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Review

Pirfenidone: Anti-fibrotic agent with a potential therapeutic role in the management of transplantation patients

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Abstract

Pirfenidone has a simple chemical structure, but may have profound implications for transplantation management. One of the leading causes of allograft failure is chronic allograft dysfunction, manifested by chronic inflammation and chronic fibrosis [Estenne, M., Hertz, M.I., 2002. Bronchiolitis obliterans after human lung transplantation. AJRCCM. 166, 440–444.]. This review summarizes the literature to date on Pirfenidone in the setting of transplantation, and those studies pertinent to the mechanisms of organ rejection and possible use of Pirfenidone in transplantation patients.

Keywords: Pirfenidone; Chronic rejection; Transplantation

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1. Introduction

Pirfenidone is an effective and novel anti-fibrotic agent with anti-inflammatory properties. The chemical structure of the drug is a substituted pyridine 5 methyl-1-phenil-2(1H)-pyridine. Pirfenidone has been tested in a variety of cellular and animal models of fibrosis and inflammation. Its anti-proliferative properties have been established in a number of studies. The exact mechanism of action is not well understood, however it is known that it regulates key fibrotic growth factors and inhibits tissue necrosis factor-alpha (TNF-α), a cytokine associated with chronic inflammation. The safety record of Pirfenidone is thus far favorable, and several studies have reported that no significant toxicity is attributable to the drug (Dosanjh et al., 2002; Azuma et al., 2005).

The management of the organ transplantation patient is one of striking a balance between maintaining immunosuppression, while avoiding infection and chronic allograft dysfunction. The management of all transplantation patients currently is hampered by the development of chronic allograft fibrosis. Chronic fibrosis is a hallmark of chronic allograft dysfunction, and is

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associated with production of both inflammatory cytokines and growth factors (Trulock et al., 2005). To date, the management of transplantation patients has been hindered by the mortality and morbidity associated with chronic fibrosis. Pirfenidone may be a uniquely suited new therapeutic agent in the management of transplantation patients. Pirfenidone has the added advantage over many other anti-fibrotic agents in interrupting the final common pathway signals for fibrosis, of such key regulators as TGF and collagen III, rather than targeting one specific regulator. Its broad actions may prevent and reverse the fibrosis associated with chronic allograft dysfunction. The purpose of this review is to highlight the use of Pirfenidone in transplantation models, and to summarize the safety profile of the drug in clinical trials.

2. Pirfenidone cellular and biochemical studies with implications for transplantation

Several studies have shown that Pirfenidone downregulates the expression of pro-inflammatory cytokines which are responsible for tissue injury and induction of fibrosis during abnormal dysregulated repair processes. In one study of RAW264 cells, a murine macrophage cell line, Pirfenidone regulated the production of TNF-α at the level of translation. The authors showed that Pirfenidone selectively suppressed pro-inflammatory cytokine induction by lipopolysaccharide (LPS). These studies showed that Pirfenidone regulation of TNF-α expression was independent of the MAPK2 system. Pirfenidone also had a potentially beneficial effect of enhancing expression of anti-inflammatory cytokine IL-10 (Nakazato et al., 2002). The authors suggest that the cellular effects of Pirfenidone are translational and therefore cause fewer clinical side effects, than drugs acting at the level of transcription.

Other interesting beneficial effects of Pirfenidone include its antioxidant effect and inhibition of lipid peroxidation. Pirfenidone may function as a scavenger of reactive oxygen species, to ultimately reduce the need for tissue repair (Giri et al., 1999).

Other studies have examined the anti-proliferative properties of Pirfenidone on lung and synovial fibroblasts. The use of Pirfenidone significantly reduced the expression of TGFβ-1 and proliferation in human lung fibroblasts in a dose dependent manner, in vitro (Dosanjh et al., 1998). Human synovial fibroblasts studied in the presence of Pirfenidone expressed less ICAM-1 (Kaneko et al., 1998).

3. Transplantation models using pirfenidone to prevent and ameliorate allograft fibrosis

Pirfenidone has both anti-inflammatory and anti-fibrotic properties with excellent oral absorption and a favorable safety profile based on currently available studies. In the setting of organ transplantation several studies have examined the use of Pirfenidone in animal transplantation models. In particular, Pirfenidone may be useful in the setting of lung transplantation. The lung is in direct contact with the environment, highly immunogenic and prone to acute and chronic allograft rejection. Obliterative bronchiolitis is one of the leading causes of morbidity and mortality in the setting of lung transplantation. There is no current effective treatment for this condition. The pathologic features of Obliterative bronchiolitis include subtotal or total fibrosis occluding the lumen of respiratory bronchioles. The exact pathogenesis is not yet fully understood, but immune mediated injury to the airway epithelium, with subsequent dysregulation of growth factor production appears to be a central mechanism (Reichenspurner et al., 1995). Pro-inflammatory cytokines and release of pro-fibrotic growth factors, such as TGFβ are found in airway tissue and lavage in these patients (Bergmann et al., 1998). The combined use of Pirfenidone and cyclosporine was described in the treatment of a 27 year old patient diagnosed with bronchiolitis obliterans. Steroid dosing was able to be reduced in this patient, and the Pirfenidone was well tolerated (To et al., 2000).

In the first study of airway transplantation using Pirfenidone to attempt to prevent or treat Obliterative Bronchiolitis, an established heterotopic tracheal rat model was used. In this model the histologic lesion of chronic progressive allograft rejection is indistinguishable from that seen in humans.

The study showed that after an initial injury there is a progressive luminal collagen deposition obliterating the lumen at day 28. With the addition of 600 mg/kg/day of nearly continual administration of Pirfenidone from the onset and delay of Cyclosporine until day 7 after transplantation, there was notably less obliteration of the airway. In addition, the authors showed that luminal supernatant airway fluids in the combined Cyclosporine–Pirfenidone group were the least fibrogenic when applied to human lung fibroblast cells. The levels of TGFβ were also significantly lower in this combined group. This study suggested that the use of Pirfenidone in combination with Cyclosporine may be useful in reversing the histologic features of Obliterative Bronchiolitis (Dosanjh et al., 2002).

In another study by McKane et al., Pirfenidone (0.5%), was used in a murine heterotopic tracheal transplantation model. The introduction of Pirfenidone was begun either at day 0, 5 or 10 days. The authors reported that the mice on a continuous regimen either from 0 to 28 days, or 5 to 28 days post-transplantation, had no evidence of obliterative airway disease. When started on day 10 however, past the early transplantation period, all mice developed obliterative lesions at day 28. This study demonstrated a delay in onset of the lesions until day 60 when Pirfenidone is given early in the course. Based on the lack of effect on anti-MHC antibodies, the authors concluded that Pirfenidone itself does not have immunogenic properties, but rather acts downstream. In the absence of an anti-rejection drug, Pirfenidone was most effective when used from day zero onward. The early prevention of the lesion may be due to its anti-inflammatory properties (McKane et al., 2004). While this study did not demonstrate that late administration could delay the onset of obliteration once established, our study did show a less organized lesion if Pirfenidone is used in combination with Cyclosporine. The studies conducted by Dosanjh et al., and McKane et al. both utilized a heterotopic tracheal transplantation model. This model has limitations since the smaller airways are not transplanted, and the lack of air in the lumen perhaps makes this model less physiologic. Nevertheless, the lesion

studied is pathologically identical to that seen in the human form of obliterative bronchiolitis.

The potential of Pirfenidone to protect against early allograft injury by curtailing the initial inflammatory response post-transplantation was further investigated in an orthotopic rat lung transplantation model. In this study isografts were compared to Pirfenidone treated and untreated allografts. Pirfenidone was started continually from day 1 until harvest. No immunosuppressive therapy was administered and lungs were harvested at day 21. The results showed that Pirfenidone decreased the resultant allograft damage, and lessened both lung myeloperoxidase enzymatic activity, and plasma tissue necrosis factor alpha (Liu et al., 2005a,b).

In another study using a heterotopic tracheal transplantation model, Pirfenidone fed mice showed less epithelial cell injury and luminal granulation tissue and fibrosis. Plasma and local TGF-β levels were less in the treated animals. The combination of Pirfenidone and rapamycin in this study showed added benefit in protecting against the development of the allograft lesion (Zhou et al., 2005). In this study, there was no benefit of late Pirfenidone administration at 9 or 16 days post-transplantation, suggesting some of the effect seen in combination with Cyclosporine, may be anti-inflammatory, rather than anti-fibrotic. While the exact mechanism of action of Pirfenidone is not fully elucidated, there are some indications based on previous studies, that Pirfenidone inhibits growth factor dependent regulation of airway fibroblast proliferation (Dosanjh et al., 1998), and downregulates arginase, an enzyme that is essential for collagen synthesis. The indirect effect of downregulating arginase, may also involve decreasing NO production (Liu et al., 2005a,b).

4. Pirfenidone attenuates fibrosis induced by calcineurin inhibitors

Calcineurin inhibitors are widely used in the setting of controlling allograft rejection. There is associated fibrotic gene induction and subsequent possible fibrosis. The presence of fibrosis can eventually lead to dysfunction of the transplanted organ.

There is current evidence that Pirfenidone, when used in combination, with a calcineurin inhibitors in animal models, attenuates the adverse effect of increased extracellular matrix production caused by this class of anti-rejection drug. In two studies, the co-administration of Pirfenidone with tacrolimus, lessened the increases in TIMP-1 mRNA expression associated with tacrolimus administration. In addition, Pirfenidone reduced expression of other pro-fibrotic genes such as collagen III. These studies suggest that Pirfenidone may limit the adverse changes in fibrotic gene expression induced by tacrolimus by reducing extracellular matrix deposition (Brook et al., 2005a,b). A recent study by Shihab et al. (2005), showed that Pirfenidone ameliorated the pro-apoptotic effect of cyclosporine in a rat model. Apoptosis may play a role in the development of fibrosis. Cyclosporine upregulated apoptotic genes, whereas Pirfenidone administration reduced the number of apoptotic cells. A previous study by the same author, Pirfenidone was shown to attenuate renal fibrosis in a model of cyclosporine nephrotoxicity. Pirfenidone was associated with a decrease in TGF-beta1 expression, and a decrease in matrix deposition. These studies suggest that Pirfenidone can be clinically useful for preventing chronic Cyclosporine associated nephrotoxicity (Shihab et al., 2002).

5. The use of pirfenidone in clinical trials and adverse events

The treatment of human subjects with Pirfenidone has been tested in a number of clinical trials, particularly in chronic pulmonary fibrosis (Azuma et al., 2005; Raghu et al., 1999) and multiple sclerosis (Walker and Margolin, 2001). In one open-label clinical trial of Pirfenidone, 20 patients with multiple sclerosis were administered Pirfenidone. At the end of the year, 18 patients were able to tolerate Pirfenidone at high doses of 2400 mg/day. Some patients had to decrease their doses due to nausea (Walker and Margolin, 2001). The safety profile of the drug demonstrates that the adverse reactions can be controlled by either temporarily stopping the drug or by lowering the dose (Azuma et al., 2005; Raghu et al., 1999). In the trial conducted by Azuma and colleagues, 73 patients with idiopathic pulmonary fibrosis were enrolled to receive Pirfenidone for 9 months. The safety profile indicated that 15% of those receiving Pirfenidone, and 5.6% receiving placebo, experienced an adverse event. This difference was not considered statistically significant and there was no difference in adherence rates between the two groups. Photosensitivity was seen in 5% of the Pirfenidone group, and none of those subjects receiving placebo. This again was not considered a statistically significant difference. In general, the drug is well tolerated by patients, since there was no difference in adherence rates (Azuma et al., 2005). There was no difference in the number of patients stopping the drug after 9 months of therapy, compared to the placebo group in this clinical trial of 73 patients treated with Pirfenidone. In a prior study of terminally ill pulmonary fibrosis patients, in a phase II clinical trial, Pirfenidone was administered to 54 patients. 87% experienced adverse events, and 11% stopped the drug due to largely GI side effects (Raghu et al., 1999). However, the patients studied were terminally ill, and there was no placebo group for comparison. In another clinical trial of patients with Hermansky–Pudlak syndrome and forced vital capacities below 75%, Pirfenidone and placebo groups reported adverse events at the same rate. The one exception was the symptom of dizziness, reported in none of the placebo patients and approximately 33% of the Pirfenidone group (Gahl et al., 2002). Pirfenidone has been used in a number of clinical trials to control fibrosis. While the drug is associated with adverse events, the largest such trial did not show that these adverse events resulted in termination of the drug or death.

6. Conclusions

Pirfenidone has unique properties, as both an anti-inflammatory agent and anti-fibrotic agent, which are promising as adjunctive therapy in the setting of transplantation. In addition,

the adverse pro-fibrotic effect of tacrolimus appears to be attenuated by Pirfenidone. Pirfenidone has been shown in animal transplantation models to both prevent development of a fibrotic lesions associated with chronic allograft rejection, and to slow progression of lesions once underway. Added antioxidant properties and attenuation of NO production are also potentially beneficial. The safety profile of the drug appears in available clinical trials to be associated with no long term morbidity or mortality. Further clinical testing of Pirfenidone in transplant recipients may be warranted to investigate its usefulness in preventing and reversing the effects of chronic allograft rejection.

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