Patterns of USPIO Deposition in Murine Atherosclerosis

In Response:

We thank Klug and colleagues for their interest in our recent publication “p38 MAPK Inhibition Reduces Aortic Ultrasmall Superparamagnetic Iron Oxide Uptake in a Mouse Model of Atherosclerosis: MRI Assessment” by Morris et al.1 Indeed the article does underscore the need for further investigation into understanding the fate of ferumoxtran-10 and its modulation of uptake in atherosclerotic plaque of the apoE<sup>−/−</sup> mouse. Additionally, investigators should be keenly aware of the complexities associated with interpreting the pattern of USPIO deposition which will be affected by numerous variables including animal model of atherosclerosis, atherosclerotic plaque location, USPIO particle size and coating, USPIO dosing paradigm, etc.

While we appreciate the continued effort in developing ferumoxtran-10 as a novel cellular readout of macrophage content or inflammation status of the plaque, one must be aware of the complexities in comparing results from experiments using similar animal models but different iron oxide MRI contrast agents. For example in Briley-Saebo et al<sup>2</sup> and Yancy et al<sup>3</sup> albeit using different USPIO contrast agents, the studies showed very similar patterns of iron deposition in the neointima of the balloon-injured rabbit with focused deposition in regions of significant macrophage accumulation both in the subendothelium as well as near the internal elastic lamina. However, although Yancy et al did not detect significant amounts of iron in the smooth muscle cap that formed over the neointima, it appears that there was significantly greater iron staining in the subendothelial layer as well as a generally more intense and uniform distribution of the Perl’s stain in the Briley-Saebo study using fractionated Feridex. Although the total iron used by Briley-Saebo et al was approximately 20% of that used by Yancy et al, the intensity of Perl’s staining was quite profound and nonspecific. This may in part be attributable to the promiscuous nature of such a small particle (~20 versus 50 nm intensity weighted mean diameter for the fractionated Feridex versus ferumoxtran-10, respectively) whereby, as the authors do suggest, such diffuse and homogeneous staining may in part be a result of luminal diffusion and migration to deeper lying macrophage.

The murine model of accelerated plaque development used by Morris et al is an MCP-1 driven model whereby angiotensin II (Ang II) is chronically administered in the apoE<sup>−/−</sup> mouse. Chronic administration of Ang II in apoE<sup>−/−</sup> mice increases recruitment of

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**Figure.** Representative histochemical analysis of atherosclerotic plaque associated with the aortic root of angiotensin II–administered apoE<sup>−/−</sup> mice. Perl’s staining was used to demonstrate colocalization of iron positive area (A through C) and Cat-S (D)-positive cells. The regions of interest in the 10× magnification images (A) are shown in B and C at 40× magnification.
macrophage and matrix metalloproteinase activity creating a “vulnerable” lesion phenotype. In our experience, the pattern of ferumoxtran-10 deposition detected in the plaque by Perl’s staining was rather heterogeneous (Figure, A) as a whole in the aortic root. However, within each section of aortic root, we typically found regions with uniformly distributed iron oxide within the intima (Figure, B) as well as regions where diffuse staining was observed predominantly in subendothelial regions with regions of focal staining adjacent to the internal elastic lamina (Figure, C), similar to the pattern of iron oxide deposition that Klug et al refer to in their observations. This is a general observation, and again it must be stressed that many factors can and will influence the pattern as well as quantity of USPIO particle uptake by plaque including, animal model, plaque location, and USPIO dosing paradigm. In another study examining Feridex deposition in atherosclerotic plaque of apoE−/− mice treated with a proinflammatory cytokine cocktail, Litovsky et al showed iron deposition to be largely intracellular and associated with subendothelial macrophages as well as occasionally found in deeper regions adjacent to the internal elastic lamina. We have also found general colocalization of the Perl’s staining with regions associated with macrophages (Figure, D). Klug et al suggest that the subendothelial uptake of USPIO may in part be a result of diffusion of the contrast agent. However it is not clear why there would be such heterogeneous diffusion as has been our experience and is reflected in Figure 1 of their letter. We have also examined regions of aorta where no lesion is present and there is no diffusion or uptake observed in the media from these regions. Nevertheless, it is difficult to determine using light microscopy the extent to which ferumoxtran-10 is located intracellularly. With increasing emphasis on using murine models of atherosclerosis, further investigation into differentiating intra- from extracellular USPIO deposition via high-resolution electron microscopy or ex vivo cellular labeling studies is warranted to gain increased confidence in using such MRI contrast agents to noninvasively image macrophage content or activity.

Disclosures

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