Difficulties in laser Doppler measurement of skin blood flow under applied external pressure

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Abstract—During the course of a study of skin blood flow under applied external pressure, it became apparent that decreasing blood flow by loading the skin surface reveals problems that are fundamental to the method of laser Doppler flowmetry. These problems have to do with the fact that the laser Doppler is extremely sensitive to red cell motion of any kind, whether associated with the ordered red cell motion of blood flow or the random red cell motion associated with changes in temperature or vessel occlusion. This effect becomes increasingly important whenever blood flow is compromised (a situation of considerable clinical significance), since the random portion of the signal then becomes significant in comparison with the diminished blood flow. Experiments have been conducted in living animals and with stationary drops of blood which clearly show the importance of these effects with regard to the interpretation of laser Doppler signals. Significant laser Doppler flow signals were repeatedly observed after manipulations which could reasonably be expected to reduce blood flow to zero.

Key words: laser Doppler flowmetry, skin blood flow, external pressure, red blood cells, vessel occlusion.

BACKGROUND

In an effort to develop a possible “marker” for distinguishing those spinal cord patients who are likely to develop pressure sores from those who are not, we have been using laser Doppler flowmetry (LDF) to study the response of skin blood flow to externally applied pressure loading. Side-lying patients were subjected to vertical loadings applied to the proximal femur at controlled levels by simply adding weights to the loading fixture shown in Figure 1. The loaded fixture rests on a cylindrical indenter which houses the fiber optic probe of the MedPacific LD 5000 laser Doppler flowmeter.

Laser Doppler flowmetry of skin blood flow is based upon measurement of the Doppler frequency shift in monochromatic laser light which is backscattered from cutaneous tissue. The frequency shift is associated with velocity of moving particles within the tissue. Because the red cells are moving at different velocities, the single laser output frequency is shifted to a spectrum of different frequencies, and is usually measured in terms of the broadening of the spectrum (2).

During these experiments, the DC level of the instrument (indicating intensity of the received laser beam) was seen to decrease dramatically as the applied load was increased. Since such changes in DC level have a large influence on the flow signal
readout of this machine, the instrument in our laboratory was subsequently modified to eliminate this change in DC level with loading.

But even with the modified laser Doppler instrument, we found that indicated skin blood flow under load remained at a level of about 30 to 40 percent of baseline flow, even at applied loads as high as 200 mmHg (Figure 2). Six subjects were tested. Repeatability was checked on 1 subject and found to be ± 5 percent.

THE PROBLEM

According to previous investigators using different techniques (1), loadings of 200 mmHg should be more than sufficient to produce blood flow occlusion. Therefore, one is left with the question of how laser Doppler measurements compare with measurements by other techniques. There have, in fact, been several studies of this question (3,4,5), and all investigators seem to agree that there is a definite Doppler signal indicating skin blood flow when the Xenon clearance technique indicates zero flow.

Some investigators (3,4) have suggested the use of an occluding arm cuff to establish a “physiologic zero,” from which one could apply an appropriate “correction” to all laser Doppler readings. We tried this technique by applying a pressure of 300 mmHg for the prescribed 2 minutes and found a “physiologic zero” of about 30 percent of baseline flow. Although such an offset would indeed be sufficient to force the indicated blood flow to zero at high loadings, we found that a similar occluding experiment carried out on a finger gave a “correction” of only 5 percent of baseline flow. It is apparent that the magnitude of a “physiologic zero” depends upon the anatomical site at which it is measured. We also know that the laser Doppler flowmeter does in fact indicate zero flow when presented with a truly stationary target, such as a flat table top. It is significant that the LD 5000 laser Doppler flowmeter is calibrated at the factory using cuvettes containing particle suspensions with no net flow. All in all, it seems quite clear that the laser Doppler may be measuring the detailed red cell motion beneath the probe, even when there is no net flow, since it cannot distinguish between random and ordered motion.

EXPERIMENTS

Human experiments (carried out at the Spinal Cord Injury Center, VA Medical Center, Palo Alto, CA)

In order to be sure that this situation is not peculiar to the particular instrument we are using, we conducted a side-by-side comparison of the LD 5000 by MedPacific (Seattle, WA) with the Periflux PF2 laser Doppler by Perimed (Stockholm, Sweden). Using a cylindrical indentor housing both needle probes, we applied high loadings (nearly 200 mmHg) to the proximal femur of a side-lying patient at bedside. The signals from the 2 instruments were indistinguishable, both indicating about 25 percent of baseline flow at equilibrium, although the Perimed instrument required about 5 minutes to equilibrate, while the LD 5000 took less than a minute. It therefore seems clear that the question of what the Doppler is measuring at low flows is not instrument-specific, but, rather, is a question of the technique itself.

Animal experiments (carried out at the Plastic Surgery Laboratory, Stanford Medical Center, Stanford, CA)

We then undertook animal experiments, using the same indentor as in the human experiments, with the
Perimed instrument at the low frequency setting (4 Hz). An island of skin connected to the animal only by the inferior epigastric vein and artery was created in an SD albino rat. This island flap was positioned on the bench and laser Doppler flowmeter (LDF) measurements were made under various loads. The preoperative LDF value was 29 volts. With the flap isolated on the bench and the probe positioned in the indentor, the LDF value fell to 14 volts. When the indentor was loaded with 300g (19 mmHg average pressure), the value fell to 3 volts (21 percent of baseline reading for the isolated flap). Addition of another 800g (71 mmHg total average pressure) produced an immediate rise to 8 volts, followed by a decline to 5 volts at 5 minutes and 3 volts at 15 minutes (still 21 percent of baseline). That is, no further change in equilibrium flow was indicated by the Doppler.

In the next experiment, a micromanipulator was used to apply the probe to the skin surface without pressure, and a shield was used to prevent ambient light from reaching the probe. With the flap isolated on the bench, a reading of 18 volts was obtained, and the pedicle attaching the flap to the animal was then clamped off. The LDF reading declined to 13 volts (72 percent of baseline) by 2 minutes and remained at that level for another 10 minutes. When the clamp was removed, the LDF reading returned to 15 volts, and when the flap was surgically severed, the measurement finally returned to the baseline value of 18 volts.

Since the flap studied above had first been subjected to loadings and then to the clamping experiments, it was probably in a nonphysiologic condition. In the next experiment, traumatization of the flap was minimized. A flap was created as before, and in this case, the preoperative value of 24 volts dropped to 4 volts (17 percent) when the flap was simply isolated on the bench. When the pedicle was then clamped, no further change occurred. Subsequently, the clamp was removed, the pedicle was washed with lidocaine solution to reduce vascular spasm, the vein was severed, the artery was severed, and finally the flap was placed at 4 degrees C for 96 hours. None of these manipulations reduced the LDF reading below 3 volts (75 percent of baseline).

In all of these animal experiments, LDF values, which were a significant percentage of baseline values, were obtained under a variety of manipula-
tions that can reasonably be expected to reduce blood flow in the flap to zero.

Static drop experiments

One way to ensure that blood flow is zero, and simultaneously to eliminate the effects of tissue physiology or optical properties, is to isolate an individual drop of blood which is held stationary in a suitable apparatus. Accordingly, guinea pig blood, anticoagulated with heparin, was obtained by cardiac puncture. Individual drops with a volume of 5 microliters or less were formed on the surface of an aluminum block. The light shield supplied with the Perimed instrument was placed over an individual drop that fit well inside the probe without contacting its sides. With this arrangement, observations on a series of isolated drops yielded LDF values ranging up to 25 volts, a value approximating that recorded from living tissue. Measurements using drops of saline, on the other hand, gave values less than 0.3 volts, and measurements with no drop in the apparatus gave a zero value.

When continuous recordings were made on individual drops subjected to the constant ambient temperature of the unheated block (about 25 degrees C), the LDF readings declined nearly linearly as the drop evaporated to dryness (Figure 3). The rate of decline and the total time to dryness varied from drop to drop. When the aluminum block was preheated to about 32 degrees C, the LDF signal remained at a high level, or even increased, for the first few minutes, and then declined more rapidly than did the unheated drops. For drops to which heat was added after an initial delay, the LDF reading first increased and then decreased rapidly, as shown in Figure 3. The absolute value of LDF readings from this series of static drops ranged up to 25 volts, comparable to those obtained on tissue in vivo.

Because of the large drop-to-drop variation in LDF measurements, 2 experiments were carried out to check the effects of blood volume. In the first, drop volume was varied from 0.5 to 5 microliters (Figure 4), indicating a general increase in LDF signal with increasing volume. In the second experiment, fresh blood drops were diluted with varying volumes of saline (Figure 5). Here is seen an increase in signal up to a value of 40 percent whole blood in saline, with an apparent gradual decline thereafter.
Figure 4.
Effect of drop volume on laser Doppler flow (LDF) readings from a stationary drop of blood.

Figure 5.
Effect of relative blood content on laser Doppler flow (LDF) readings from a stationary drop of blood and saline.
DISCUSSION

Many questions arise in using the laser Doppler to measure skin blood flow, not the least of which is, "What do we really mean by skin blood flow in the first place?" There are the several discrete layers of the skin, the varying directions of the vessels within them, and the varying velocities of the blood within the vessels. Since the laser beam necessarily includes a large number of vessels in its line of sight, one must be receiving some sort of "lumped" indication of blood cell motion. As one applies external loading to the skin, the question is further complicated by the following facts: 1) the laser beam reaches to an unknown and varying depth depending on the skin displacement; and, 2) the associated tissue deformation must cause changes in vessel distribution and/or geometry which in turn affect the calibration of the instrument (2).

The present investigation has concentrated on the single issue of, "What is it that the laser Doppler flowmeter is detecting at reduced flow levels produced by partial or full occlusion of the vessels?" We have investigated both occlusion by external pressure and occlusion by clamping of the feeding vessel. In both cases, when all net flow is stopped, there remains a clear and sizable signal generated by the laser Doppler that is evidently associated with the motions of individual red blood cells. To simply cancel this signal out as being "not useful" flow, or to apply a simple "correction" to the output signal, is to ignore the fact that the laser Doppler flowmeter cannot distinguish between directed (or net) flow and random red cell motion. This distinction becomes increasingly important as the flow velocities decrease, which is a situation of considerable clinical interest.

For the limiting use of zero net flow, the laser Doppler signals from single drop experiments have shown that the instrument responds sensitively to changes in the random kinetic energy of individual blood cells caused by temperature changes within the physiologic range. The flow signal is also sensitive to changes in the relative blood content, and therefore to local hematocrit changes which may occur in the microcirculation. How significant the contribution of random kinetic energy is to the indicated flow at different absolute levels of blood flow is not known.

CONCLUSION

Perhaps the only conclusion to be drawn from this investigation is that the laser Doppler flowmeter, when used in accordance with the manufacturer's instructions, cannot distinguish between random and ordered motion of red cells at low flows. Therefore, the zero offset of the correlation with other methods does not represent a simple "error" to be discounted or corrected for, but rather indicates a fundamental difference in what is being measured. At high flow rates, this distinction becomes relatively unimportant.

The variation of the laser Doppler flow signal with relative volume of blood in a single drop indicates further that there is also a dependence on hematocrit. Here again, especially in the microcirculation, it is not clear that hematocrit will remain constant if the flow becomes compromised.

All in all, the observations presented here should serve as a caution to those who would use laser Doppler flowmetry to assess microcirculatory changes under conditions in which skin blood flow is compromised to the point that the contributions of random red cell motion to the Doppler signal may become significant.

REFERENCES