Exact Assessment of Perfusion and Collateral Vessel Proliferation in Small Animal Models

To the Editor:

Quantification of blood flow restoration and neovascularization poses a significant challenge, especially in small animal models. Although descriptive as well as functional methods of assessment exist, perfusion measurement has become a key point of current angiogenesis/arteriogenesis and stem cells studies. Cheng et al, in their recent article, provide interesting data on the role of MMP-2 in ischemic angiogenesis. Considerable efforts are made to correlate angiographic collateral imaging with perfusion as assessed by laser Doppler flowmetry in a murine MMP-2+/− hindlimb model. However, the article raises important technical questions regarding the exact quantification of blood flow restoration and neovascularization.

First, in Figure 3, the authors compare microangiographies from MMP-2+/− mice to wild-type controls. The resolution obtained seems insufficient for quantitative analysis. Although the MMP-2+/− phenotype (Figure 3A) is associated with lesser numbers of vascular connections (Figure 3A upper versus lower left), differences between unaffected and affected limbs in wild type controls are hardly discernible (Figure 3A upper left versus right). Although “collateral vessel formation” is being described, no typical collateral vessel morphology such as distinctive “corkscrew patterns” are detectable in the angiographies presented.

When choosing time points for angiographic collateral vessel imaging, it would be interesting to learn if the effects of “pruning” have been taken into account, as the number of collateral vessels detectable depends on the time interval after femoral artery ligation, their absolute numbers decreasing over time because of the rarefication process collateral vessels undergo once their growth stimuli decrease.

Second, laser Doppler flowmetry has been chosen to quantify hindlimb perfusion restoration, one major advantage of the method being its applicability for serial measurements. Although hindlimb perfusion is clearly visible, and consistent with the overall results, noticeable differences, inconsistencies and even perfusion deficits are to be observed when comparing the perfusion of the limbs to the tails and the lower abdomen in Figure 1 and Figure 2. Perfusion in those areas should be constant and unaffected by femoral artery ligation.

This indicates important drawbacks of the method, in part relativizing its read-out (compare). Interferences of fur and skin pigmentation with the LDF signal together with its proven low intrinsic depth of penetration allow examination of skin circulation only. Thus, LDF is feasible for assessment of extremities only and solely in conjunction with additional functional studies.

Moreover, LDF assessment at resting conditions disregards any influences on perfusion because of adrenergic tonus in the animals between different time points, in contrast to, eg, measurements under vasodilatation. To obtain vasodilatation in vivo, Terjung et al have successfully applied exercise assessments, which are, although excelled in specificity by complete vasodilatation, feasible for serial studies. Microsphere perfusion therefore remains the gold standard for the assessment of collateral flow. Its most prominent drawback, ie, its limited feasibility for serial studies, becomes less significant with increasing numbers of labels available.

Third, the authors apply histological methods to evaluate neovascularization. Therein the assessment of proliferation markers (Ki-67, PCNA) seems to be missing. Given the ischemia-mediated mitogenic action of the master regulator of ischemic angiogenesis, VEGF, on endothelial cells, the mere assessment of capillary/muscle fiber ratios after GS lectin I staining seems less substantive for exact identification and experimental targeting of developing neovessels.

Moreover, although the authors focus on ischemia-induced angiogenesis, it should be noted that after femoral ligation vascular proliferation occurs simultaneously in the foot (angiogenesis) as well as the upper thigh (arteriogenesis). Thus, an assessment of arteriogenesis/collateral artery proliferation seems to be missing. This is of importance in two contexts: On the one hand, the choice of the arterial ligation site is highly relevant for the predominant mechanism of revascularization. As outlined in the article, the sites of arterial occlusion directly determines if angiogenesis, arteriogenesis or both dominate the model. Regrettably, the technique is not outlined to that detail. We therefore suggest a closer look at the arteriogenic response, given that on the other hand the impact of growing arterial collaterals often outweighs that of ischemia-mediated capillary network formation on the restoration of perfusion with regards to the law of Hagen and Poiseuille.

In conclusion, whereas angiographic technique, functional perfusion measurements and the role of arterial collateral vessel proliferation remain as issues to be addressed in further studies, the results presented by Chen et al constitute a major contribution to the clarification of pathways of neovascularization and remodelling in limb ischemia.

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Circ Res. 2007;100:e82-e83
doi: 10.1161/01.RES.0000266608.88805.8b

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