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# Cell-specific delivery of biologicals: problems, pitfalls and possibilities of antifibrotic compounds in the liver

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**Liver fibrosis is a complex disease affecting millions of people world-wide. It involves the activation of several cell types whose activities are tightly controlled by endogenous mediators. No pharmacotherapy is available for this disease, despite the fact that many experimental drugs are very effective *in vitro* and the liver is easily accessible for most drugs. Our review provides arguments showing that cell-selectivity is essential for most antifibrotics. Several cell-specific drug carriers targeting the key pathogenic liver cells are discussed with special focus on hepatic stellate cells and fibroblast-like cells. Since endogenous mediators represent a powerful set of tools to modify the pathogenic process, this review focuses on these mediators as therapeutics and the problems and pitfalls associated with the use of such biologicals.**

## The aiming points

We have entered the era of biologicals [1]. Although new chemical entities are still produced and successfully reach the market, many new biological products like antibodies and their derivatives, siRNA, cytokines, enzymes and other therapeutic peptides are now being developed. Already a third of all new therapeutic products in 2011 were biologicals rather than chemical derivatives [1].

These biologicals provide many new exciting opportunities but also new challenges. New opportunities include manipulation of complex biological processes using endogenous substances with potent pleiotropic activities. Cytokines for instance provide powerful tools that are effective in the picomolar range within the key effector cells of diseases [2–4]. Within the liver field Interferon α2a and α2b (Pegasys respectively PegIntron) have revolutionized the treatment of Hepatitis B and C, providing an unmet medical need at the time of their introduction [5,6].

However, native endogenous substances are subjected to endogenous clearance mechanisms that affect their pharmacokinetic profile considerably. Most cytokines and lipid mediators are locally acting mediators due to rapid inactivation and degradation in plasma, renal clearance or uptake by different cell types. Their limited action radius is important because receptors for these mediators are often expressed throughout the body. Systemic administration either leads to lack of efficacy due to the aforementioned clearance mechanisms or, after increased dosing compensating for these mechanisms, cause multiple adverse effects. Pegylation of cytokines [7], leading to increased plasma stability and reduced renal clearance, is an efficient way to overrule these clearance mechanisms, but although the efficacy of pegylated or other long-circulating biologicals is in many cases significantly enhanced, adverse effects may also be enhanced.

In addition, it is becoming increasingly clear that the pleiotropic effects of cytokines often lead to a combination of local effects depending on the cell types present. It may be difficult, if not impossible, to modulate this overall effect simply by increasing the concentration of a cytokine in the diseased tissue. For instance, Il-10 has powerful anti-inflammatory effects in dendritic cells, B-cells, CD4+ and Cd8+ cells and M1 macrophages [8], thus preventing fibrogenesis but it also exerts pro-fibrotic effects in M2c macrophages [9,10] thus stimulating fibrogenesis. In a mixed cell population the outcome of treatment will depend on the dominant cell type that is present in the tissue. An exciting new line of compounds in the field of biologicals is siRNA. SiRNA has been very effective in blocking pathological responses in effector cells *in vitro* but its intracellular delivery and delivery to the target cells *in vivo* are key problems that significantly hamper their clinical development [11–13].

In this review we aim to summarize the possibilities to deliver anti-fibrotic agents to the fibrotic liver. We specifically focus on the use of biological products because these represent an exciting

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new group of compounds whose therapeutic application becomes within reach after modification of their *in vivo* distribution profile.

## Options for improvement

To prevent the rapid clearance of biologicals, long-circulating compounds have been developed. In particular pegylation of cytokines has proven to be a valuable strategy. Interferonα has been pegylated (Pegasus and PegIntron) leading to a prolonged circulation time; the circulation time of Interferon α, which is only a few hours [14], was increased to several days after Pegylation [15] allowing dosing schedules of once or twice a week [14,15]. This was associated with an increased therapeutic efficacy compared to native Interferon α. The long circulation time led to an increased uptake into hepatocytes, the target cells for this disease. These compounds provided therefore a substantial improvement over standard therapies with chemical drugs, although adverse effects of these long circulating compounds were still evident [14]. Recently, new direct-antiviral drugs with high efficacy against Hepatitis B and C and less adverse effects have been developed [16,17], demonstrating that the chemical approach is not made redundant. Nevertheless, pegylation of cytokines is nowadays a widely pursued approach. Another approach is the coupling of compounds to albumin or incorporation in liposomes which prolongs the plasma half-life and thereby the efficacy of drugs and biologicals [18]. Doxil (Caelyx) is based on doxorubicin incorporated in pegylated liposomes and this compound has less adverse effects than the free drug in cancer patients [19]. The use of pegylated liposomes also has been explored for antifibrotic therapies and successful delivery of Hepatocyte Growth Factor to the fibrotic liver has been achieved in rats [20], but this approach has not led to any follow up yet.

This strategy of prolonging plasma half-life is particularly successful in diseases characterized by an increased local vascular permeability. Angiogenesis in tumors, associated with poorly developed microvessels, or acute and chronic inflammatory processes, associated with local release of many vasoactive compounds, are characterized by local extravasation of compounds [21,22]. The low local extravascular pressure will subsequently lead to a relative retention of blood-derived compounds, in particular of high-molecular weight compounds like immunoglobulins, albumin-bound substances and exogenous products like liposomes, polymers, or pegylated compounds. Due to this enhanced permeability and retention (EPR) effect, a prolonged plasma half-life of such compounds leads to higher drug concentrations in diseased areas and therefore to higher efficacy of drugs.

However, this approach is not always a proper solution. A prolonged circulation-time also leads to a prolonged exposure of non-target cells to the drug, and may thus also lead to enhanced adverse effects. In addition, a prolonged circulation time by increasing size generally means reduced renal clearance. This will result in higher non-specific uptake by other cells endowed with multiple receptor-mediated uptake mechanisms, such as hepatocytes or antigen presenting cells, including macrophages. *Via* this mechanism, immune responses were elicited against PEG in long-circulating Pegylated liposomes [23–25].

As stated above, many cytokines have pleiotropic activities with effects in one cell type being counterbalanced by effects in a neighboring cell type. These balanced effects cannot be modulated by longer circulation times. The differential effects of Il-10 have been mentioned already but many other therapeutic cytokines may encounter the same pitfall. Interferon-γ (INF-γ) for instance inhibits fibroblast-like cells [26], it stimulates polarization of macrophages into an antifibrotic phenotype [9,27], and it enhances NK-mediated apoptosis of hepatic stellate cells [28], all leading to reduced fibrogenic activity. However, it also stimulates production of the macrophage chemoattractant MCP-1 [29], thereby stimulating inflammation. In addition, INFγ-stimulated NK-cells in non-alcoholic steatohepatitis (NASH) seem to drive the progression of hepatitis towards fibrosis [30]. So, *via* effects on different cell types a mixed result on fibrogenesis is achieved upon systemic administration of Interferon-γ. Similarly, TGFβ is one of the most potent stimulators of fibrosis in fibroblasts [31], yet it also has anti-inflammatory effects on macrophages [8,9]. In these cells TGFβ induces an enhanced IL-10 and PGE2 production, both of which can induce subsequently pro- and antifibrotic effects [32,33], thus balancing TGFβ-mediated effects. Thus, for all these cytokines and mediators the local effects seem to depend on the composition of local cell types that respond to the mediator. Therefore even enhanced local delivery of cytokines and other mediators to diseased tissues, that is, by prolongation of their circulation time, may yield mixed responses and hence low efficacy, despite their potency *in vitro*.

In addition, some diseases are not characterized by an enhanced vascular permeability. A hallmark of fibrotic and sclerotic diseases is an increased deposition of extracellular matrix constituents. And although enhanced angiogenesis is found during for instance liver fibrosis [34], this disease is associated with a reduced size of endothelial fenestrae and capillarisation of hepatic sinusoids caused by the collagen deposition in the space of Disse and thus to a reduced vascular permeability in the diseased areas [34]. Similarly, some solid tumors have increased pressure inside the mass due to rapid cell proliferation, thus limiting the advantage of long circulating compounds.

## Liver fibrosis

In order to overcome these problems, cell-specificity may be essential for therapeutic success. For anti-tumor therapies, cell-specific approaches are widely pursued but also for liver fibrosis, such an approach may be quite relevant. It is a complex disease, induced by the concerted action of many cell types. It can be induced by viruses (Hepatitis B and C), genetic disorders (*e.g.* Wilson disease), autoimmune-mediated disorders (Primary Biliary Cirrhosis, Primary Sclerosing Cholangitis), toxins, alcohol and obesity (NASH) and it affects millions of people world-wide. To date, there is no pharmacotherapy available to treat liver fibrosis or its end-stage cirrhosis [35]. The only options are liver transplantation or removal of the inciting stimulus.

As outlined above, much progress has been achieved with potent new antiviral drugs thus preventing the cause of this disease, yet liver fibrosis as such cannot be treated yet [36,37]. This is also true for other fibrotic and sclerotic diseases like Idiopathic pulmonary fibrosis and renal fibrosis. The reversal of fibrosis seen in some rodent models and in patients with sustained viral reduction [38], demonstrates that scar tissue formation is not an irreversible process, which provides opportunities for therapeutics.

The key cells in the pathogenesis of liver fibrosis are hepatic stellate cells and portal fibroblasts [39]. These cells proliferate upon



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