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BRIEF COMMUNICATION

Capillary Blood Cell Velocity in Human Skin Capillaries Located Perpendicularly to the Skin Surface: Measured by a New Laser Doppler Anemometer

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INTRODUCTION

In general, capillary loops of the skin are perpendicularly oriented to the skin surface. Only in a few areas of the human body, the lips, the nipple, or the nailfold, do the capillary loops run parallel to the skin surface. In the latter area capillary blood flow can be measured by means of modern noninvasive techniques like the so-called videocapillary microscope with frame-to-frame analysis (Bollinger *et al.*, 1974), the flying spot technique (Tyml and Ellis, 1982), or the cross-correlation method (Fagrell *et al.*, 1977a). However, there has been almost no technique available to measure skin blood flow in capillary loops located at a 90° angle to the skin surface until recently, when a new laser Doppler anemometer with a very small sample volume was introduced. The aim of this study was to evaluate this new device in a clinical situation, to control reproducibility, and to compare our results with results obtained from previous studies using other microscopic techniques.

MATERIALS AND METHODS

Laser Doppler Anemometer

In our investigations the laser Doppler anemometer CAM1 (KK Technology, England) was used. A technical description is given schematically in Fig. 1. The CAM1 includes a laser source (1.5 mW Laser Diode, wavelength 780 nm), focused by a microscope objective lens to a spot size of approximately 10-μm diameter. This results in a very small sample volume, so that the velocity in capillaries of 9.8- to 32.1-μm diameter can be singled out. A CCD camera (Model XC-75CE; Sony, Japan) is focused

[1] This paper contains results of the dissertation of Volker Baier.

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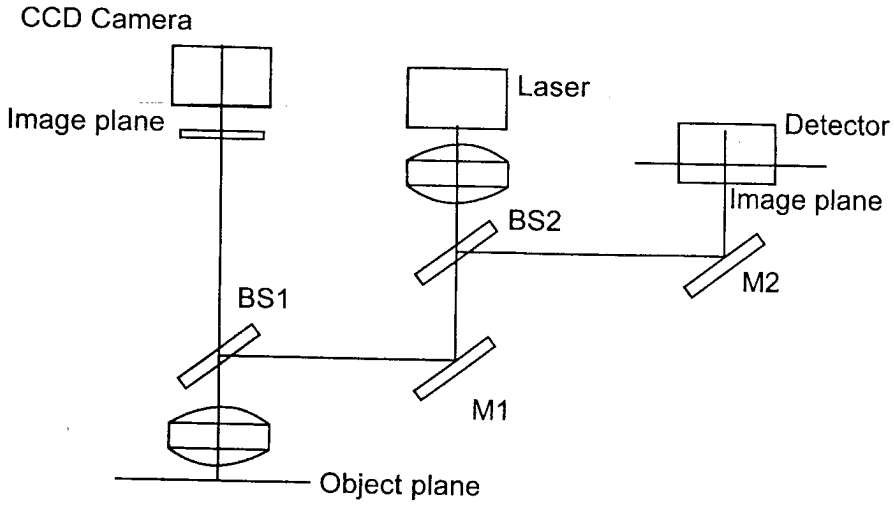

FIG. 1. Schematic diagram of laser Doppler anemometer CAM1. M, mirror; BS, backscatter.

so that the object plane and the laser focal point are the same. The output from the camera is used to identify the location of capillaries within the field of view. The operator then adjusts and maintains the position of the CAM1 so that the laser beam is positioned on a suitable capillary. An acoustic control with sounds from the Doppler shifts provides a good position for the device during the entire measurement. If a blood cell is moving with a velocity component perpendicular to the object plane then the laser radiation will be reflected with a Doppler shift. Laser radiation is also scattered by the vessel wall and surrounding tissue without being Doppler shifted. The CAM1 objective L2 collects some of the Doppler shifted and unshifted laser radiation. The wavelength-dependent beamsplitter BS1 separates the collected laser radiation from the CCD image and routes it via mirrors M1 and M2 and beamsplitter BS2 to the photodetector. The photodetector detects the mixing or heterodyning of the two optical signals. The mixing produces a signal containing the sum and difference frequencies, and the difference frequency, or actual Doppler shift, generates an electrical current in the photodetector. This is then amplified and processed, and the Doppler shift frequency detected. Since the Doppler shift is proportional to the velocity of the reflecting blood cells, the velocity is readily calculated.

Subjects

All measurements were performed in healthy volunteers. Subjects suffering from diseases influencing the microcirculation of the skin such as arterial hypertension, Raynaud's syndrome, arterial occlusive disease, atopic dermatitis, or collagen vascular disease were excluded. Every measurement was carried out at the dorsal aspect of the proximal index finger. The subjects were investigated in a sitting position with the hand at heart level after acclimatization for at least 30 min. The finger under investigation was stabilized by a special finger holder. Moreover the forearm was immobilized by a cuff slightly inflated at 5 mmHg. To make the skin transparent and to further minimize the reflections from the skin, surface oil was applied. The laboratory temperature was maintained at 21°C.

Measurement of Resting Capillary Blood Cell Velocity

Resting capillary blood cell velocity (rCBV) was studied in 20 subjects (10 male, 10 female, mean age 28). In order to detect intraindividual differences of rCBV within a patient, resting blood velocity was measured in five capillaries in each individual.

Postocclusive Hyperemic Response (PRH)

The postocclusive hyperemic response was investigated in 19 subjects (9 male, 10 female, mean age 27). For this a cuff was placed at the upper arm of each subject to perform suprasystolic arterial occlusion. At the beginning of the procedure rCBV was recorded for 3 min, after which the cuff was inflated for 3 min at 250 mmHg. When the pressure was released CBV was measured until resting blood flow could be registered. This procedure was repeated after 30 min. The percentage increase of CBV during PRH (PRH%) was calculated (Östergren and Fagrell, 1985).

Statistics

To control reproducibility a regression analysis was performed. Differences between the values before and after the test procedures were tested using Student's t test for paired samples (SPSS for Windows, Germany).

RESULTS

Resting Blood Velocity

The mean CBV during rest was 0.47 mm/sec (SD ± 0.37 mm/sec, range 0.14 to 0.93 mm/sec). The average intraindividual difference between max rCBV and min rCBV was 0.30 mm/sec (SD ± 0.18 mm/sec). The maximum difference between the capillaries of a single subject ranged up to 0.63 mm/sec.

Postocclusive Reactive Hyperemia

In every volunteer a postocclusive hyperemic response could be observed. CBV values increased from rCBV = 0.47 mm/sec (SD ± 0.37) at rest to 0.90 mm/sec (SD ± 0.46 mm/sec) peak blood cell velocity. The peak occurred after 24.9 sec (tpCBV) (SD ± 9.2 sec). PRH was 118%. The reproducibility of pCBV ($r = 0.67$; $P \leq 0.002$) and tpCBV ($r = 0.97$; $P \leq 0.0001$) was high, but the reproducibility of PRH was weak ($r = 0.09$; $P \leq 0.002$).

DISCUSSION

Laser Doppler anemometry has not been used for quantification of blood flow in capillaries *in vivo* in human so far. A laser Doppler anemometer was used for the first time by Kreid and Goldstein. They measured velocity profiles in capillaries *in vitro* using the heterodyne technique (Kreid and Goldstein, 1971). Riva *et al.* used the same technique to determine CBV in retinal arteries (Riva *et al.*, 1979). All the previous literature on laser Doppler anemometers for measuring blood velocities in single vessels were designed to measure velocities in the object plane. Also many of these devices used the dual beam, or fringe laser Doppler technique (Einav and Berman, 1988). In order to obtain satisfactory signals a minimum number of fringes are required, thus limiting the minimum measurement volume. Devices not utilizing the dual beam method required larger beam areas to increase signal levels. By seeking to measure velocities in vessels perpendicular to the object plane, it has been possible to reduce the laser spot size.

In the present study first experiences with a new laser Doppler anemometer are reported. For the first time it was possible to perform *in vivo* measurements of capillary

blood cell velocity by means of a laser Doppler anemometer in capillaries smaller than 65 μm diameter (Koyama *et al.*, 1975).

The mean rCBV of 0.47 mm/sec is slightly lower than has been reported in the literature (0.84 mm/sec, Bollinger *et al.*, 1974; 0.8 mm/s, Butti *et al.*, 1975; 0.65 mm/sec, Fagrell *et al.*, 1977a; 0.66 mm/s, Jacobs, 1985). We consider that the primary reason that the rCBV from the CAM1 measurements is lower compared to previous studies is the different measurement sites. In the present study we measured blood cell velocity in capillaries of the dorsal aspect of the proximal phalanx and not in the capillaries of the nailfold. The nailfold capillaries surely have unique thermal and nutritional conditions different than the rest of the skin, being such a thin layer of skin. Sometimes in our device it is difficult to determine which side of the capillary apex is the arterial side. Another reason may be that velocity mainly was assessed in the venous limb of the capillary loop, whereas usually CBV is lower than in the arterial limb, as in our device the venous and arterial limbs of the capillary loop are sometimes relatively hard to distinguish.

Absolute values of CBV, obtained in measurements with an environmental temperature of 21°, ranged from 0.14 to 0.93 mm/sec in different individuals. Differences in velocity of up to 0.63 mm/sec in adjacent capillaries of the same subject could be found. Inter- and intraindividual differences in CBV have already been reported in the literature (Bollinger *et al.*, 1974; Butti *et al.*, 1975; Fagrell *et al.*, 1977b).

The PRH response after suprasystolic occlusion is a well-established determinant in blood flow parameters and was expected to have high reproducibility (Fagrell *et al.*, 1977b; Fagrell and Intaglietta, 1977). As in other studies a good reproducibility of the pCBV and the tpCBV value could be shown while the variation of PRH% and rCBV is larger, which has been described already in studies using the cross-correlation technique (Östergren and Fagrell, 1986).

In our opinion, laser Doppler anemometry has three advantages:

1. For the first time a device is available to measure rCBV in human capillaries oriented perpendicular to the skin surface.
2. Almost every blood cell velocity is measurable. The range goes from 0.1 to 14 mm/sec. There are many limitations in other techniques.
3. All measurements are performed online and it is comparatively convenient to use it in a clinical situation.

Altogether measurements of high reproducibility are possible assessing both capillary morphology and capillary blood cell velocity. So this new device is a very innovative technique and appears to be particularly valuable to assess dermal microcirculation noninvasively.

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