although bubbles provoke vasoconstriction, the evoked change in vessel diameter must also account for the mechanical behavior of the gas–liquid interface of the bubble. There is a force balance across the gas–liquid interface dictating the final interfacial shape that

Table 2. Predicted and Observed Absorption Time of Parent Bubbles and Remnants in Embolized Vessel

Subject	Pretreatment	Bubble Volume nl	$T_{predicted}$ min	$T_{observed}$ min	ΔT% Equation 1
Animal 1	Saline	6.28	16.6	17.4	−4.8
Animal 2	Saline	5.38	14.7	14.5	1.4
Animal 3	Saline	9.08	21.8	23.1	−6.2
Animal 4	Saline	4.23	15.8	14.6	7.7
Animal 5	Saline	6.76	16.9	17.2	−1.9
Mean	—	—	—	—	−0.7
SD	—	—	—	—	5.5
Animal 6	Surfactant	6.85	17.0	12.1	28.9
Animal 7	Surfactant	5.86	15.4	10.2	33.7
Animal 8	Surfactant	9.97	23.1	14.8	36.0
Animal 9	Surfactant	5.84	15.3	11.6	24.1
Animal 10	Surfactant	5.57	15.0	11.4	23.8
Mean	—	—	—	—	29.3*
SD	—	—	—	—	5.5

$T_{predicted}$ calculated from measured bubble dimensions using theoretical model[7]; $T_{observed}$ measured from experimental video recording.

* $P < 0.00003$ compared with saline.

must also be considered. The law of LaPlace governs this force balance. The law of LaPlace, $P_i = P_T + (2\sigma/R)$, is a balance between P_i, the pressure internal to the bubble, P_T, the external pressure, σ, the surface tension, and R, the radius of curvature of the bubble end caps. As an example, if the pressure external to the bubble remains constant but the surface tension is reduced, the radius of curvature of the end caps can decrease while a constant internal pressure is maintained. As the lumen narrows, the bubble must elongate to preserve volume.

Our measurements indicated that the surfactant did not increase the muscle force of contraction generated by the vessel wall to allow the generation of a greater external pressure outside the bubble. Instead, it appears that surfactant permits a smaller radius of curvature to form at the bubble end caps. Thus, the bubble becomes more deformable and this, in turn, allows the active vasoconstrictor response elicited by the bubble to proceed to a smaller vessel diameter. The surfactant, in effect, facilitates vessel constriction around the bubble by reducing resistance to a decrease in the radius of curvature. In the absence of the surfactant, the vasoconstriction is still active, but the bubble is stiffer and the vessel can only narrow by a few percent.

To illustrate how the force balance accounts for the observed behavior, we inserted the estimated values for surface tension (converted from 50 mN/m to 0.0375 mmHg · cm) and bubble radius at the time of entrapment, just before vasoconstriction. This gives an internal bubble pressure of:

$$P_i = P_T + \frac{2\ (0.0375\ \text{mmHg} \times \text{cm})}{(0.0031\ \text{cm})} = P_T + 24.2\ \text{mmHg} \quad (2)$$

After embolization, there is no stimulus to cause a change in bubble internal pressure (it will elongate instead) or external pressure (equal to the arteriolar pressure); therefore, the only components of the force balance, which can change, are the radius and the surface tension. Solving for the value of the radius, R, which will preserve the internal bubble pressure with a decreased surface tension of 34 mN/m (or 0.0255 mmHg cm, the estimated value of surface tension with 1.5% Antifoam present) gives:

$$P_i = P_T + 24.2\ \text{mmHg} = P_T + \frac{2\ (0.0255\ \text{mmHg} \times \text{cm})}{(R,\ \text{cm})} \quad (3)$$

in which R is equal to 0.0021 cm or a diameter of 42 μm. The average vessel diameter in the surfactant-pretreated animals was decreased to 45.6 μm after embolization, showing excellent agreement with our predicted value. Thus, the presence of the surfactant enables a reduction in vessel diameter as a result of the vasoconstriction stimulated by the bubble.

An additional potential benefit is that as the radius of curvature shrinks, the surface area of the end cap falls, and the surfactant molecules become more tightly packed. This increases the local surfactant concentration, which lowers surface tension further and creates a feed-forward loop for bubble constriction and elongation. The data support the notion that the surface tension in the control animals remained sufficiently high to limit the degree of vessel narrowing achieved despite stimulation of arteriolar constrictor mechanisms. In contrast, the surface tension reduction in the surfactant group permits greater vessel narrowing to occur, accompanied by the bubble shape change and breakup observed (fig. 3).

The shape change facilitated by surfactant also transiently increased the total bubble surface area. This provides a greater interface for gas diffusion outward and, in

theory, increases the speed of bubble reabsorption.[7] Reabsorption is further enhanced by parent bubble breakup into multiple smaller bubbles, a feature of surfactant, but not saline, pretreatment (fig. 3). van Blankenstein *et al.*[3] proposed that surfactant would increase the surface area of the bubble and speed up absorption, which our observations support; however, we have shown this elongation to occur only after bubble entrapment and not during lodging, as previously speculated.[3]

Theoretical calculations have predicted that a bubble will either relax into a spherical shape or break up into smaller bubbles, depending on the aspect ratio.[15] These effects are attributed to a surface tension instability exacerbated by an increasing aspect ratio. In our experiments, the bubbles in the surfactant group undergo a large increase in the aspect ratio (fig. 4, location 3), and eventually breakup (fig. 3), agreeing well with this theory.[15]

The relation between bubble size and dislodgment, however, is unclear. Smaller bubbles have less surface contact area for adhesion with the endothelium, especially as the vessel wall transiently goes back to its original diameter (fig. 4, location 4) and the newly created bubbles become separated from one another (fig. 3). With a reduction in the contact area, the adhesive forces between the activated blood constituents and the endothelium are theoretically decreased.

The relation between bubble size and dislodgment, however, is unclear because the molecular structure of the adhesion elements between the bubble surface and the vessel wall is unknown. *In vitro* work has shown that larger bubbles dislodge at lower flow rates than smaller bubbles, despite the greater contact area of larger bubbles with the wall.[16] However, those experiments have no other effects such as clot formation or macromolecule adsorption to the interface that could influence the adhesion force.

Our experiments indicate that the addition of exogenous surfactant to blood can sufficiently alter interfacial forces so that bubble dynamics after deposition are changed in a manner associated with reduced absorption times.[7] This study, then, provides some proof of concept, which may help to guide rational drug design of a compound selective for these desired interfacial effects. Other important effects of the surfactant we used were not investigated. These include immune, hemostatic, and toxic effects, as well as mechanisms of clearance and metabolism. All such topics would have to be addressed for an experimental compound to have a clinical application.

Although it was not specifically studied in the current work, the changes induced in bubble conformation could potentially minimize the degree of tissue damage incurred by blood flow obstruction. The nearly 30% reduction of the duration of blood flow obstruction achieved in this study could be associated with a reduction in ischemic tissue damage as well as minimization of bubble-induced endothelial injury. Exogenous surfactants introduced into the vasculature may therefore provide end-organ protection for individuals experiencing cerebral gas embolism.

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