International Journal of Pharmaceutics, 45 (1988) 263–265
Elsevier

IJP 01556

Letter to the Editor

Laser Doppler velocimetric measurements of skin blood flow: a reply

Andreas J. Bircher, Kathleen V. Roskos, Howard I. Maibach and Richard H. Guy

Departments of Pharmaceutical Chemistry, Pharmacy and Dermatology, University of California, San Francisco, CA 94143 (U.S.A.)

(Received 16 November 1987)
(Revised version received 16 February 1988)
(Accepted 24 February 1988)

Dear Editor:

We write with observations pertinent to the recent article of Kohli et al. (1987) who confirmed that laser Doppler velocimetry (LDV) is a useful method for studying the effects of topically applied vasoactive drugs. Their results also imply, however, that vehicles as simple as distilled water and some short-chain alcohols can produce a highly inconsistent effect on cutaneous blood flow determined by LDV. The authors further suggest that this 'solvent effect' on blood flow has not been adequately addressed in previous publications which have reported LDV-assessed changes in skin blood flow.

Research in our laboratory, on the other hand, supports the conclusion that a response to topically applied distilled water may be detectable by LDV, but that the magnitude of the blood flow response is small in comparison to the response elicited by a topically applied vasoactive compound, such as methyl nicotinate (MN). Additionally, a spontaneous change in baseline blood flow may also occur over the duration of a typical experiment and must be taken into account during data analysis.

Our experiments were performed using a Periflux PF 1d laser Doppler flowmeter (LDF) (Perimed KB, Stockholm, Sweden) interfaced to a YEW flatbed pen recorder (Yokogawa Hokushin Electric Corporation, Tokyo, Japan). LDF settings used were $\times 10$ gain, 4 kHz frequency limit and a 1.5-s time constant; chart recorder speed was 1 cm/min. The output signal from the LDF, which is proportional to skin blood flow (Holloway et al., 1977; Watkins et al., 1978; Stern et al., 1977; Bonner et al., 1981), is displayed as a fluctuating voltage (in mV) on the recorder. The volunteer subjects studied were males and females aged 19–85 years. The experiments were performed in a single well-ventilated room under constant temperature and humidity conditions ($T = 23° \pm 2°C$, $RH = 50–70\%$). The subjects were supine for the duration of the experiment and the measurements were made on the ventral forearm.

In the first series of experiments, measurements of basal skin blood flow at the same skin site were made before and after a 45–90 min interval of quiet resting. Accurate repositioning of the probe for the second measurement was facilitated by aligning marks on the probe with marks drawn on the skin with a felt-tip pen. Prior to the initial determination of perfusion, the subjects tested were acclimated to the measurement environment for at least 20 min. The results of this part of the investigation are in Fig. 1. The data are a complete compilation of observations from 17 volunteers and include several concomitant measurements on bilateral, symmetric sites on left and

Correspondence: R.H. Guy, Box 0446, School of Pharmacy, U.C.S.F., San Francisco, CA 94143, U.S.A.

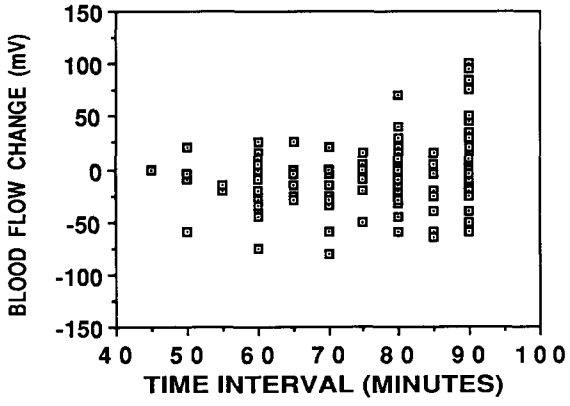

Fig. 1. Spontaneous skin blood flow changes plotted as a function of the time interval between observations. This data set is a complete compilation of observations from 17 volunteers and includes several concomitant measurements on bilateral, symmetric sites on left and right arms.

right arms. Fig. 1 shows first that spontaneous skin blood flow changes can occur and that the changes are not obviously related to the arm used or to the interval between observations. Next, we find that these apparently unprovoked alterations can be as large as 100 mV. It is important, therefore, when using LDV to follow, for example, topically applied drug-induced changes in skin blood flow, to record 'basal' measurements of perfusion from a control site before and after the deliberate provocation of vasodilatation.

In the second part of the study, forearm skin blood flow response to the topical application of distilled water was followed. Water was applied for 15 s via a saturated filter paper disk (Al-test, Imeco-ab, Sweden) and blood flow was monitored continuously thereafter for the next 30–55 min. As suggested by the results of the first component of this investigation, control baseline measurements of blood flow at a non-treated skin site were made so that spontaneous fluctuations in perfusion could be subtracted from those induced by the application of water. The results of 8 separate experiments are collected in Fig. 2. The maximum change in blood flow, which could be attributed to water was 70 mV. Typically, however, responses less than 20 mV were observed. Thus, like Kohli et al. (1987), we do see changes in skin blood flow that may be water-induced. The onset time of the 'reaction' can be obtained from our data. The key point of our work, though, is the magnitude of the response, which is generally very small. We have shown that changes can be spontaneous and that, once such fluctuations have been acknowledged, the additional pertubation (both size and duration) putatively caused by water is rather insignificant. The latter conclusion is particularly true when the 'effect' of water is compared to that of a 10 mM aqueous solution of methyl nicotinate (Guy et al., 1984). In our experience, the maximum response following a 15 s application of the vasodilator in this form is typically in excess of 300 mV.

The possible explanations for spontaneous skin blood flow changes (and those perhaps caused by water), even under the reasonably well-controlled conditions of our studies, are manifold. No single reason can be concluded to be more likely on the basis of the experiments performed. Certainly a cooling/hydration/temperature disturbance proposed by Kohli et. al. (1987) is plausible. Other environmental changes (e.g. a draught, conversation) or physiological/psychological perturbations (e.g. yawning, mental activity) can also induce short-term peripheral vascular changes of the order of magnitude seen. We conclude that the caution implicit in the paper of Kohli et al. (1987) is

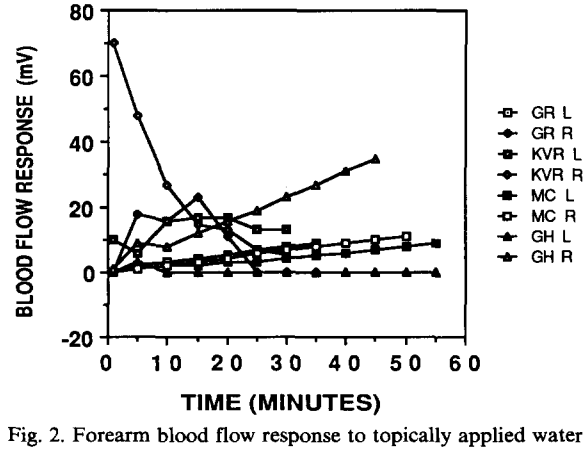

Fig. 2. Forearm blood flow response to topically applied water in 4 volunteers. These measurements were made on bilateral, symmetric sites on left and right arms. Legend markers include subjects' initials and indicate whether the left (L) or right (R) arm was used for measurement.

appropriate. However, the scale of the perceived problem is, in our opinion, quite small and the effect can be minimized by acquisition of appropriate pre- and post-test basal perfusion data. LDV is a valuable tool for the assessment of local drug-induced changes in skin blood flow. It provides a unique, non-invasive method to record useful pharmacodynamic data in vivo in humans. While normal biological variability in the results must be accepted, careful experimental design, including measurement of proper control information, will yield good quality data in skin pharmacology.

Acknowledgement

Supported by grants from the National Institutes of Health (GM-33395 and HD-23010) to R.H.G.

References

Bonner, R.F., Chen, T.R., Bowen, P.D., Bowman, R.L., Laser-Doppler continuous real-time monitor of pulsatile and mean blood flow in tissue microcirculation. In Chen, S.H., Chu, B., Nossal, R. (Eds.), *Scattering Techniques Applied to Supramolecular and Non-equilibrium Systems.* Plenum, New York; NATO, ASI Ser. B73 (1981) 685–702.

Guy, R.H., Tur, E., Bugatto, B., Gaebel, C., Sheiner, L.B. and Maibach, H.I., Pharmacodynamic measurements of methyl nicotinate percutaneous absorption, *Pharm. Res.,* 1 (1984) 76–81.

Holloway, G.A. and Watkins, D.W., Laser Doppler measurement of cutaneous blood flow. *J. Invest. Dermatol..,* 69 (1977) 306–309.

Kohli, R., Archer, W.I., Li Wan Po, A., Laser velocimetry for the non-invasive assessment of the percutaneous absorption of nicotinates. *Int. J. Pharm.,* 36 (1987) 91–98.

Stern, M., Lappe, S.L., Bowen, P.D., Chimoskey, J.E., Holloway, G.A., Keiser, H.R., Bowman, R.L., Continuous measurement of tissue blood flow by laser Doppler spectroscopy. *Am. J. Physiol.,* 232 (1977) H441–H448.

Watkins, D. and Holloway, G.A., An instrument to measure cutaneous blood flow using the Doppler shift of laser light. *IEEE Trans Biomed Eng.,* 25 (1978) 28–33.