Simultaneous Assessment of Blood Flow in UVB-inflamed Human Skin by Laser Doppler Flowmetry and the 133-Xenon Washout Technique

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The purpose of the study was to compare skin blood flow by laser Doppler flowmetry (LDF) and the 133-Xenon washout technique in UVB-inflamed human skin. Six healthy subjects participated in the study. Forearm skin blood flow was measured prior to irradation and then 8, 24, 48 and 72 h after the exposure to twice the minimal erythema dose of UVB. Baseline blood flow as measured by the 133-Xenon washout method was 5.2 (range 3.0 to 10.4) ml/100g/min and LDF flux was 3.1 (range 2.7 to 5.7) arbitrary units. Following irradiation, maximum blood flow increase as evaluated by the 133-Xenon washout was 10.8 (95% confidence interval 3.7 to 11.3) times baseline blood flow, corresponding to an absolute blood flow of 47.5 (range 33.8 to 56.3) ml/100g/min. Maximum increase after induction of inflammation as assessed by LDF was 34.6 (95% confidence interval 24.6 to 56.5) times baseline flux. The two methods showed comparable time courses in all subjects. A significant correlation between the two methods was found, Spearman's rho = 0.54, $p = 0.006$. The relative LDF blood flow increase was 4.2-fold (95% confidence interval 2.7 to 5.0) greater than the increase measured by the 133-Xenon washout method. These results are at variance with previous comparative studies of the two methods. Some explanations are discussed. *Key words: UVB-inflammation; Skin blood flow; Laser Doppler flowmetry; 133-Xenon washout.*

Laser Doppler flowmetry (LDF) has been introduced for non-invasive assessment of total skin blood flow. The method has previously been used to measure blood flow in UVB-inflamed human skin in areas free from arteriovenous anastomoses (1, 2). Comparisons of LDF and the established 133-Xenon washout technique have previously been per-

formed and good correlations found (3, 4). As the 133-Xenon method measures total skin blood flow in areas without large numbers of shunt vessels, the results validated the LDF technique.

However, the experimental set-up can be criticised. The 133-Xenon washout method has been used without curve resolution and blood flow has been calculated following intradermal installation of the indicator, i.e. during an injection trauma phase (5). Previously LDF has been used without correction for the laser signal in non-perfused tissue (biological zero) and filter settings have rarely been stated.

Consequently, the purpose of the present study was to compare cutaneous blood flow obtained by simultaneous application of the 133-Xenon washout method and LDF, taking criticisms raised into account.

MATERIALS AND METHODS

Subjects

The study included 6 healthy, non-medicated Caucasians (4 men and 2 women, aged 21–49 years). The study was approved by the local Medical Ethics Committee and informed consent was obtained from each subject.

UVB inflammation

A set of two Philips TL 20W/12 tubes was used as the source of UVB light, which peaked at 310 nm (spectral flux: UVB 2.1 W; UVC 35 mW). Minimal erythema dose (MED) was determined for each subject using $\sqrt{2}$ increments from 0.05 to 4.0 J/cm^2 measured by an UVX–Digital Radiometer with an UVX-31 sensor (Ultra-violet products, Inc, California, USA). The test site was the upper part of the volar aspect of the right forearm. The MED was defined as the minimal dose necessary to produce a distinct, well-defined erythema 24 h after irradiation.

Measurement of blood flow by 133-Xenon washout

The atraumatic, epicutaneous labelling technique was used (6, 7). A chamber (10 mm in diameter) formed by double-sided adhesive tape and a gas-tight Mylar membrane was attached to the skin. 133-Xenon in saline was injected into
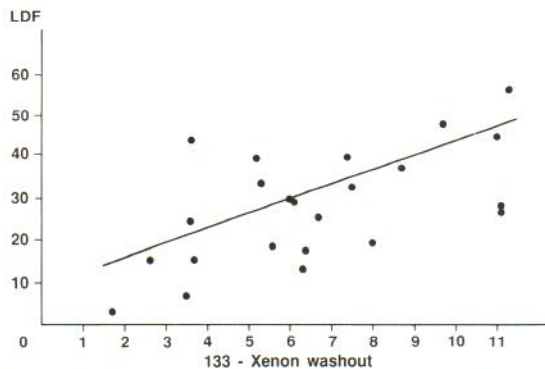
Fig. 1. Relative blood flow increases as measured by the two techniques are presented. The parametric linear correlation line is plotted to indicate the relation between the methods.

the chamber and diffusion was allowed to take place for 3 min. Then the saline was drawn back into the syringe, the chamber was removed and surplus liquid wiped off with cotton pads. The decline in activity was monitored with a NaI(Tl) scintillation detector placed 20 cm above the labelled area. The pulses were fed into a gamma-spectrometer printer and printed out every 20 s.

Measurement of blood flow by LDF

A Pf1d laser Doppler (Perimed, Sweden) was used. The probe type was a Pf 102d. Initially the probe was directed at a white reflecting surface and the pen recorder was set at zero (electrical zero). Then the probe was attached to the skin by a probe head holder. Upper frequency limit was 12 kHz and the time constant was set at 10 s. For a comprehensive review on LDF, refer to Shepherd & Öberg (8).

Experimental set-up

On the volar aspect of the left forearm, a circular area (25 mm in diameter) was marked with an ink pen. In the exact centre of this area, the skin was labelled with 133-Xenon and the washout was followed for at least 1 h. Second, the LDF probe was attached on the skin. The probe was repositioned five times to rule out spatial variation in skin blood flow. The LDF measurements were performed inside the 133-Xenon labelling area. Finally, a cuff was placed on the upper arm and inflated to 250 mmHg and the LDF signal was recorded (biological zero). Following these measurements, the circular area was exposed to 2 × MED of UVB and the measurements were repeated 8, 24, 48 and 72 h after irradiation. During the period of measurement, the subject lay supine, with arm held at heart level and immobilized. Room temperature was 22–23°C.

Calculation of blood flow by 133-Xenon washout

The counts were corrected for background activity and plotted versus time in a semilogarithmic diagram. The washout of 133-Xenon from skin is biexponential as the lipophilic indicator accumulates in the subcutaneous adipose tissue. Therefore, isolation of the cutaneous clearance curve was obtained by curve resolution as discussed in

detail elsewhere (6, 7, 9). Blood flow from dermis was calculated by the formula:

$$f = k \cdot \lambda \cdot 100$$

were f is the dermal blood flow rate (ml/100 g/min), k the washout rate constant (min^{-1}) and λ the tissue-to-blood partition coefficient (ml/g). A λ value of 0.7 (ml/g) was used (6).

Calculation of blood flow by LDF

Blood flow values in arbitrary units (AU) were read directly from the pen recorder. The biological zero was subtracted and the median value of five measurements in different positions was used for the calculations.

Statistics

Non-parametric statistics were used. Median and 95 percentiles of the median are used unless otherwise stated. Analytical statistics comprised Spearman correlation analysis.

RESULTS

133-Xenon washout results

After induction of inflammation and throughout the study period, the dermal washout curves were convex, with fast initial washouts. Re-labelling and subsequent counting during arterial occlusion demonstrated a pronounced decrease in counts when the cuff was inflated *prior* to the labelling. When a cuff was inflated immediately *after* the labelling procedure, no decline was seen except for the first 20 s. It was concluded that the leaky skin barrier did not influence the measurements when a time lapse of 20 s was interposed between the removal of the labelling chamber and the beginning of the counting. Consequently, cutaneous blood flow was calculated from the initial part of the dermal washout curve.

Prior to irradiation, blood flow was 5.2 (range 3.0 to 10.4) ml/100g/min. Maximum increase following irradiation was 10.8 (3.7 to 11.3) times baseline blood flow corresponding to an absolute blood flow of 47.5 (33.8 to 56.3) ml/100g/min.

LDF results

The repeated LDF measurements demonstrated a coefficient of variation of 15.2 (12.2 to 18.2) %. No difference in reproducibility between normal and inflamed skin was found.

Prior to irradiation, LDF flux was 3.1 (range 2.7

to 5.7) AU. Maximum increase after induction of inflammation was 34.6 (24.6 to 56.5) times baseline flux.

The biological baseline was not constant throughout the study; biological zero was raised by a factor of 1.9 (1.5 to 3.3) compared to biological zero prior to irradiation.

133-Xenon-LDF comparison

The time courses of blood flow increase following irradiation were comparable as measured by the two methods. Five of 6 subjects demonstrated peak blood flow 8 to 24 h after irradiation and 1 subject showed a diphasic blood flow increase with a maximum 72 h after exposure to UVB. Both methods detected this deviation. A significant correlation was found between the relative blood flow increase as assessed by the two methods (Spearman's rho = 0.54, 95% confidence interval 0.21 to 0.77, p = 0.006). The relative blood flow increases are plotted in a scatter diagram in Fig. 1. The relative LDF blood flow increase was 4.2-fold (2.7 to 5.0) greater than the 133-Xenon increase. The difference was independent of the 133-Xenon increase (p = 0.38), confirming a linear relationship between the blood flow increases as measured by the two methods.

DISCUSSION

The main finding in the present study was a major difference in the relative blood flow increase in an inflammatory condition as assessed by the two methods. Such a difference was unexpected, as both methods are believed to measure total skin blood flow.

Washout of 133-Xenon from skin is an established method for the measurement of *total* skin blood flow – provided that only small numbers of shunt vessels are present (6). The method is theoretically well supported (6, 10) and in other tissues experimentally proven, as blood flow estimates are comparable with venout outflow measurements (11).

During the initial part of the labelling procedure, the indicator is distributed not only in the skin but also in the underlying subcutaneous adipose tissue. Therefore, the washout curve is biexponential, reflecting the sum of two monoexponential washout curves: total skin and subcutaneous adipose tissue blood flow (6). In the early literature, blood flow was calculated from the initial slope of the total washout curve (3, 4). Such calculations are obsolete, as skin perfusion is underestimated by this technique

(9). This statement is also valid in high blood flow situations, especially when the subcutaneous blood flow is increased. Such an increase has recently been described (12).

In inflamed skin, the dermal component was not monoexponential. A possible loss of 133-Xenon by diffusion out through the epidermis was ruled out in our control measurements. Consequently, the fast washout reflected perfusion in the cutaneous tissue. The non-linear curve was most likely due to an increasing disequilibrium in the distribution of the indicator between tissue and blood in the dermal vascular bed. We therefore argue that only the first part of the curve represents total skin blood flow. Similar results have been shown in skeletal muscle preparations (13).

LDF flux changes demonstrated results quite different from the 133-Xenon washout results. The maximum blood flow increase was approximately four times greater as assessed by the LDF technique than measured with the 133-Xenon method. Our LDF measurements demonstrated the relative LDF increase to be even more pronounced than previously described (1, 2). However, as we used biological zero whereas previous studies have used electrical zero, the disparity might be caused by this difference in the baseline used.

When the blood flow was stopped by a proximally applied cuff, LDF flux decreased to 44% (range 37 to 60%) of the perfusion value in normal skin. A difference between electrical and biological zero has been demonstrated in several kinds of tissues. The biological zero represents laser signals not related to net perfusion, but the origin of these signals is a riddle. In microvascular research, the trend is to use the biological zero (14). Our findings of increased biological zero in inflamed skin stress the importance of the one reading-one zero concept. The increase was negligible, however, when compared with the LDF flux in perfused, inflamed skin.

The maximum blood flow as assessed by 133-Xenon washout was approximately 45 ml/100g/min. This figure corresponds to previous measurements of forearm blood flow in maximally dilated skin vessels (15). The increase as assessed by LDF was 35 times baseline flux and 4-fold greater than the 133-Xenon results. Such a huge increase seems unlikely. Hence we performed studies on postocclusive reactive hyperaemia on normal forearm skin in 2 subjects. Maximum blood flow was found following 12 min of occlusion with no further increase with pro-

longed occlusion. In these experiments, the relative increase was 14–16 times baseline flux. Similar results were obtained by upperarm occlusion versus local counter pressure (to avoid muscle steal phenomenon). These results suggest that the huge increase seen in inflamed skin might be caused partly by factors not related to increased skin blood flow.

It is not possible from the present study to determine which method represents the true estimate of total skin blood flow. No golden standard exists and comparative studies with selective venous outflow measurements from cutaneous tissue are certainly impossible. Both methods have advantages and disadvantages, assumptions and pitfall. The trend in microvascular research is to take an interest in LDF (16) but the easy handling of the laser Doppler equipment must not overshadow the importance that the exact nature of the LDF flux is unknown.

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