PUTTING A BRAKE ON INFLAMMATION BY TARGETING GENES OR DRUGS TO ENDOTHELIAL CELLS

Jan A.A.M. Kamps, Sigridur A. Ásgeirsdóttir, Joanna M. Kuldo, Hidde J. Haisma and Grietje Molema

Department of Pathology and Laboratory Medicine, Medical Biology section, Laboratory for Endothelial Biomedicine & Vascular Drug Targeting Research, University Medical Center Groningen (UMCG), University of Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands.
(j.a.a.m.kamps@med.umcg.nl)

At this time, application of many of new molecular entities (MNE) is hampered by toxicity and lack of selectivity. Formulation in cell specific carrier systems can overcome these problems. Endothelial cells play a pivotal role in the pathophysiology of chronic inflammation (*1*). They are fully accessible for any compound transported by the blood. In addition activated endothelial cells present in areas of inflammation selectively (over)express adhesion molecules, among others E-selectin and VCAM-1, which are absent in non diseased tissue. There is an enormous potential for new nano-technology-based formulations of pharmacologically potent drugs that are targeted to endothelial cells in inflammatory diseases (*2*). In our laboratory we followed two approaches to pharmacologically interfere with endothelial cell behavior to inhibit inflammation. 1. We investigated whether targeted delivery of transgene that inhibits nuclear factor κB signal transduction could silence the proinflammatory activation status of endothelial cells. For this, an adenovirus encoding dominant-negative κB (dnIκB) as a therapeutic transgene was employed. Selectivity for the endothelial cells was introduced by antibodies specific for inflammatory endothelial adhesion molecules E-selectin or VCAM-1 chemically linked to the virus via polyethylene glycol (*3*). *In vitro* the retargeted adenoviruses selectively infected activated endothelial cells to express functional transgene. The comparison of transductional capacity of both retargeted viruses revealed that E-selectin based transgene delivery was superior. *In vitro* targeted dnIκB transgene expression in endothelial cells inhibited the expression of various inflammatory genes. *In vivo*, in mice suffering from glomerulonephritis, E-selectin-retargeted adenovirus selectively homed in the kidney to glomerular endothelium. Consequent downregulation of endothelial adhesion molecule expression two days after induction of inflammation demonstrated pharmacological potential of this gene therapy approach. 2. In the same mouse model for glomerulonephritis we selectively delivered the corticosteroid dexamethasone into activated glomerular endothelial cells by anti-E-selectin immunoliposomes to decrease renal injury while preventing systemic side effects. After intra venous injection, anti-E-selectin liposomes were specifically taken up by the diseased glomerular endothelial cells in the affected kidney, whereas liposomes with an irrelevant antibody coupled were not (*4*). Site selective delivery of anti-E-selectin liposomes encapsulated dexamethasone reduced glomerular proinflammatory gene expression without affecting blood glucose levels, a side effect of administration of free dexamethasone. The anti-E-selectin liposomes containing dexamethasone were effective in inhibiting the progression of renal injury (*5*). Our data show that carrier mediated targeted drug or gene delivery to glomerular endothelium engaging in the pathology presents a powerful strategy for treatment of glomerulonephritis. The general phenotypic features of inflammatory diseases, i.e., endothelial cell activation and leukocyte recruitment, imply that the approach can also be used for therapy of other inflammatory diseases. An important next step will be to relate the molecular consequences of therapeutic intervention to local gene expression and disease activity.

References

1. Kuldo, J. M., Ogawara, K. I., Werner, N., Asgeirsdottir, S. A., Kamps, J. A., Kok, R. J., and Molema, G. (2005) Molecular pathways of endothelial cell activation for (targeted) pharmacological intervention of chronic inflammatory diseases, *Curr. Vasc. Pharmacol. 3*, 11-39.

2. Kamps, J. A. A. M. and Molema, G. (2007) Targeting Liposomes to Endothelial Cells in Inflammatory Diseases., in *In Liposome Technology, Volume III: Interactions of Liposomes with the Biological Milieu.* (G.Gregoriadis, Ed.) pp 127-150, CRC Press.

3. Kuldo, J. M., Kamps, J. A. A. M., Asgeirsdottir, S. A., Zwiers, P. J., Bellu, A. R., Rots, M. G., Schalk, J. A. C., Ogawara, K. I., Trautwein, C., Haisma, H. J., and Molema, G. (2008) Targeted adenovirus mediated inhibition of NF-B-dependent inflammatory gene expression in endothelial cells *in vitro* and *in vivo*., *submitted*.

4. Asgeirsdottir, S. A., Zwiers, P. J., Morselt, H. W., Moorlag, H. E., Bakker, H. I., Heeringa, P., Kok, J. W., Kallenberg, C. G., Molema, G., and Kamps, J. A. (2007) Inhibition of proinflammatory genes in anti-GBM glomerulonephritis by targeted dexamethasone-loaded AbEsel liposomes, *Am. J. Physiol Renal Physiol in press*.

5. Asgeirsdottir, S. A., Kamps, J. A., Bakker, H. I., Zwiers, P. J., Heeringa, P., van der, W. K., van, G. H., Petersen, A. H., Morselt, H., Moorlag, H. E., Steenbergen, E., Kallenberg, C. G., and Molema, G. (2007) Site-specific inhibition of glomerulonephritis progression by targeted delivery of dexamethasone to glomerular endothelium, *Mol. Pharmacol. 72*, 121-131.