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Editorial

Targets in Fibrotic Disorders

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Fibrosis is a serious medical problem affecting a large number of patients worldwide. Two of the hallmarks of fibrosis are excessive deposition of extracellular matrix and the formation of fibrotic scar tissue. These processes further disarrange the functioning of normal cells within the affected organ and evoke a further progression of the fibrotic process. Eventually, this vicious cycle needs to be stopped or life-saving measures like organ transplantation become inevitable. Existing antifibrotic drugs only slow down the progression of the disease. Therefore, the development of more effective agents that can stop or even revert fibrosis is a major challenge. The special section on *Fibrotic Disorders* in this issue of Pharmaceutical Research encompasses four papers that report on interesting developments in the area of renal fibrosis. The selected contributions open perspectives on novel potential targets for antifibrotic therapy. The section encompasses papers on (1) *pharmacological targets* for antifibrotic therapy, (2) *target cells* which play a pivotal role in the fibrotic process and which may be the target of novel or existing drugs (3) *drug targeting* for creating improved responses of antifibrotic agents in the kidney. Taken together, the papers highlight attractive targets for more effective antifibrotic therapies.

The review by Nguyen and Goldschmeding addresses the delicate balance between profibrotic and antifibrotic factors, and how its disturbance can lead to fibrosis (1). The importance of transforming growth factor-β1 (TGF-β1) as profibrotic growth factor is widely acknowledged, and its inhibition has been pursued by a variety of therapeutic approaches ranging from neutralizing antibodies, receptor antagonists or agents that interfere in signaling cascades downstream of the TGF-β receptor. More recently, the regulatory activity of antifibrotic growth factors which oppose fibrogenesis has been valued. Bone morphogenetic protein-7 (BMP-7), also known as osteoprotegerin-1 (OP-1), is one of the best-described representatives of this class of proteins. During fibrosis, down-regulation of BMP-7 and over-expression of TGF-β1 tilts the normal homeostasis of tissue repair. Recombinant BMP-7 is already used in the clinic for the treatment of bone disorders, and may be used (locally) to treat fibrosis.

Another mechanism contributing to exaggerated fibrotic responsiveness is the increased expression of modulator proteins that enhance or diminish the activity of growth factors. Connective tissue growth factor (CTGF) has a dual role in this, since it both enhances the activity of TGF-β1 and reduces BMP-7 activity, most likely *via* direct binding to the factors. By this, profibrotic responses are further enhanced. Inhibition of CTGF is also an appealing approach, for which both neutralizing antibodies and antisense approaches are under investigation.

Motazed *et al.* explored the antifibrotic activity of BMP-7 in proximal tubular cells (2). The canonical pathways activated by BMP-7, Smad 1/5/8, lead to the formation of transcription complexes that suppress the transcription of fibrotic genes by TGF-β1. This process was confirmed in studies in which proximal tubular cells were treated with BMP-7 at normal doses. A different action of BMP-7 was detected when proximal tubular cells were treated with BMP-7 at very low concentrations. Those conditions did not activate Smad signaling, but activated the p38 MAP kinase pathway. More interestingly, the authors observed that BMP-7 at normal doses silenced the p38 pathway, and they postulated that activated Smad1 was responsible for this. Thus, BMP-7 at normal doses may exert part of its antifibrotic activity *via* inhibition of MAP kinase signaling pathways. This notion is important when considering the development of BMP-7 mimetics as potential antifibrotic agents.

The tubular epithelial cell is the second *target* in the section on Fibrotic Disorders. This cell type plays an important role in tubulointerstitial fibrosis but has also a central role in other types of renal disorders. Activation of tubular epithelial cells is one of the early events that occur in tubulointerstitial fibrosis. Loss of the epithelial phenotype and transformation into mesenchymal cells, so-called epithe-

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lial mesenchymal transition or EMT, results in the deterioration of functional nefrons. Eventually, the cells will turn into matrix-producing myofibroblasts. The group of Ruiz-Ortega explores fibrotic mechanisms that occur in proximal tubular cells and experimental models of renal fibrosis. Their paper by Rodrigues-Diez *et al.* reports on the profibrotic activity of Angiotensin II (Ang II), a vasoconstrictive peptide well-known for its role in cardiovascular disorders (3). Stimulation of cultured tubular cells with Ang II induced EMT, *via* activation of both mitogen activated protein (MAP) kinase and RhoA/ROCK signaling pathways. One of the downstream mediators that was activated in the cells was CTGF.

Angiotensin receptor blockers and other inhibitors of the renin–angiotensin system (e.g. ACE inhibitors) are first-choice treatment for progressive kidney diseases. The presently reported activity of Ang II in proximal tubular cells illustrates the importance of local effects of these compounds within the kidney. However, novel therapeutic agents are needed since angiotensin blockers only slow down the progression of renal disease. Kinase inhibitors that can block the activity of MAP kinases and ROCK seem interesting candidates for this, and the present study demonstrates the potential of such experimental inhibitors. In addition, a more established class of therapeutics, statins, was explored for this purpose. These compounds are able to block signal transduction events and partially prevented the induction of EMT by Ang II in tubular cells.

The fourth and last contribution to the section on *Fibrotic Disorders* addresses drug targeting as a novel approach to treat renal fibrosis. Prakash *et al.* employed a renal-selective carrier for the targeting of antifibrotic agents to proximal tubular cells (4). As discussed above, this cell type plays a pivotal role in progressive renal disease and offers an interesting druggable target. During fibrosis, one of the early events is the activation of tubular cells by TGF-β1 which can be inhibited by kinase inhibitors specific for this pathway. An inhibitor of the TGF-β receptor kinase was conjugated *via* a novel linker approach to the low molecular weight protein lysozyme. The resulting conjugates accumulated efficiently in the proximal tubular cells of the kidneys *via* receptor-mediated endocytosis, which is the normal fate of renally filtered proteins. The tubular targeted kinase inhibitor conjugates showed antifibrotic effects in the unilateral ureteral obstruction model of renal fibrosis. This study nicely demonstrated that fibrotic responses can be counteracted locally, by specific intervention in fibrotic signaling cascades.

To conclude, the special section reports on interesting developments within the field of renal fibrosis that may lead to novel therapeutic approaches. We hope that the reader will appreciate the selected manuscripts.

APPENDIX

INTERVIEW

Interview Questions for Dr. Robbert J. Kok

1. *What do you think holds the key to your success as a pharmaceutical scientist?*
   One of the aspects of the pharmaceutical sciences I like best is its multidisciplinary character. It combines the best of biology, chemistry and medicine. I think that this is also reflected in my scientific expertise: I specialized in medicinal chemistry in my Master, became expertized in pharmacology and pharmacokinetics during my PhD and I have lectured in pharmaceutics, analytical chemistry and pharmacokinetics. This broad expertise enables me to communicate well with both medical scientists and biochemical or organic chemists. I can translate between the medical needs and chemical possibilities.
2. *What do you consider to be your key research accomplishments?*
   I consider myself still a young scientist, so it feels strange to talk about my 'key research accomplishment'. My major contribution to the field of drug targeting is that I investigate different classes of drugs. The majority of the researchers in this field stick to classical 'proof-of-concept' drugs like doxorubicin or paclitaxel. Such compounds kill target cells, while my goal is to conduct pharmacology with cellular targeted drugs. In the past—during my PhD and my first post-doc project—access to potent drug candidates and the actual preparation of the drug targeting conjugates have been the major limitation. The synthesis of drug targeting conjugates is no longer a problem with the new linking technology that I have developed, and I have established collaborations that provide me with a wealth of interesting drug candidates. At present, we have prepared a multitude of drug-carrier conjugates and the major challenge in our research is the actual selection between the compounds we have prepared.
3. *What was the turning point in your career?*
   This has been the moment when I started to work on the development of new linker technology for the coupling of drugs to carrier systems and later on applied this to the cellular targeting of kinase inhibitors. Together with our own expertise on drug targeting, the biotech companies and my university possessed all the essential elements for the successful preparation of drug targeting conjugates and for the evaluation of these products in cells and animal studies.
4. *Who are the individuals who most influenced your research career?*
   Here I will name my promoter, Dirk Meijer of the Department of Pharmacokinetics and Drug Delivery at the University of Groningen. He showed me that you can always find an optimistic element in experiment results that may seem disappointing at first. For instance, he would turn a poorly bioreversible drug-carrier conjugate into a slow-release product. A pessimist will focus on the failure of a certain experiment (and we all have failed experiments), while an optimist will consider a new application for the established system.

   Another person who influenced me greatly is Grietje Molema of the University Medical Centre of Groningen. She has been a very inspiring supervisor during my post-doc and afterwards.

5. *Pharmaceutical scientists are faced with the dilemma of having to publish in biomedical or basic science journals. Does it mean cutting edge science will not likely be featured in the Pharmaceutical Research?*
   I think this statement is true. Cutting edge results will be sent first to journals with the highest impact factor. But it also depends on the type of study and the audience that needs to be reached with a certain paper. Especially for manuscripts that cover multiple areas, e.g. ranging from chemistry to pharmacology, Pharmaceutical Research is a well-esteemed journal.
6. *Where is the field of Fibrotic Disorders going? How do the articles in the theme section fill the gap?*
   I think this question is nicely addressed in the editorial. The use of new drugs that evoke specific responses is a major step forward. Both small molecules (kinase inhibitors) and recombinant proteins will find their way in the treatment of fibrosis in the coming years.
7. *What are the challenges for Fibrotic Disorders and how can they be overcome?*
   The major challenge is that fibrosis is a chronic disease, which requires life-long treatment once diagnosed. Antifibrotic drugs therefore need to be safe, also when used chronically. This is a major difference as compared to cancer. Chronic administration will also be an issue with respect to the use of drug targeting preparations. We will have to find solutions for immunogenicity, repeated administrations etc.
8. *What is the key to developing successful collaborative relationships?*
   The collaboration should be beneficial to all parties. One should keep in mind why the other party is involved, and communicate openly which goals should be pursued. Hidden agendas are fatal to a successful collaboration.
9. *What is your philosophy of educating graduate students?*
   Graduate students should be open-minded and willing to explore. Textbook knowledge is the basis for a scientific opinion, but the scientific truth is not static, it evolves in time. A graduate student should be able to explain his own scientific convictions. As a teacher, we can show them the different resources and techniques that are needed in pharmaceutical sciences.
10. *What are the challenges facing the pharmaceutical sciences?*
    In my view, the field is evolving from formulation sciences to a more biotech-like science, in which pharmaceutical scientists apply novel technologies developed by molecular biologists and chemical scientists to the development of therapeutics.
11. *What is the place for collaboration with industry in academia?*
    Collaborations are essential. I especially appreciate my collaborations with smaller biotech companies, since we communicate very directly and scientific achievements within the projects contribute directly to the scientific know-how of the companies.

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Robbert J. Kok studied Pharmacy at the University of Groningen, The Netherlands. He graduated in 1993 as a pharmacist and obtained his PhD in 1998. The topic of his thesis was Renal targeting of Angiotensin Converting Enzyme inhibitors. From 1998-2002 he worked as a postdoctoral scientist at the University of Groningen on the targeted delivery of anti-inflammatory and anti-angiogenic drugs to endothelial cells. In 2002, he became Principal Investigator at an EU-funded project dedicated to the development of novel linker technology for the preparation of drug targeting conjugates. The developed linker systems have been applied for targeted delivery of kinase inhibitors to the kidney, liver and tumor vasculature. Dr Kok took up his present position as Assistant Professor at the department of Pharmaceutics of Utrecht University in 2006. Focus areas in his present research are renal delivery of kinase inhibitors for the treatment of renal fibrosis and tumor directed drug delivery. His expertise area ranges from medicinal chemistry to pharmacokinetics and preclinical pharmacology.