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Microheterogeneity of myocardial blood flow

Abstract  Myocardial blood flow exhibits the most marked heterogeneity at the microvascular level. Its within-layer spatial distribution can be described from subepi- to subendocardium with resolutions of 0.1 x 0.1 to 1 x 1 mm² by quantitative digital radiography based on the technique of desmethylimipramine deposition. In the subendocardium, flow heterogeneity is the highest, whereas local flow randomness is the lowest, showing the clustered pattern of high- or low-flow regions. The resolution-dependence of flow heterogeneity is characterized by its fractality, which holds consistently down to the microvascular level through the vascular structural transition from the treelike arteriolar to the non-treelike capillary network. Flow heterogeneity is adjustable in a transmurally different manner to local metabolic changes. The redistribution of flow is considered as a result of adaptive coordination of microperfusion between adjacent microcirculatory units, which are perfused by a single precapillary arteriole.

Key words  Molecular flow tracer – digital radiography – transmural difference – microvascular unit – fractality

Introduction  Regional myocardial blood flow shows marked spatial heterogeneity even within a single myocardial layer (6, 20). Although flow heterogeneity matches well with heterogeneity of myocardial O₂ requirements (5, 40, 45), the flow- or O₂-supply limited conditions may lead to the regional mismatch between flows and O₂ requirements (15, 30, 31, 42). Such a regional O₂ deficiency was recognized as the patchy pattern of high NADH fluorescence in epimyocardium (11, 53). The size of those patchy zones was distributed around a few to several hundreds of micrometers, comparable in size to a region perfused by a single precapillary arteriole (24) or a microvascular unit. Therefore, the distribution of myocardial perfusion is likely to be determined by microvascular anatomical or functional minimal units (24, 35). The distributed pattern of regional flows will differ between the outer and the inner layers because there are transmural differences in O₂ consumption (19, 48) and extravascular mechanical conditions (18, 21, 51). Accordingly, it is of great importance to evaluate regional myocardial flow at the microvascular level and its microheterogeneity transmurally for understanding the mechanism of myocardial perfusion.

In this article, we introduce our high-resolution tracer autoradiography, which is the only technique allowing us to visualize myocardial flow distribution at the microvascular level, and the current knowledge concerning microheterogeneity of flow under pathophysiological conditions.
Measurement of regional flow distribution at the microvascular level

Regional myocardial flow has been measured mainly by the microsphere method. Recent detection techniques of microsphere deposition using monochromatic synchrotron radiation (38, 39) or an automated imaging cryomicrotome (9) has refined the microsphere method; however, the flow disturbance due to microsphere embolization itself makes it difficult to measure regional flows at the microvascular level. As mentioned above, tracer autoradiography is the only method to visualize and quantitate regional flows with the region size comparable to the microvascular unit. As a molecular flow tracer, desmethylimipramine (DMI) labeled with $^3$H is ideal. DMI is delivered to tissue in proportion to local flow, nearly completely extracted during a single pass, and stably deposited at $\alpha_2$ receptors almost exclusively in capillaries and therefore, the local DMI depositions in small capillary tissue units are proportional to the flow (33, 34). In addition, the autoradiographic image of $^3$H-DMI precisely reflects the two-dimensional distribution of relative regional flow because the path length of $\beta$ particles emitted from $^3$H is only a few micrometers, resulting in a negligible blurring effect on autoradiographic imaging. Thus, autoradiography combined with $^3$H-DMI deposition can resolve the flow distribution into regions smaller than the microvascular unit without any microembolization. In Fig. 1, a digital radiographic image of flow within a rabbit myocardial slice is exemplified (100 pixels/mm$^2$).

Recently, we developed double-tracer digital radiography using $^{125}$I-DMI along with $^3$H-DMI (37). Blur in $^{125}$I-DMI deposition imaging due to the radiation spread is not negligible and accordingly limits the spatial resolution of this method; however, the regions resolved are just comparable in size to the microvascular unit (400 x 400 µm$^2$). This method allows two-time measurement of myocardial flow distribution, e.g., before and after intervention. By this technique, we have also revealed that the 15-µm microsphere embolization, mimicking a coronary microvascular disease, increased microheterogeneity of regional flow.

Spatial microheterogeneity of myocardial flow

■ Transmural variation of within-layer flow heterogeneity

Figure 2A shows a typical example of flow images (i.e., $^3$H-DMI deposition distributions) within subendocardial and subepicardial layers of the rabbit left ventricular free wall. The flow distribution was resolved into 100 x 100-µm$^2$ regions. In Fig. 2B, the coefficient of variation of flows (CV = standard deviation/mean) are plotted against pixel area (100 x 100 µm$^2$ to 1 x 1 mm$^2$) on a double logarithmic scale. CV was higher in subendocardium than in subepicardium, indicating higher heterogeneity in

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Fig. 1 Digital radiogram of a rabbit left ventricular myocardial layer sliced in parallel with the epicardial surface. The within-layer flow distribution was visualized in 256 levels of black and white gradations with a spatial resolution of 100 pixels/mm$^2$.

Fig. 2 A Myocardial flow images of the rabbit left ventricular subendocardium (Endo) and subepicardium (Epi) resolved into 100 x 100-µm$^2$ pixels. Shading is proportional to local flow. B Coefficient of variation of flows (CV) vs. pixel area in subendocardium and subepicardium on a double logarithmic scale. The overall difference between the layers was significant (P < 0.05, nonparametric ANOVA). The logarithm of CV decreased linearly with the logarithm of pixel area. C Correlation between adjacent regional flows (CA) vs. pixel size in subendocardium and subepicardium. The overall difference between the layers was significant (P < 0.05, nonparametric ANOVA). Error bars, SD.