Loaded Leukosomes:

A Smart Bomb to Halt Vascular Inflammation

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Even today, a therapy designed to delay, or completely prevent, the ravages of systemic atherosclerosis remains a holy-grail for translational medicine.[1] Since every therapy has a potential downside, an approach to target that therapy with precision to an area and thereby reduce off-target complications of any agent is an essential requirement. A step toward this lofty dream appeared in the report from Boada et al[2] in *Circulation Research*, where the authors used a novel approach to produce leukosomes, biomimetic nanoparticles generated by combining lipids with membrane proteins derived from lipopolysaccharide-stimulated macrophages, to target the inflamed vasculature and inhibit disease pathogenesis. These leukosomes were loaded with rapamycin (Leuko-Rapa) and delivered for a relatively short therapeutic course (once a day for 7 days) into hypercholesterolemic, ApoE$^{-/-}$, mice. The authors observed decreased macrophage proliferation in the aorta of Leuko-Rapa-treated animals when compared with vehicle treatment or the systemic administration of rapamycin, the latter of which is known to have undesirable side effects including dyslipidemia and interstitial lung disease.[3,4] Moreover, Leuko-Rapa treatment reduced production of inflammatory cytokines and decreased MMP (matrix metalloproteinase) activity in the atherosclerotic aorta. These results are important, as increased production and activity of MMPs play a key role in arterial remodeling, plaque destabilization, rupture, and atherothrombotic vascular disease.[5]

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The over-riding premise of this report was that the increased endothelial expression of adhesion molecules, proinflammatory cytokines, and chemokines, within an inflamed aorta can be exploited to selectively attract nanoparticles. The authors integrated membrane proteins from the lipopolysaccharide-stimulated J774 mouse macrophage cell line into lipid vesicles and loaded the nanoparticles with rapamycin, an inhibitor of the mTOR (mammalian target of rapamycin) signaling pathway. This creative approach did not change the overall size of the nanoparticles but decreased their negative ionic charge, which has the potential to increase the leukosomes' interaction with the negatively charged glycocalyx of inflamed endothelium and foster their delivery to atherosclerotic lesions.[6] Despite the overall positive findings in the study, several questions remain. For instance, how did these bioengineered particles traverse through the endothelial layer? Although it is known that monocytes use the actin cytoskeleton to transmigrate after initial binding to traverse across the vessel wall, whether the leukosomes work through an active (endocytosis) or passive (permeability) transcellular or paracellular transfer process is unknown. Additional and more direct confirmation that the bioengineered leukosomes enter atherosclerotic arteries but not noninflammatory vasculature in control mice would have been ideal. The dynamics of leukosome transfer into the arterial wall also needs to be further delineated and may be a limitation, as the bulk of rapamycin is released from the leukosomes within 6 hours. This rapid release may lead to systemic effects after administration, depending on the agent and the mass delivered. Moreover, the shell of the leukosomes alone was not administered, and whether rapamycin alone is the active part of the smart bomb remains to be determined. The study also did not investigate whether the vessels are better functioning in the active treatment group and information such as vascular reactivity would have been useful translational data. Finally, other tissues (ie, the lungs, which will be described in additional detail below) appear to have been affected by the leukosome treatment, which itself brings into question the selectivity of the nanoparticles. Regardless of these limitations, the article remains a significant advance for pharmacological targeting of the inflamed vasculature, irrespective of whether rapamycin is the final chosen agent.

Since targeted delivery was a key part of the article, information on potential effects, good, bad, or neutral, on a leukosome only control group would have been helpful. Although rapamycin was studied systemically as one control, the protein/lipid milieu of the leukosomes clearly could be inducing effects independent of the rapamycin. Appreciating the complexity of the leukosome production, some of the group numbers in the study, while fine for a proof of concept, were low. The end points within the study focused on MCP-1 (monocyte chemoattractant protein 1), IL (interleukin)-1 β, and MMP activity, and while appropriate, are a bit limited. The role of IL-1 β has, moreover, conflicting results between mice[7] and humans.[8] Moreover, data on levels of anti-inflammatory cytokines, such as IL-10, would have given more information on the anti- to proinflammatory cytokine balance.

Additional aspects also were worthy of mention. The study used a relatively short treatment course (1x per day for 7 days) and treated the mice during an active state of disease. The extent to which this degree of targeting will occur in a more chronic human disease state is open to question. Of course, the short treatment time-course can be advantageous if the effects of the treatment are durable. Importantly, cardiovascular outcomes in studies ranging from statins to antiplatelet agents demonstrated that short term-treatment courses can lead to

long-term clinical benefits. Should any benefit from the protein/lipid mixture occur, then at least a loose analogy can be made to the use of exosomes from cells for cell therapy, as was recently shown in models of acute lung injury.[9]

A final part of the study was assessment of the lung tissue. Patients treated with free rapamycin are at risk for developing interstitial lung disease (pneumonitis) within 1 year, an issue which is mostly resolved upon withdrawal of the medication.[10] Although the short duration of the Leuko-Rapa (rapamycin-loaded leukosomes) treatment (7 days) in this study did not allow for a thorough evaluation of long-term effects, the authors reported a promising observation that lung pathology, assessed by alveolar wall thickness and lung cell counts in the ApoE$^{-/-}$ mice fed a high-fat diet for 12 weeks, was significantly reduced compared with control or free rapamycin treatments. Moreover, the active leukosome-treated group seemed to have lower circulating LDH (lactate dehydrogenase) levels compared with control and free rapamycin treatments, although results did not reach statistical significance. Future studies should address the mechanism(s) by which leukosomes do not merely lack toxicity but may even exert protective actions in the lungs and liver, taking into account their potential residual off-target effects or the release of protective circulating mediators upon their interaction with the arterial wall.

As for all potential therapies, advancing from mouse studies to human treatment will require crossing the translation valley of death. A major component of the study was the use of protein mixes from lipopolysaccharide-activated cells, but preparation to preparation variability will be an issue for large scale studies. This will occur even with a cell line used at a constant passage number. The isolated components are still going be heterogeneous and will be expected to vary, to some degree, from preparation to preparation. Given the nature of the future approach of a biologic/pharmacological study to limit atherosclerosis, a translational biomarker of activity will be absolutely essential. Although systemic measures are possible, additional information on vascular reactivity or vascular permeability would be ideal, as would easy to obtain assessments of the microvasculature. Despite the treachery of the therapeutic journey, Boada et al[2] have made an important contribution to the field and set a pathway forward to limiting atherosclerosis and its sequelae.

Acknowledgments

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