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Anti-fibrotic Activity and Enhanced Interleukin-6 Production by Hepatic Stellate cells in Response to Imatinib Mesylate

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Abstract

Objective—To examine imatinib mesylate's effects on stellate cell responses *in vivo* and *in vitro*. The hepatic stellate cell (HSC) is a key target of anti-fibrotic therapies. Imatinib mesylate is a small molecule receptor tyrosine kinase (RTK) inhibitors indicated for treatment of chronic myelogenous leukemia and GI stromal tumors.

Design—Because imatinib inhibits β -PDGFR signaling, which stimulates HSC proliferation, we assessed its activity in culture and *in vivo*, and examined downstream targets of its activity in a human stellate cell line (LX-2) using cDNA microarray.

Methods and Results—Imatinib inhibited proliferation of LX-2 cells (0.5 mM – 10 mM) but not primary human stellate cells, with no effect on viability, associated with attenuated β -PDGFR phosphorylation. Mitochondrial activity and superoxide anion production were decreased in response to imatinib. cDNA microarray uncovered up-regulation of 29 genes in response to imatinib, including interleukin-6 (IL-6) mRNA, which was correlated with progressive IL-6 secretion. Imatinib also decreased gene expression of collagen $\alpha_1(I)$, alpha SMA, β -PDGFR, TGF β_1 receptor type 1, matrix metalloproteinase 2 (MMP2) and tissue inhibitor of metalloproteinase 2 (TIMP2). *In vivo*, imatinib administered to rats beginning 4 weeks after starting thioacetamide led to reduced collagen content, with significant reductions in portal pressure and down-regulation of fibrogenic genes in whole liver. Importantly, hepatic IL-6 mRNA levels were significantly increased in TAA-treated animals receiving imatinib. **Conclusions:** These findings reinforce the anti-fibrotic activity of imatinib and uncover an unexpected link between inhibition of HSC activation by imatinib and enhanced secretion of IL-6, a regenerative cytokine.

Keywords

Hepatic regeneration; PGDF receptor; tyrosine kinase; LX-2 cells; microarray

INTRODUCTION

Elucidation of pathogenetic mechanisms underlying hepatic fibrosis is yielding fertile targets for anti-fibrotic therapies in patients with chronic liver disease. Most experimental studies have focused on the hepatic stellate cell to identify targets driving ‘activation’ of this non-parenchymal cell type, a transdifferentiation characterized by enhanced fibrogenesis, contractility, proliferation and adoption of an inflammatory phenotype (1). Among these pathways, inhibition of stellate cell proliferation is appealing as a therapy because pathways driving proliferation are well characterized (2). Many proliferative pathways involve signal transduction through receptor tyrosine kinases (RTKs), for example, the receptors for epidermal growth factor receptor, vascular endothelial growth factor (3–5), and platelet derived growth factor (6–8), among others (3, 9, 10). Among RTK pathways, PDGF signaling is the most potent mitogenic pathway in stellate cells, and is therefore an especially appealing target (7). Moreover, the dominant receptor subtype, the β -PDGFR isoform, is expressed minimally in normal liver, but rapidly induced only in hepatic stellate cells, providing an inherent targeting of antagonists aimed at this receptor (6).

RTKs are also a major target of therapeutic antagonism in both cancer and non-malignant, fibroproliferative diseases (11–13). Among several compounds, imatinib mesylate, (gleevec® or STI571; Novartis Pharmaceuticals) has been the most successful RTK inhibitor to date. This molecule is indicated for the treatment of both chronic myelogenous leukemia (CML) based on its inhibition of the RTK activity of the Bcr-Abl oncogene product (14), and in gastrointestinal stromal tumors based on its inhibition of the c-kit RTK (15, 16). The antagonistic activity of imatinib extends to other RTKs, including β -PDGF receptor (17). Given this feature, the drug is an attractive therapeutic candidate for hepatic fibrosis. Indeed, several recent studies have exploited this possibility in rodent models of cardiac (18) and hepatic fibrosis (19, 20). In liver, imatinib reduces the features of hepatic fibrosis including hyaluronic acid content, serum fibrosis markers, and suppression in procollagen I, alpha smooth muscle actin, TIMP I and attenuated phosphorylation of PDGFR- β and MEK (21).

At the same time, attention has focused not only on the fibrogenic response, but also on mechanisms that link fibrogenesis to preservation of hepatocellular mass. Interestingly, stellate cells provide key regenerative signals (22), including hepatocyte growth factor (23, 24) and neurotrophin signaling (25–27). Among other factors derived from stellate cells, interleukin-6 (IL-6) is also critical, as IL-6 knockout mice lack regeneration after partial hepatectomy (28). IL-6 is also hepatoprotective following toxic liver injury (29).

Our study had two major aims. First, we sought to extend earlier animals studies to define at which point during the progression of hepatic fibrosis imatinib exerts its anti-fibrotic effects, and whether this was associated with improvement in portal hypertension. Second, we wanted to broaden our understanding of imatinib’s effects on hepatic stellate cells beyond growth inhibition. In doing so, we have uncovered a new link between imatinib’s ability to attenuate stellate cell activation and potentially enhance hepatic regeneration by stimulating IL-6 production.

MATERIALS AND METHODS

Culture Studies

LX-2 cells (30) and primary human stellate cells (31) were maintained in medium containing 10% fetal bovine serum. Cells were treated with imatinib mesylate (Novartis Pharmaceuticals, New Jersey) for 12 or 24 hours at concentrations of 0.5, 1.0, 2.0, 5.0 μ M and 10 μ M in medium with 0.2% fetal bovine serum. For some studies cells were incubated

with the β -PDGF receptor tyrosine kinase inhibitors JNJ-10198409 (Sigma) or AG 1296 (Sigma) (5 nM).

Cell Proliferation—DNA synthesis was assessed by assaying ^3H -thymidine incorporation (32).

Cell Viability—Viability was assessed in 6 well plates using an MTT assay (Sigma). For final data, triplicate OD values from three independent experiments were combined.

Phosphorylated β -PDGFR analysis—Immunoblot analysis of phosphorylated β -PDGFR in LX-2 cells extracts was performed as previously described (33), with modifications. LX-2 cells were treated with imatinib or PBS containing 0, 1, or 10 nM PDGF-BB (BD Biosciences, San Jose, CA) for 10 seconds and the cells were harvested using RIPA buffer for protein assay. Blots were incubated with anti-phospho- β -PDGFR (Y716) antibody (Upstate Biotechnology) and anti-total β -PDGFR antibody (Upstate), diluted 1:500 for 2 hours at room temperature. Immunoreactivity was revealed by enhanced chemiluminescence using an ECL kit (Amersham). Blots were normalized to alpha tubulin protein detected using a polyclonal antibody (Santa Cruz), diluted 1: 200.

Analysis of oxidative stress—LX-2 cells were cultured for 24 and 48 hours with or without imatinib 2 μM . Mitochondrial activity was measured using MitoTracker® Red (Molecular Probes). Superoxide anion was quantified using 2 μM dihydroethidium (hydroethidine) (DHE), according to the manufacturer's protocol (Molecular Probes) and quantified using a FACS Caliber (Becton Dickinson).

Fibrosis-related mRNA quantification—Primers used are listed in Supplemental Table 1. Total mRNA from LX-2 cells or 100 mg of rat whole liver tissue were extracted and purified (Qiagen RNeasy Kit). Concentrations were measured (ND-100 Spectrophotometer, Nanodrop Technology), and 1 μg of total mRNA was reverse transcribed to complementary DNA (cDNA) using the Reverse Transcription System (BD Sprint). Real-time quantitative PCR was performed in triplicate with a 384 well format Lightcycler® 480 System (Roche Diagnostics, USA). We used GAPDH as a reference gene. Relative quantification was calculated using the comparative threshold cycle (CT) method (ABI PRISM 7700 user's manual).

Microarray Analysis—Total RNAs from LX-2 cells were prepared using RNeasy Micro Kit and integrity assessed with an Agilent Bioanalyzer 2100. Then, 5 μg RNA were reverse-transcribed using a poly (dT) oligonucleotide (GeneChip®, Invitrogen). Hybridization was conducted using a human genome U133 plus 2.0 array (Affymetrix). The genechip data were analyzed using R (www.r-project.org) and Bioconductor (34). Raw data were first normalized across the chips by log scale robust multi-array analysis (RMA)(35) method. To identify significantly differentially expressed genes between groups, Significance Analysis of Microarray (SAM, version2.1)(36) was employed. Genes with a q value <0.05 are considered to be significantly different.

IL-6 protein assay—Human IL-6 was assayed using the Quantikine ELISA Kit and protocol (R&D Systems) in 200 μl of medium from LX-2 cells.

***In vivo* Studies**

Male Sprague-Dawley rats (350 g or 6–8 weeks old), were maintained according to NIH guidelines, and approved by the Mount Sinai IACUC Committee. *In vivo* studies employed 'prophylactic', 'therapeutic' and 'reversal' treatment schedules (see Supplemental Figure 1).

In Group 1 ('prophylactic'), rats were administered 150 mg/kg of TAA intraperitoneally (IP), three times weekly for 6 weeks to induce cirrhosis. Ten animals were treated either with imatinib 20 mg/kg/day IP or placebo using the same volume of saline. Following treatment, imatinib-treated and control rats were sacrificed and analyzed. In Group 2 ('therapeutic'), TAA was administered in the same pattern, however imatinib or placebo began from the 4th week of TAA and was administered for 6 weeks thereafter. In Group 3 ('reversal'), imatinib or placebo was administered beginning only after 6 weeks of TAA were completed for an additional six weeks.

At the time of sacrifice portal pressure was measured using a 16G angiocatheter introduced into the portal vein to measure the height of a water column. Next, blood samples were obtained for AST, ALT and bilirubin, and the liver was removed and processed.

Liver histology—Liver sections (15 μ m) were stained in 0.1% Sirius red in saturated picric acid (both from Sigma). In addition hematoxylin & eosin sections were analyzed blindly by an expert pathologist (M.I.F.), by scoring for the presence of ballooning, portal inflammation, lobular inflammation, ductular reaction, atypical ductal structures, steatosis and fibrosis. Data were derived from blinded analysis of 5 sections from each of 10 animals in each group. Fibrosis scores were included in displayed data in Supplemental Table 3, although this variable was also quantitated assessed using morphometry.

Fibrosis quantification—Relative fibrosis area was assessed by analyzing 36 Sirius red-stained liver sections per animal using a computerized Bioquant Life Science® morphometry system. From each group analyzed, the average fibrosis area was expressed as a percentage of total area.

Statistical analysis—Data from all experiments were analyzed for median, standard deviation, standard error, and statistically significant differences by Student t-test and SAM test.

RESULTS

Effects of Imatinib on the Human Stellate cell line, LX-2

We first confirmed imatinib's inhibition of LX-2 cell proliferation, based on its ability to inhibit the β -PGDF receptor (30) (37) (Figure 1A). Interestingly, inhibition of proliferation by imatinib was inconsistently observed in primary human stellate cells (data not shown). Viability was unaffected at concentrations of 1 μ M or 2 μ M, with some toxicity at 10 μ M, by MTT assay (not shown). Western blot confirmed reduced phosphorylation of the β -PDGFR at 2 and 10 μ M imatinib, similar to a recent report (20) (Figure 1B).

We next assessed the impact of imatinib on a panel of fibrogenic mRNAs assessed by real time PCR (Figure 2). In cells treated with imatinib for 12 hours with 0.5, 1.0 or 2.0 mM, there was consistent down-regulation of mRNAs encoding collagen 1a1, β -PDGFR, and MMP2 mRNAs were down-regulated at several concentrations of imatinib treatment while that of alpha-SMA, TGF β R1 and TIMP2 mRNAs were unaffected. Interestingly, there was a modest but consistent up-regulation of TIMP 1 ($p < 0.05$). While the mechanism is not certain, a previous report identified a unique regulatory element, UTE1, in the TIMP1 promoter in hepatic stellate cells (38), whose regulation by imatinib merits direct analysis in future studies.

Oxidant stress is a key stimulus to hepatic stellate cell activation and fibrosis during chronic liver injury (2, 39). Thus, we examined the effects of imatinib on mitochondrial integrity in LX-2 cells, using a commercial reagent (MitoTracker). As shown in Figure 3, there was less

accumulation of fluorescence in the imatinib-treated cells compared to control cells, indicating reduced mitochondrial activity and oxidation. These effects are in contrast to a deleterious effect of imatinib on mitochondrial integrity in cancer cells (40) and cardiomyocytes (41), but the mechanism for these divergent effects in stellate cells is not known.

Imatinib's targets include not only the Bcr-Abl oncogene and β -PDGR, but several other RTK receptors. Moreover, stellate cells express many receptor tyrosine kinases, not all of which have been fully characterized in this cell type (3). In addition, imatinib has the potential to elicit 'off-target' effects, for example its blockade of calcium channels (42). With these considerations in mind, we sought to identify any downstream targets of imatinib in stellate cells that might not be predicted based on its known molecular targets. To accomplish this, microarray was performed on LX-2 cells; five samples and arrays each were used for each of two conditions (cells with or without imatinib, 2 μ M, for 12 hours), and the SAM (Significance Analysis of Microarray) method was used to identify mRNAs affected by imatinib (Supplemental Figure 2; Supplemental Table 2). Twenty nine genes were induced by imatinib and seven were validated by real time PCR (not shown) but subsequent studies focused solely on IL-6.

Among transcripts affected by imatinib in stellate cells, interleukin-6 (IL-6), a potent regenerative cytokine (43), was consistently up-regulated. To confirm an effect on IL-6 protein as well, ELISA demonstrated time-dependent induction in secreted IL-6 between 24–72 hours of incubation with imatinib (Figure 4).

Because imatinib inhibits several receptor tyrosine kinases, we next examined whether a similar effect on IL-6 would be evident when stellate cells were treated with other selective β -PDGF receptor kinase inhibitors, or alternatively whether IL-6 induction might be due to inhibition of other tyrosine kinase targets of imatinib. To address this question, LX-2 cells and primary human stellate cells were incubated with either the PDGF tyrosine kinase inhibitor JNJ-10198409 or AG-1296. Both JNJ and AG suppressed LX-2 proliferation to the same extent as imatinib (not shown). As shown in Figure 5A, both of these inhibitors significantly repressed expression of collagen I and β -PDGFR mRNAs, and induced IL-6 mRNA expression, similar to imatinib. In primary human stellate cells, the inhibitors modestly repressed collagen I mRNAs and imatinib and JNJ markedly induced IL-6 mRNA, while AG-1296 had no effect.

Anti-fibrotic Effects of Imatinib on Hepatic Fibrosis in vivo

To establish the potential benefit of imatinib as an anti-fibrotic, we performed *in vivo* studies using the thioacetamide (TAA) model of liver injury and fibrosis (44). This model is particularly well suited to studies of anti-fibrotic drugs because it is much less necrotic than CCl₄, and importantly, spontaneous improvement in fibrosis is minimal after 5–6 weeks of IP administration (data not shown). Three different dosing schedules were used in which all animals were administered TAA for six weeks and either imatinib or vehicle control for six weeks (Supplemental Figure 1): 1) a 'prophylactic' regimen in which both TAA and imatinib were administered concurrently; 2) a 'therapeutic' regimen in which imatinib was initiated only 4 weeks after the beginning of a six-week TAA dosing and continued for another 4 weeks thereafter, and; 3) a 'reversibility' regimen in which imatinib was administered for six weeks only after completing 6 weeks of TAA. Animals were extensively characterized including serum transaminases and bilirubin, portal pressure, standard histology and collagen morphometry and real time PCR analysis of key fibrogenic genes. Moreover, we also analyzed IL-6 gene expression based on results obtained from the microarray analysis.

The most potent effects of imatinib *in vivo* were seen in Group 2, in which the drug was administered beginning 4 weeks after TAA was begun. Anti-fibrotic activity was also observed in Group 1 (data not shown). In contrast, in Group 3 ('reversibility' regimen) there was less fibrosis but not a statistically significant difference between imatinib and control treated animals, as both had extensive fibrosis to the same extent (not shown), indicating that imatinib did not reverse well established fibrosis, as previously reported (21).

In Group 2 animals there were no differences in serum liver tests however portal pressure was reduced by 35%, associated with significantly decreased collagen content in imatinib-treated animals as assessed by morphometry in Sirius red-stained liver sections (Figure 6A & B). These changes were associated with reduced inflammatory and fibrotic scoring as assessed blindly by a hepatic pathologist (Supplemental Table 3). Interestingly, ductular reaction, which is associated with increased fibrosis in human disease (45), was reduced in these animals. Fibrogenic mRNA expression also correlated with the histologic results. Specifically, there was significant down-regulation in whole livers of collagen Type I (Coll1) 0.5 fold, β -PDGFR, and metalloproteinase 2 (MMP2). Interestingly, gene expression of Type I TGF β R was increased significantly. No significant changes were detected in the expression of alpha SMA, TIMP1 or TIMP2 (Figure 7).

Based on the finding of IL-6 induction by imatinib in LX-2 cells and human primary stellate cells, we examined whether IL-6 was also induced *in vivo* in TAA-dosed animals treated with imatinib. Indeed, induction of total mRNA from whole liver tissue, IL-6 was present in all three groups (Figure 8). Interestingly, the increase was greatest in animals from Group 3, even though there was no significant impact on fibrosis by imatinib in these animals treated after fibrosis was well established.

DISCUSSION

Our findings reinforce the potential value of imatinib as an anti-fibrotic in the treatment of fibrosing liver injury, and add to the evidence justifying exploration of RTK antagonists as anti-fibrotics (46) (11, 47). Imatinib displayed a significant inhibition of experimental fibrosis due to TAA when administered in a 'therapeutic' schedule, partially in a prophylactic regimen, but not in a reversal regimen, corroborating the findings of Neef *et al* (21). Of significance, this anti-fibrotic effect in a 'therapeutic' regimen was associated with significant reductions in portal pressure, attesting to the functional importance of its anti-fibrotic activity. The lack of an effect in reversing established fibrosis suggests that it does not sufficiently stimulate or unmask protease activity required for matrix degradation, which depends on either up-regulation of interstitial collagenase activity or down-regulation of inhibitory TIMPs (48, 49). This is not entirely surprising, since antagonism primarily of stellate cell proliferation might not affect pathways of matrix degradation. On the other hand, in an activated stellate cell line, imatinib significantly down regulated several fibrosis-related transcripts, including collagen α 1 (I) (Coll1) and β -PDGF Receptor.

The *in vivo* findings indicate that a more refined approach to anti-fibrotic therapies may be required, in which combination therapies will need to target different components of the fibrogenic response, and possibly different cell types. Also, the required combinations might differ depending on the fibrosis stage, for example, patients with earlier stages of fibrosis would likely benefit more from drugs like imatinib that prevent expansion of fibrogenic cell populations, whereas established cirrhosis might be directed primarily to enhance degradation of existing scar. Given its safety and expanding use in clinical practice, imatinib remains an appealing anti-fibrotic.

Our findings have also uncovered an unexpected link between inhibition of receptor tyrosine kinase activity by imatinib and enhanced production of a major regenerative cytokine, IL-6. In cultured human stellate cells, imatinib at modest doses significantly increased IL-6 gene expression, which was accompanied by increased secretion of the cytokine as determined by ELISA. The relevance of these findings was underscored by evidence of increased IL-6 *in vivo* in animals with TAA fibrosis treated with imatinib compared to animals receiving TAA alone. However, since sources of IL-6 in liver also include Kupffer cells in addition to stellate cells, imatinib's stimulation of IL-6 *in vivo* may extend to more than one cell type. Nonetheless, the findings greatly strengthen the emerging role of stellate cells in preserving hepatocellular mass. Of equal importance, they suggest that stellate cell activation may lead to loss of vital signals required for liver integrity. Therapies that augment these signals, including imatinib, may achieve the dual effect of both inhibiting fibrosis and enhancing liver function. On the other hand, since IL-6 may also contribute to hepatocellular carcinoma (50), induction of IL-6 through inhibition of stellate cell activation by imatinib could provide a permissive signal that enhances hepato-carcinogenesis in conditions where there is a risk of hepatocellular carcinoma.

At present, it is uncertain whether stimulation of IL-6 by imatinib is mediated through its effects solely on PDGF receptor signaling. While the mechanisms underlying the induction of IL-6 by imatinib, one intriguing candidate is p53, since imatinib induces p53 (51), which has been identified as a repressor of IL-6 gene expression, and (52). Regardless of the mechanism, the findings raise the important possibility that therapeutically down-regulating stellate cell activation in liver injury may directly lead to restoration of hepatocellular function through upregulation of vital regenerative cytokines. Whether this is a generalized feature of inhibiting stellate cell activation, or is relatively unique to pathways inhibited by imatinib should be a focus of future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

HSC	hepatic stellate cell
IL-6	interleukin-6
cDNA	complementary DNA
RTK	receptor tyrosine kinase
PDGF	platelet derived growth factor
SMA	smooth muscle actin
TIMP	tissue inhibitor of metalloproteinase
MMP	matrix metalloproteinase

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Figure 1A

Figure 1B

Figure 1. Effect of imatinib on stellate cell (LX-2) cells

A. Proliferation. LX-2 cells were incubated with 0.5, 1.0, 2.0 or 10.0 μM imatinib for 12 and 24 hours. ³H-thymidine was added 4 hour prior to assessment of incorporation and final data expressed as relative proliferation compared to cells incubated with medium (set at 1.0). Imatinib significantly inhibited DNA synthesis at all three concentrations for 12 and 24 hours (all p values indicate comparison to cells treated with control medium (ie., no imatinib); at 12 hours, p < 0.01 for 0.5, 1.0, 2.0 μM imatinib; p < 0.001 for 10 μM imatinib; at 24 hours, p < 0.05 for 1.0, 2.0 μM imatinib and p < 0.001 for 10 μM imatinib).

B. β-PDGFR phosphorylation. LX-2 cells were treated with PDGF-BB ligand for 10 seconds (1 – 10 nM), and then exposed to imatinib (2 or 10 μM). Expression of both total and phosphorylated β-PDGFR was assessed by Western blot, as described in Methods. Whereas PDGF BB stimulated receptor phosphorylation, imatinib at both doses inhibited phosphorylation of the β-PDGFR.

Figure 2. Effect of imatinib on fibrogenic mRNA expression in LX-2 cells

LX-2 cells were incubated with 0.5, 1.0, 2.0 or 10.0 μM imatinib for 12 hours. Imatinib down-regulated mRNAs encoding collagen 1 α (I) (Coll 1 α (I)), alpha smooth muscle actin (α SMA), β -PDGF receptor, type I transforming growth factor β receptor (TGF β R1), matrix metalloproteinase 2 (MMP2), and tissue inhibitor of metalloproteinase type 2 (TIMP 2). Interestingly, there was a modest but consistent up-regulation of TIMP 1, $p < 0.01$, whereas all other genes were down-regulated. Data expressed relative to mRNA expression for medium-treated cells (controls). All experiments were repeated at least three times in triplicate.

A. Control **B. Gleevec (2 μ M)** **Figure 3. Inhibition of mitochondrial activity by imatinib**

LX-2 cells were incubated for 24 hours with or without imatinib, 2 μ M, and mitochondrial activity was assessed using a commercial reagent (MitoTracker). Phase and fluorescent images of the same fields are shown for both control and imatinib-treated cells. Reduced accumulation of fluorescence in the imatinib (Gleevec) treated cells was evident, indicating diminished mitochondrial activity due to reduced oxidant stress.

Figure 4. Effect of imatinib on Interleukin-6 (IL-6) secretion by ELISA

LX-2 cells were incubated with or without imatinib for 24, 48 and 72 hours. IL-6 concentrations in the medium (200 μ l) were analyzed using an ELISA. In cells treated with imatinib, concentrations of secreted IL-6 were significantly increased at all time points.

Figure 5 A

Figure 5B

Figure 5. Inhibition of β-PDGF receptor is specifically linked to IL-6 induction in LX-2 cells and primary human stellate cells

A. LX-2 cells were incubated for 24 hours in the presence of control medium or either imatinib (2 μM) or the multi-targeted receptor kinase inhibitors JNJ-10198409 (“JNJ”) or AG-1296 (“AG”), 5 nM each, then mRNA expression of mRNAs for collagen I (Coll 1α

(I)), β -PDGF receptor or IL-6 were quantified by real time PCR. All three agents inhibited collagen I and β -PDGF receptor mRNA expression, and significantly induced IL-6 mRNA expression.

B. Primary human stellate cells were incubated for 24 hours in the presence of control medium or either imatinib (2 μ M) or the multi-targeted receptor kinase inhibitors JNJ-10198409 ("JNJ") or AG-1296 ("AG"), 5 nM each, then mRNA expression of mRNAs for collagen I (Coll 1 α (I)), β -PDGF receptor or IL-6 was quantified by real time PCR. All three agents inhibited collagen I and induced IL-6 and β -PDGF receptor mRNA expression.

Figure 6A***TAA + PBS*** ***TAA + Gleevec***

Figure 6B

Figure 6. Effects of imatinib (Gleevec) on collagen accumulation in TAA-induced fibrosis in rats
a) Histology of rat liver with H&E (A & C) and Sirius Red (B & D) in representative liver sections from animals in group 2. Prominent septae (blue arrows) in the control mice are greatly reduced in animals treated with imatinib; b) quantification collagen in 10 livers each as assessed by Bioquant morphometry. A statistically significant difference in fibrosis is evident in the imatinib-treated animals.

Figure 7. Down-regulation of fibrosis-related mRNAs by imatinib in TAA-induced fibrotic rat liver

Total mRNA was extracted from 70~100 mg of liver from Group 2 rats, and mRNAs were assessed by real time PCR. There was significant down-regulation of mRNAs for collagen α1 (I), (Coll1), β-PDGFR, and matrix metalloproteinase 2 (MMP2). In contrast, gene expression for the type I TGFβR was increased significantly. No significant changes were detected in the expression of alpha SMA, TIMP1 or TIMP2.

Figure 8. IL-6 mRNA induction by imatinib in liver of TAA-induced fibrosis

IL-6 mRNA up-regulation in all three treatment regimen groups was detected. Interestingly, the increase was greatest in Group 3 animals, even though there the effect on fibrosis was not significant in this group (not shown).