COMMENT

Human forearm venous occlusion plethysmography: methodology, presentation and analysis

Forearm venous occlusion plethysmography is widely used for the study of arterial function in vivo in humans. In their timely paper on the effect of the angiotensin-converting enzyme inhibitor lisinopril on endothelial function in hyperlipidaemic patients, Lee et al. [1] raised several points of methodology relating to the correct analysis and presentation of data.

Blood flow was measured in both forearms and the test compound infused into one arm. Results were expressed as the ratio of flow in the infused over the control arm. It is argued by Benjamin et al. [2] that since the mean ratio of blood flow of both arms approaches unity at rest, it follows that the change in ratio of blood flow in the infused arm is the direct result of the test compound. This method has been used previously [3,4], and has the distinct advantage of taking into account small alterations in arterial pressure or sympathetic activation. Although many other practitioners measure blood flow from both arms, most do this to exclude a systemic response during each drug infusion and do not include in their presentation and analysis the ratio of infused/non-infused arm. Rather, results are expressed either as absolute or percentage forearm blood flow (ml·min⁻¹·100 ml⁻¹), where the basal flow is that obtained from the test arm either pre infusion or during a saline infusion [5–7]. An alternative is the use of measurements from a single arm with the ratio of responses obtained pre and post test compound [8–11]. Exclusion of a systemic response to the drugs infused is confirmed by measuring blood/arterial pressure. Of relevance to this is the debate surrounding the pertinence of measurements of forearm vascular resistance. It is the contention of Benjamin et al. [2] that the formula used to calculate vascular resistance (perfusion pressure/blood flow) is not applicable to blood flow as it is driven through a distensible system by a pulsatile pressure rather than a fixed resistance under a steady driving pressure. Although this is true and measures of forearm vascular resistance are not arithmetically precise, they nevertheless provide a simple approximate guide to the contractile state of the arteries and are commonly, and informatively, used as such [8,10,12].

Another issue complicating the interpretation of the results of Lee et al. [1] was the fall in blood pressure after 6 months of lisinopril, but not placebo, and whether this was associated with the fall in baseline blood flow in this group. This potential confounding factor has been well discussed by the authors but highlights the difficulties in interpreting data when values of basal blood flow and/or blood pressure differ. We agree with Benjamin et al. [2] that, under these circumstances, expression of data as flow rather than resistance and as a ratio between the test and the control arm minimizes error. These measures, however, do not neutralize the differences in basal conditions and one suggestion has been to study several drugs with differing mechanisms of action but with a similar functional outcome within each group [2]. A within-group interpretation of data can then be more usefully made. Another possibility is to study, where feasible, blood vessels isolated from the same population where starting conditions (e.g. resting tone) can be manipulated [13,14].

The correct analysis of these data is also debatable. We, and others [5–7,10,11], feel that analysis of variance for repeated measures followed by post-hoc t-tests with the appropriate corrections is most suited for the experimental approach. A simple alternative is to obtain a single-point summary of the data set from both groups, i.e. the active versus placebo patients. This can be done either as area under the curve [15] or as a within-group difference, e.g. in change of maximal response to acetylcholine from baseline. The latter method is the least sensitive and therefore least satisfactory as it does not take into account the full data set available.

Finally there is the question of whether correlations of drug responses should be made with the concentration or the dose of drug delivered. Investigations of vascular reactivity using forearm venous occlusion plethysmography have usually related the response, blood flow or vascular resistance, to the mass of drug delivered per unit time [5–7,16]. It has, however, been suggested that correction should be made for the drug-induced increase or decrease in blood flow [17]. It is our contention that a correction more securely based on the underlying principles of plethysmography is by compartment volume. In the absence of detailed compartment dose–response relationships, the next best correction is by limb volume.

Plethysmographic assessment of relative change in forearm volume is by derivation of arterial inflow at the time of outflow occlusion, representing a situation in which volume inflow persists during a period where interstitial pressure has not yet appreciably changed. Under these prevailing conditions the steady-state
concentration of an infused agonist with a given half-life is determined by the mass per unit time infused and the total volume of distribution into which the agonist is infused. An alternative condition in which the amount of drug reaching the tissue is corrected for changes in flow, is the circumstance where no obstruction to outflow occurs and no reservoir volume into which the inflow is mixing transpires – conditions which are not applicable to plethysmographic studies. Human forearm venous occlusion plethysmography in fact employs a closed-loop system such that blood flow out (venous outflow) does not occur due to the inflation of the venous occlusion cuff and blood flow in (arterial inflow) is the major, if not the only, contributor of limb volume increments (measured by the strain gauge). This is true at least until the pressure achieved within veins equals the venous occlusion cuff pressure, in which case no further limb volume increases are obtained.

Forearm venous plethysmography is a non-invasive and therefore indirect means of measuring vascular reactivity in vivo. It is important to acknowledge that it is not possible to measure accurate volumes of distribution and precise drug concentrations using this method. For vasoactive agents one would expect forearm intravascular volume to be the major compartment affected. However, the relative contributions of conduit arteries, resistance arteries, the venous circulation as well as the interstitial fluid compartment are unknown. Where possible, efforts should be made to control for the potentially confounding distribution effects by matching for forearm length and volume. In practical terms, when data are obtained from individuals with grossly varying forearm volume, for example in males and females or in obese and lean individuals, adjustment by forearm volume may play an important role and should be considered.

In summary, forearm venous occlusion plethysmography is a simple and convenient method for studying forearm arterial bed responses to differing agents. Although limitations apply, these can be minimized using care with methodology, presentation and analysis.

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REFERENCES