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A STUDY ON HEPATOPROTECTIVE AND ANTI-FIBROTIC AGENTS

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Abstract

Hepatic fibrosis and cirrhosis cause strong human suffering and necessitate a monetary burden worldwide. Therefore, there is an urgent need for the development of therapies. Pre-clinical animal models are indispensable in the drug discovery and development of new anti-fibrotic compounds and are immensely valuable for understanding and proofing the mode of their proposed action. In fibrosis research, inbreed mice and rats are by far the most used species for testing drug efficacy. During the last decades, several hundred or even a thousand different drugs that reproducibly evolve beneficial effects on liver health in respective disease models were identified. However, there are only a few compounds (e.g., GR-MD-02, GM-CT-01) that were translated from bench to bedside. In contrast, the large number of drugs successfully tested in animal studies is repeatedly tested over and over engender findings with similar or identical outcome. This circumstance undermines the 3R (Replacement, Refinement, Reduction) principle of Russell and Burch that was introduced to minimize the suffering of laboratory animals. This ethical framework, however, represents the basis of the new animal welfare regulations in the member states of the European Union. Consequently, the legal authorities in the different countries are halted to foreclose testing of drugs in animals that were successfully tested before. This review provides a synopsis on anti-fibrotic compounds that were tested in classical rodent models. Their mode of action, potential sources and the observed beneficial effects on liver health are discussed. This review attempts to provide a reference compilation for all those involved in the testing of drugs or in the design of new clinical trials targeting hepatic fibrosis.

Keywords: hepatic fibrosis, 3R principle, therapy, animal experimentation, collagen, α-smooth muscle actin, clinical trials, translational medicine.

Introduction

The last statistical report on the number of animals used for experimentation and other scientific purposes in the member states of the European Union was published in 2011. It contains data collected in 26 member states in 2011 and in France in 2010. In summary, this report shows that about 11.5 million animals were used for experimental and other scientific purposes in the EU, of which mice (60.9%) and rats (13.9%) were by far the most used species. Over 46% of these animals were used for biological studies of a fundamental nature and 8.75% in the area of toxicology and other safety evaluations. These are particular the two research areas in which novel test drug candidates for future human trials are pre-clinical screened for their safety and efficacy. In hepatology research, there is a mandatory need for novel anti-fibrotic therapies and many different in vivo and in vitro rodent models were introduced during the last decades. Mice and rats are relatively inexpensive and can be bred in large quantities, their inbred character helps to establish reproducible results, and their anatomy, genetics and biology is similar to humans. Most importantly, the pathogenesis of experimental hepatic disease in rodents closely resembles the disease progress in humans. In this process, hepatic stellate cells (HSC) and portal fibroblasts are major collagen-producing cells. The proliferative activity is triggered by numerous pro-fibrogenicchemokines and cytokines that in liver are produced by residental cells or infiltrating blood cells. This complex network of cellular interactions and the great diversity of different mediators offer a wealth of potential drug targets for targeting disease progression.

(B) In these models, a time-dependent progress of liver damage occurs in which inflammation, fibrosis, and cirrhosis time-dependently follow each other.

Using pre-clinical rodent models, many hundred (or thousands) pharmacological active ingredients with presumed fibropreventive, fibrostatic, or fibrolytic spectrum were discovered. However, the translation of these encouraging findings to humans and the initiation of human trials is perennially hampered by many factors. Consequently, there are no effective treatments for hepatic fibrosis to date. Instead, many of the identified substances are tested in regular intervals in other cell systems or animal models confirming previous reports. Although the authors of these confirmatory studies will compile some nice publications, the novelty of these studies is rather low. In addition, all these studies are expensive, cause needless pain, and suffering to animals and subvert the ethical framework for conducting scientific experiments with animals that was first proposed by Russell and Burch (1959). These guidelines encourage the replacement, reduction and refinement of animals used for scientific purposes and testing. Currently largely ignored, this so called 3R principle is the basis of the new animal welfare rules that have been implemented in the member states of the EU by the EU Directive 2010/63 and had turned into law at the beginning of year 2013. Nevertheless, in future this regulation predicts that new animal studies initiated with the aim to test drugs that were already tested before will not be approved in the Member states of the EU. Moreover, applying for a new animal study will require a concise review on what is done so far and what was not tested yet.

In the present review, a comprehensive synopsis on experimentally tested anti-fibrotic compounds is given. The chemical structure, potential sources, mode of action, molecular target and their experimental pharmacological activity in hepatic fibrogenesis of each drug is discussed.

Antioxidants/radical scavengers

Reactive oxygen species (ROS) formation is one key driver of hepatic inflammation and fibrosis. Under normal physiological conditions, oxygen-containing reactive molecules control key physiological activities such as cell growth, proliferation, migration, differentiation, and apoptosis. However, elevated intracellular ROS concentrations induce damage to cell structures (DNA, RNA, protein, lipids, and cofactors), oxidative stress and inflammation. In the liver, ROS induce apoptosis and necrosis of hepatocytes, stimulate the production of profibrogenic mediators by Kupffer cells and recruitment of circulating inflammatory cells, and leads to direct activation of HSC. Accumulating evidence suggest that beside multiple other mechanisms, the upregulation of different NADPH oxidases (NOX) subtypes in liver fibrogenesis is majorly the cause for increased intracellular ROS concentrations. This assumption was recently confirmed in NOX1- or NOX4-deficient mice. When these mice were treated with carbon tetrachloride (CCl4) to induce liver fibrosis, they showed reduced hepatic inflammation than wild type mice. Moreover, culture-activated HSC derived from these mice had overall reduced expression of pro-fibrogenic genes and the dual NOX1/4 inhibitor GKT137831 suppressed ROS production and expression of inflammation-associated genes. Intracellular ROS formation also affects the activity of pro-fibrogenic genes and vice versa. Of particular interest in hepatic fibrogenesis is the interrelation of ROS and transforming growth factor-β (TGF-β). In cultured HSC, TGF-β increases the production of H_2O_2, which in turn induces the expression of α1(I) procollagen mRNA. Consequently, catalase, an enzymatic scavenger of H2O2, abrogated TGF-β-mediated type I collagen gene expression. Numerous agents available prevent or even interfere with ROS formation, or alternatively eliminate or scavenge elevated intracellular ROS traces. Based on their chemical composition, they can be divided into sulfur-containing and non-sulfur containing antioxidants. In the following, some examples of both groups and their beneficial effects in hepatic fibrosis are summarized.

Glutathione, N-acetyl-L-cysteine, S-Nitroso-N-acetylcysteine, S-adenosyl-L-methionine, S-allylcysteine

There are a large number of sulfur-containing antioxidants with beneficial effects on hepatic inflammation and fibrosis. Glutathione (GSH) is an essential nutrient that is synthesized in the body from amino acids L-cysteine, L-glutamic acid, and glycine. It exists in both reduced from (GSH) or in a dimer oxidized (GSSG) form. In the reduced form, the sulfhydryl group of the cysteine residue is able to donate a reducing equivalent and serve as a proton, give rise to its activity as an antioxidant. GSH and its structurally related compounds N-acetyl-L-cysteine (NAC), S-Nitroso-N-acetylcysteine (SNAC), S-adenosyl-L-methionine (SAM) and S-allylcysteine (SAC) have been used in clinics for the treatment of fibrotic diseases. NAC, SNAC, SAM, and SAC either serve as direct GSH precursors, nitric oxide (NO) donors, or serve as methyl group donors required for methylation of nucleic acids, phospholipids, histones, biogenic amines, and proteins. In addition, NAC dose-dependently blocked TGF-β signaling in fibrogenic cells by monomerization of the biological active TGF-β dimer. NAC attenuated hepatic oxidative stress and prevented increases in cytochrome P450 2E1 apoprotein, TNF-α expression, and induction of auto-antibodies associated with lipid peroxidation in a dietary polyunsaturated fat model of non-alcoholic steatohepatitis (NASH) in rats. Likewise, NAC prevented cirrhosis by reducing oxidative stress and TGF-β expression. The oral SNAC administration resulted in a reduction in collagen α1, increased matrix metalloproteinase (MMP)-13 activity, and a significant suppression of TIMP-2 and TGF-β1. It further induced de-differentiation of the immortalized murine hepatic stellate cell line GRX. SAM is a modulator of cellular apoptosis, suppressor of tumor necrosis factor (TNF)-α and inducer of interleukin (IL)-10 expression. It further inhibits cellular proliferation, adhesion, migration and invasion of human HSC in vitro. The efficacy of SAC was proven in a porcine serum-induced hepatic fibrosis model in rats in which this compound attenuated hepatic fibrosis and suppressed α-smooth muscle actin (α-SMA) expression.

Bucillamine

This substance is a cysteine derivative that contains two donatablethiol groups thereby acting as a potent sulfhydryl donor rendering a potent antioxidant activity. It is particular efficacious in acute settings characterized by inflammation and oxidative stress. It further sequesters iron (II) and copper (II) that are both involved in oxidative stress-induced damage. Bucillamine also inhibits neutrophil activation during hepatic injury and modulates the Bax/Bcl-2 ratio without affecting the tissue GSH levels.

Lipoic acid

Lipoic acid (or better α-lipoic acid) contains two sulfur atoms that are connected by a redox-sensitive disulfide bond. It functions as a cofactor for some enzyme systems involved in acyl group transfer. Independent studies have shown that α-lipoic acid and its reduced form dihydrolipoic acid inhibit liver fibrosis in rats chronically treated with thioacetamide (TAA), most likely by preventing ROS generation and ROS-mediated signaling in HSC. In addition, lipoic acid prevented the development of BDL-induced hepatic fibrosis and effectively attenuated TGF-β-stimulated PAI-1 expression through inhibition of the TGF-β-associated mediators Smad3, AP1, and SP1. Similarly, this substance prevented fibrosis development, inflammation and cellular apoptosis in rats that were subjected to a high fat diet.

Taurine

This compound is a derivative of cysteine that is a major constituent of bile and has many biological activities in bile acid conjugation, membrane stabilization and calcium signaling. It further acts as a direct antioxidant and protects against the toxicity of different metals. Taurine administration in drinking water prevented the activity of lipid hydroperoxides, improved mitochondrial enzyme activities, and regulated iron and calcium levels in experimental rat liver fibrosis that was induced by simultaneous application of ethanol and iron. In the same model system, taurine lowered the levels of IL-6, TNF-α and peroxidation products, as well as the expression of α-SMA, desmin, and TGF-β1 and further improved the antioxidant status. Other studies showed that this β-amino acid is neither a classical scavenger nor a regulator of the anti-oxidative defenses but more likely serves as a regulator of mitochondrial protein synthesis.

α-tocopherol and trolox

The non-sulfur-containing lipid-soluble benopyranol α-tocopherol (vitamin E) and its water-soluble analogTrolox are effective peroxyl radical scavengers and absorber of oxygen radicals. Moreover, these substances were found to act as non-competitive inhibitors of cyclooxygenase, suppressor of vascular endothelial growth factor (VEGF) and TGF-β gene transcription. The importance of the antioxidant capacity of vitamin E in preventing fibrosis was proven in animals studies and clinically confirmed.

Ascorbic acid

This soluble essential nutrient (vitamin C;) is an essential cofactor in enzymatic reactions, involved in many biochemical activities, and as a electron donor acting as a direct free radical scavenger. Vitamin C alone or in synergy with other agents decreases lipid peroxidation directly or indirectly by regenerating vitamin E. In line with this assumption, the pre-treatment with vitamin C prior exposure to TAA is sufficient to prevent hepatic cirrhosis in rats. However, ascorbic acid also induced pro-fibrotic effects in hepatic fibrogenesis in mice that lacked regucalcin (SMP30), a gene that is critically involved in hepatic Ca2+ homeostasis.

Melatonin

This substance, also known as N-acetyl-5-methoxytryptamine, is a free-radical scavenger and antioxidant that protects against nuclear and mitochondrial DNA damage and interacts with the immune system. It has also the capacity to form complexes with different metals. In dimethylnitrosamine (DMN)-induced liver fibrosis in rats, melatonin functions as a potent fibrosuppressant and antioxidant by preventing the decrease of GSH and superoxide dismutase levels. This substance has recently renewed interest because it is an effective drug that inhibits autophagy, necroptosis, and endoplasmic reticulum stress in CCl4-induced hepatic fibrosis in mice. In the same model, it attenuated liver injury and inhibited the expression of collagens types I and III, TGF-β, PDGF, connective tissue growth factor (CTGF), amphiregulin, and activation of Smad3, while the MMP-9 activity decreased and the expression of nuclear factor erythroid-2-related factor 2 (Nrf2), representing is a central regulator of anti-oxidative response, increased.

Caffeic acid, rosmarinic acid

The natural phenol caffeic acid is present in modest concentration in coffee. However, it is unrelated to caffeine and is composed of a phenol ring carrying an unsaturated carboxylic acid side chain. Like its ester, rosmarinic acid, caffeic acid has a high rate constant to react with hydroxyl radicals. Rosmarinic acid inhibited proliferation and induced apoptosis in HSC-T6, partly due to the inhibition of phosphorylation in STAT3. The intraperitoneal application of phenethyl ester of caffeic acid was recently shown to evolve hepatoprotective effects and to suppress HSC activation by inhibiting oxidative stress in rats that were injected subcutanly with CCl4, feed with high fat forage, and administered with alcohol orally.

Genistein, luteolin, quercitin, apigenin, naringenin, and other polyphenolic compounds

Genistein is a naturally occurring phytoestrogicisoflavone that is present in high concentration in soy and many other plants. Similar to many other polyphenolic substances, this compound acts as a direct antioxidant. It reduces damaging effects of free radicals, prevents the release of cytochrome c from mitochondria and has further the ability to modulate the activity of the nuclear receptor Peroxisome proliferator-activated receptor (PPAR)-γ and the estrogen receptor. Dietary supplementation of genistein down regulated the augmented gene expression associated with hepatic inflammation and fibrosis in a methionine-choline-deficient (MCD) diet in leptin receptor deficient db/db mice. Likewise, genistein ameliorated developing liver injury and fibrosis that was induced by repeatedly intragastric administration of alcohol. Luteolin is a flavone that is found in high concentrations in leaves of the yellow myrobalan (Terminaliachebula), avocado, celery, olive oil, chamomile, peppermint and many aromatic plants. It was supposed that this compound and some of its derivatives act as dopamine transporter activators. Although the precise activity is not fully understand, this drug increased the expression of MMP-9 and Metallothionein thereby promoting extracellular matrix (ECM) degradation in established hepatic fibrosis that was induced by administration of CCl4. More recently, it was demonstrated that luteolin inhibited DEN-initiated alcohol-promoted hepatic inflammation by stimulating hepatic sirtuin 1 activity that is a master regulator in hepatic lipid metabolism. Other reports have suggested that luteolin prevents progression of liver fibrosis through a multitude of different mechanisms that include inhibition of fibrosis-related genes in HSC, induction of HSC apoptosis and cell arrest, and inhibition of cytokine signaling pathways (TGF-β and PDGF;). Quercitin is a pentahydroxyl flavonoid occurring for example in onions, apples, broccoli and green beans. In CCl4-treated rats, this substance prevented oxidative stress, lipid peroxidation and increased levels of GSH, SOD, catalase, GPx, and GST levels. Similarly, quercitin decreased ALT, AST, GGT and LDH levels and increased expression of albumin in a high fat diet model in rats. This drug was also effective in a MCD diet model in mice in which it attenuated pro-fibrotic and pro-inflammatory gene expression. Strong antioxidant activity, anti-apoptotic and hepatoprotective effects were also reported for the flavone apigenin that occurs in garden parsley (Petroselinumcrispum), celery (Apiumgraveolens), and different chamomiles. The efficacy of this drug was proven in different toxic liver injury models. Naringenin occurs in high concentrations in grapefruit and other citrus fruits in form of conjugated glucosides such as naringenin-7-rhamnoglucoside or naringenin-7-

glucoside. The oral administration of naringenin prevented DMN-induced hepatic fibrosis in rats. A subsequent study in vitro study performed in HSC-T6 cells showed that naringenin dose-dependently exerts its anti-fibrogenic activity by down-regulation of Smad3 protein expression and activation. Furthermore, this substance reduced the plasma fat and the hepatic expression of pro-inflammatory mediators such as TNF-α, IL-6, IL-1β, iNOS, MMP-2, and MMP-9 in rats that were fed with a high cholesterol diet. Likewise, there are many other natural plant-derived polyphenolic compounds with anti-oxidative activity including acids (3,4-OH-benzoic, gallic, O-, and P- coumaric, syringic, vanillic), alcohols (tyrosol and OH-tyrosol), theobromine, rutin, catechine, and apigenin. All these substances are in the pipeline of researchers and are worth to be tested in experimental models of hepatic fibrogenesis.

Nicotinic acid, nicotinamide, and nicotinamide adenine dinucleotide (phosphates)

Nicotinic acid (niacin) and its amide (vitamin B3) serve as the most fundamental nicotinamide adenine dinucleotide precursors (Supplementary). They are critical cofactors in a wide variety of intracellular oxidation-reduction reactions and serve as antioxidants. Nicotinamide adenine dinucleotides consist of either oxidized or reduced unphosphorylated (NAD+ or NADH) and phosphorylated (NADP+ and NADPH) forms. These compounds are indispensable for a multitude of dehydrogenase enzymes, act as mitochondrial electron carriers and electron donors for GSH, thioredoxin, and NADPH oxidases (i.e., the NOX enzymes). They further act as ADP donors in ribosylation reactions, substrates for NAD+-dependent enzymes (e.g., PARPs and Sirtuins), and redox regulators that modify ion channel function. Since the cellular concentration of oxidized and reduced forms of these intracellular ROS modulators is itself influenced by countless factors (e.g., total ROS concentration, enzymatic activities of ROS producers and scavengers), this axis offers a plenitude of possibilities to modulate the intracellular ROS concentration. In TAA-induced hepatic fibrogenesis, the co-administration of nicotinic acid prevented fibrosis by its antioxidant properties and reduction of TGF-β expression. As mentioned above, NOXs play crucial roles in hepatic fibrogenesis and HSC express a non-phagocytic form of NOX, which plays a critical role in activating fibrosis-associated pathways. The deficiency of NOX1 and NOX4 in CCl4-treated mice reduced liver injury, inflammation, and activation of stellate cell thereby attenuating fibrosis. The importance of NOX1 and NOX4 in HSC was further underpinned by the fact that the dual NOX1/4 inhibitor GKT137831 not only suppressed ROS production but also prevented HSC activation by inhibition of inflammation- and proliferation-associated signaling. Likewise, the application of the coumarin derivative decursin that blocks the expression and activity of NOX1, NOX2, and NOX4 reduced the quantities of ROS and fibrogenesis in a CCl4-induced liver injury model in mice. Another possibility to reduce the hepatic ROS content and fibrogenesis is the overexpression of the ubiquitously expressed thioredoxin. This protein acts as an antioxidant by facilitating the reduction of other proteins by cysteine thiol-disulfide exchange. In line with this attributed, thioredoxin transgenic mice were protected against TAA-induced hepatic fibrogenesis and HSC isolated from these mice were less proliferative than those isolated from wild type littermates.

HMG-CoA reductase inhibitors

The central and rate-controlling enzyme in the synthesis of cholesterol is the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase that NADH- or NADPH-dependently forms mevalonate from HMG-CoA. There are a large number of drugs, i.e., the statins, which inhibit the activity of this enzyme. A growing number of statins such as lovastatin, atorvastatin, simvastatin, pravastatin, fluvastatin, pitavastatin, rosuvastatin, and others and combinations thereof are on the market. They should prevent cardiovascular disease in liver-diseased patients. Atorvastatin attenuated ongoing and established hepatic fibrogenesis in BDL rats by inhibiting HSC activation or turnover suggesting that this statin has both protective and therapeutic potential. In accordance with its therapeutic potential, it was shown that atorvastatin decreased cytokine and collagen production in myofibroblasts (MFB) in vitro and initiated apoptosis. Anti-proliferative activity in cultured primary HSC was also demonstrated for lovastatin and simvastatin that both inhibited proliferation and collagen expression. Rosuvastatin successfully improved hepatic steatosis in a high-fat and high-cholesterol diet-induced NASH model in rats and further improved hepatic fibrosis via improved peroxisomal β-oxidation. Simvastatin administered intragastrically in a model of high fat diet in rats significantly reduced expression of inducible nitric oxide (iNOS) synthase, α-SMA, TGF-β1, and collagen, while inducing the expression of endothelial nitric oxide (eNOS) synthesis that evolves protective function in the cardiovascular system. However, there are an increasing number of reports that somewhat questioning the beneficial effects of statins in therapy of hepatic disease. In a BDL model performed in rats, the administration of rosuvastatin in early stages of cholestasis decreased α-SMA expression and inhibited NF-κB activation but also increased hepatocytolysis, oxidative stress formation, and hepatic inflammation and sustained increased levels of TGF-β1. Likewise, in the TAA model in rats both atorvastatin and rosuvastatin failed to inhibit liver cirrhosis or oxidative stress formation and had no effect of HSC proliferation.

Hepatoprotective substances affecting fat metabolism

Excessive alcohol consumption, high caloric intake, or several metabolic disorders predispose for fatty liver in which triglycerides accumulate in liver cells. This process termed steatosis is accompanied by inflammation that on long term ends in fibrosis. Therefore, substances that prevent intracellular fat uptake or increase the turnover and metabolization of triglycerides have anti-inflammatory and anti-fibrotic impact.

Polyenylphosphatidylcholine (PPC)

This highly purified mixture of different phospholipids is marked as “Essentiale” with indications in acute and chronic hepatitis, fatty degeneration, toxic liver damage and dyslipoproteinemia. This preparation should evolve several hepatoprotective activities such as recovery of hepatocytes, improvement of lipid and glycogen metabolism, correction of mitochondrial failure and activation of RNA synthesis. Its main phosphatidylcholine species dilinoleoylphosphatidylcholine is highly effective in blocking TGF-β1-induced collagen and TIMP-1 expression in rat HSC, while Palmitoyl-linoleoylphosphatidylcholine, the second most abundant component in PPC had no effect on expression of these pro-fibrogenic genes.

Hepatoprotective substances affecting sugar metabolism

Elevated fasting glucose is a risk factor for non-alcoholic fatty liver disease that is associated with NASH, fibrosis, and cirrhosis. Insulin resistance and diabetes contribute to the progression from NASH to fibrosis through the development of a pro-fibrotic environment in the liver. Therefore, drugs with insulin-sensitizing or anti-hyperglycaemic activity are one option that protects for hepatic inflammation and fibrosis. Several drugs are available that either has one or both of these activities.

Pioglitazone (actos) and rosiglitazone (avandia)

Based on its insulin-sensitizing and anti-hyperglycaemic activity, this drug is clinical used for the treatment of adult-onset diabetes. Chemically, it belongs to the large group of thiazolidinediones also known as glitazones (Supplementary Figure 4) that activate the nuclear PPAR receptors. In experimental hepatic steatosis and fibrosis in rats induced by feeding of a choline-deficient L-amino-acid-defined diet, pioglitazone reduced the expression of TIMP-1, TIMP-2, and prevented the activation of HSC. These experimental findings were also confirmed in a rat model of high fat-induced steatosis in which pioglitazone reduced excess hepatic fatty degeneration and fibrosis, serum levels of transaminases, triglycerides, free fatty acids, glucose, insulin, and expression of hepatic collagen I and α-SMA. In humans, a detailed meta-analysis of pioglitazone activity in 137 patients that suffered from NASH showed that patients taken this drug had significant lower grade of fibrosis, lower body weight fat and improvement of ballooning degeneration, lobular inflammation, and steatosis than the placebo group that contained 134 individuals. However, in some rat models the therapeutic anti-fibrotic efficacy of this thiazolidinedione was limited. It failed to interrupt progression of BDL-induced fibrosis suggesting that the etiology leading to fibrosis, the duration of the underlying liver disease, and/or the severity of fibrosis at the time of initiation of piaglitazone treatment significantly influence the therapeutic potential this drug. Rosiglitazone, another thiazolidinedione, showed highly beneficial effects in prevention and ameliorating nutritional fibrosingsteatohepatitis in rodents. In contrast this drug on long-term id associated with increased expression of pro-inflammatory genes in humans that however has no effects on collagen I or TGF-β expression. In summary, all these findings indicate that not all glitazones might be entirely suitable for treatment of NASH or uniformly useful in other kind of hepatic injuries.

Inhibitors of cytokine signaling

Cytokines are produced by a broad range of cells. They and act in an autocrine or paracrine manner by binding to specific cell-surface receptors thereby initiating intracellular signaling cascades commonly resulting in modulation of gene transcription. Over the last decades, there has been increasing knowledge on the involvement of different cytokines and their pathways in the pathogenesis of hepatic fibrosis. Some cytokines that seems to be extremely important for initiation and progression of hepatic fibrosis represent good drug targets that were comprehensively explored during the last decades.

Conclusions

Preclinical work from many laboratories has identified and evaluated hundreds of substances or mixtures of components showing highly beneficial effects in therapy of experimental hepatic fibrosis. They counteract intracellular ROS formation, prevent hepatic infiltration with circulating blood cells, or target pro-inflammatory and pro-fibrotic signaling pathways or mediators involved in ECM generation or turnover. Scientists and clinicians agree that it is now the time to estimate if these encouraging findings can be translated to the clinic. In particular, a first screen for safety and efficacy would provide urgently needed data on pharmacodynamics and pharmacokinetics of these "pipeline drugs." Moreover, it will be necessary to test in volunteers if the beneficial effects of these drugs can be reproduced in humans. Of course, the transfer to human is somewhat hampered because most of these drugs were only tested in models of ongoingfibrogenesis and information about the curative effects are still missing. Therefore, in future it would be more sensible if drug candidates are tested in models of established fibrosis than to confirm their hepatoprotective or anti-fibrotic effects in other disease models. This would not only expand the knowledge of the usability of a specific drug but further helps to make science in fibrosis research more cost-effective and minimize the number of animals used in preclinical experiments requested by the 3R guiding principles proposed by Russell and Burch.

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