Halofuginone to Treat Fibrosis in Chronic Graft-versus-Host Disease and Scleroderma

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ABSTRACT

Chronic graft-versus-host disease (cGvHD) and systemic sclerosis (scleroderma [SSc]) share clinical characteristics, including skin and internal organ fibrosis. Fibrosis, regardless of the cause, is characterized by extracellular matrix deposition, of which collagen type I is the major constituent. The progressive accumulation of connective tissue results in destruction of normal tissue architecture and internal organ failure. In both SSc and cGvHD, the severity of skin and internal organ fibrosis correlates with the clinical course of the disease. Thus, there is an unmet need for well-tolerated antifibrotic therapy. Halofuginone is an inhibitor of collagen type I synthesis in cells derived from various tissues and species and in animal models of fibrosis in which excess collagen is the hallmark of the disease. Halofuginone decreased collagen synthesis in the tight skin mouse (Tsk) and murine cGvHD, the 2 experimental systems that show many features resembling those of human GvHD. Inhibition of collagen synthesis by halofuginone is achieved by inhibiting transforming growth factor β–dependent Smad3 phosphorylation. Dermal application of halofuginone caused a decrease in collagen content at the treated site of a cGvHD patient, and reduction in skin scores was observed in a pilot study with SSc patients. The results of the human studies provide basis for using halofuginone treatment for dermal fibrosis. As a first step toward future treatment of internal organ involvement, an oral administration study was performed in which halofuginone was well tolerated and plasma levels surpassed the predicted therapeutic exposure.

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KEY WORDS:
Collagen • Smad • Tsk

INTRODUCTION

Tissue fibrosis, including pulmonary, renal, hepatic, and cardiac fibrosis, is manifested as excessive production of connective tissues that result in destruction of normal tissue architecture and function. This review focuses mainly on the antifibrotic potential of a novel and promising agent, halofuginone [1-3], with emphasis on chronic graft-versus-host disease (cGvHD) and systemic sclerosis (SSc) that share clinical characteristics including skin and internal organ fibrosis.

FIBROSIS

Fibrosis represents the response of the organ to diverse acute and chronic insults. Fibrosis, regardless of the cause, is characterized by an increase in extracellular matrix (ECM) constituents, of which collagen type I is the major one. Although the synthesis and systemic accumulation of collagen and noncollagen matrix proteins are essential for normal tissue development and wound repair, excessive matrix accumulation leads to pathologic fibrosis. The pathogenesis of the fibrotic disorders is similar, regardless of the tissue, and the cellular mechanism of fibrosis is shared among the various insults. This mechanism in many aspects mirrors the scarring and wound-healing processes, but the persistent activation of the genes encoding for the ECM proteins distinguish controlled wound repair from the uncontrolled connective tissue deposition that results in fibrosis. In addition to the
ECM synthesis and deposition, and not less important to the progression of fibrosis, there are inadequate degradation, removal, and clearance of the ECM. In the extracellular space, matrix degradation occurs predominantly as a consequence of the actions of the matrix metalloproteinases (MMPs) and the tissue inhibitors of metalloproteinases (TIMPs) that closely regulates MMP activity. The MMPs and TIMPs play a crucial role in the fine regulation of the ECM turnover that is altered in most pathologic states associated with fibrosis [4,5].

The fibrotic reaction is thought to involve the stimulatory response of tissue cells that results in increased cell proliferation as well as ECM deposition. In some cases, such as skin, pulmonary, and kidney fibrosis, fibroblasts are thought to play a pivotal role in collagen synthesis [6,7]. In the liver and pancreas, other resident cells, the stellate cells, were found to be the cellular source of collagen and other ECM molecules [8,9]. Regardless of their origin, and in response to the fibrogenic stimulus, the cells that are usually quiescent with low proliferation rates differentiate into myofibroblast-like cells, with high proliferative capacity and an ability to synthesize large numbers of ECM molecules, especially collagen type I [10,11].

CGVHD AND SSC

cGvHD is a major late complication of allogeneic stem cell transplantation (alloSCT) and is a principal cause of morbidity and nonrelapse mortality. As a result of increasing numbers of unrelated and mismatched alloSCT and of the change from bone marrow to peripheral blood grafts, the number of patients with cGvHD has increased [12]. Chronic GvHD may develop in 25% to 45% of recipients of alloSCT from matched, related siblings depending upon age, and its frequency may increase to 40% to 70% in patients receiving alloSCT from matched, unrelated donors [13,14]. The incidence is significantly lower in patients receiving human umbilical cord blood grafts or haploidentical CD34+ purified grafts [15,16]. Chronic GvHD may develop as an extension of acute GvHD (progressive onset), after resolution of acute GvHD (quiescent onset), or without preceding acute GvHD (de novo onset). Chronic GvHD is categorized as either limited (localized skin and/or hepatic involvement) or extensive (diffuse skin and/or multorgan involvement); the latter is associated with a more severe prognosis [17]. The introduction of low-intensity conditioning (LIC) and nonmyeloablative alloSCT has not resulted in a reduction in the frequency of cGvHD [18]. cGvHD usually develops more than 100 days after transplantsations, with increased frequency in patients receiving peripheral blood stem cell grafts and in those undergoing LIC alloSCT [12,18]. Chronic GvHD typically resembles connective tissue autoimmune-like immunologic disorder such as SSc characterized by lichenoid or sclerodermoid lesions of the skin, along with joint contractors with eosinophilia, circulating autoantibodies, hypergammaglobulinemia, and plasmacytosis of viscera and lymph nodes [17].

The skin includes papulosquamous dermatitis, plaques, desquamation, depigmentation and vitiligo, and thickened reticular dermis because of increased synthesis of collagen [19]. Cutaneous appendages become encased in collagen and tend to disappear. There is occurrence of a variable perivascular and interstitial inflammatory cell infiltrate, composed predominantly of lymphocytes and occasional plasma cells [20]. Less-frequent skin findings include poikiloderma, reticulated hyperpigmentation, alopecia, dystrophic nails, leucoderma bullae, discoid lupus erythematosus, and photosensitivity. The pathophyslogic mechanism involves autoreactive lymphocytes and cytokine dysregulation [21,22]. Current therapeutic options are limited. Fewer than 20% of patients with untreated extensive cGvHD with Karnofsky performance scores ≥70% survive [17]. Currently, corticosteroids, alone or in combination with cyclosporine, remain the treatment of choice for established cGvHD. Other therapeutic options that have been tried include thalidomide, mycophenolate mofetil, tacrolimus, rapamycin, clofazimine, etretinate, hydroxychloroquine, ursodeoxycholic acid, penicillamine, cyclophosphamide, and nedocromil sodium, as well as medical procedures such as total lymphoid irradiation, phototherapy (PUVA), and extracorporeal phototherapy [23].

SSc is a connective tissue disease with an unknown cause. As an autoimmune disease involving cellular and humoral immunity, it affects multiple organ systems, including skin, gastrointestinal tract, lungs, kidneys, and heart. The severity of skin and internal organ involvement correlates with the clinical course of the disease [24]. According to the degree of skin involvement, SSc can be classified as limited cutaneous SSc or diffuse cutaneous SSc. Limited cutaneous SSc, which is slowly progressive and frequently associated with anticientromere antibodies, is characterized by sclerodactyly or acrosclerosis, with distal involvement of the extremities with or without facial involvement, Raynaud’s phenomenon, dysphagia, calcinosis cutis, and telangiectasia [24]. The most severe systemic complications are pulmonary hypertension and biliary cirrhosis. Diffuse cutaneous SSc is more severe and shows proximal involvement of the extremities, trunk, or both, and is associated with pulmonary interstitial fibrosis, renal crises, and gastrointestinal involvement (dysphagia, hypomotility, and other disorders). Diffuse cutaneous SSc is frequently associated with Scl-70 (antitopoioseromase) and nucleolar autoantibodies.
(polymerase I and III, fibrillarin). The mechanism of fibrosis in SSc is not fully understood, although it is known that soluble mediators (transforming growth factor β [TGF-β], platelet-derived growth factor, interleukin-4 and -6, tumor necrosis factor α [TNF-α]) can be secreted by lymphocytes, monocytes, and endothelial cells and can affect the behavior of fibroblast growth, proliferation, collagen synthesis, and chemotaxis [25,26]. The excessive tissue fibrosis is caused by expansion of fibrogenic clones of tissue fibroblasts, which behave relatively autonomously and overexpress genes encoding ECM components, leading to excessive deposition of collagen and other ECM proteins in skin and internal organs and blood vessels [27]. Present therapies are directed to improve peripheral blood circulation with vasodilators and antiplatelet aggregation drugs, to prevent the synthesis and release of harmful cytokines by means of immunosuppressants, and to inhibit or reduce fibrosis with agents that interfere with collagen synthesis or enhance collagenase production. Autologous stem cell transplantation was performed after immunosuppressive pretreatment in diffuse SSc patients with involvement of 1 or more internal organs [28]. The mortality rate was 17%, in part caused by interstitial pneumonitis, possibly as a result of an exaggerated response to total body irradiation. Seventy percent of patients had over 25% improvement in their skin score and stabilization of lung function. This is still an experimental therapy and currently limited to patients with severe, progressive, life-threatening disease [29]. The treatment of SSc remains a challenge to the clinician, and there is an unmet need for new therapies [30].

HALOFUGINONE

For centuries, the roots of Dichroa febrifuga, a saxifragaceous plant, have been employed in China in the treatment of malarial fever. Febrifugine and its stereoisomere, isofebrifugine, were identified as the active components against malaria. Febrifugine analogues bearing a modified or unmodified 4-quinazolinone ring are active, whereas those produced through the modification of the side chain attached to the N-3 position of the 4-quinazolinone are ineffective [31]. Halofuginone (7-bromo-6-chloro-3-[3-(3-hydroxy-2-piperidinyl)-2-oxopropyl]-4(3H)-quinazolinone) is one of the febrifugine analogues used worldwide in commercial poultry production for coccidiosis [32].

HALOFUGINONE AND THE COLLAGEN CONNECTION

Serendipity was the driving force beyond the finding that halofuginone has an inhibitory effect on collagen synthesis [1-3]. In vitro halofuginone was found to inhibit collagen type I synthesis but not that of type II [33] or III [34]. Halofuginone inhibited collagen synthesis in a variety of cells, including primary cultures of mouse skin fibroblasts, avian growth plate chondrocytes, a transformed rat embryo cell line, vascular smooth muscle cells, bovine aortic endothelial cells, and rat liver stellate cells [33-36], all of which produce and secrete collagen type I. In animal models of fibrosis in which excess collagen is the hallmark of the disease, administration of halofuginone prevented the increase in collagen synthesis and collagen α(I) gene expression. These models include rat postoperative abdominal and uterine horn adhesions [37,38], rat urethral strictu-tration formation [39], thioacetamide and dimethylnitrosamine-induced rat cirrhosis [36,40], rat pulmonary fibrosis after bleomycin treatment [41], and tight skin (Tsk) + and cGvHD-afflicted mice [42-44]. Inhibition was independent of the route of administration (intraperitoneally, administered locally, or given orally).

The reduction in collagen synthesis by halofuginone appears not to be a direct effect of halofuginone on the collagen α(I) promoter but rather dependent on new protein synthesis, because simultaneous treatment of fibroblasts with protein synthesis inhibitors such as cycloheximide or actinomycin D blocks the suppressive effect of halofuginone on collagen α(I) mRNA gene expression [45]. The nature of the newly synthesized protein(s) and how they function to decrease procollagen mRNA levels is unknown.

HALOFUGINONE IN ANIMAL MODELS OF CGVHD AND SSC

Although there is no animal model that exhibits all aspects of cGvHD or SSc [46], 2 experimental systems are available that show many features resembling those of the human disease [47]. The first is murine cGvHD, in which changes develop that are similar to human cGvHD and SSc, including cutaneous fibrosis, loss of dermal fat, atrophy of dermal appendages, deposition of mast cells, and mononuclear cell infiltration [48]. The second model is the Tsk mouse possessing a single gene mutation in the fibrillin-1 gene on chromosome 2 [49]. The mutation is transmitted as an autosomal dominant trait, whereas the heterozygous Tsk/+ mice develop coetaneous hyperplasia and connective tissue abnormalities in skin, heart, and lungs [50,51] associated mainly with an increase in collagen type I [52,53]. These mice produce autoantibodies characteristic of SSC patients [54]. In vitro halofuginone inhibited collagen production by Tsk skin fibroblasts by inhibition of the collagen gene expression [44]. The reduction in collagen synthesis preceded any effect on cell proliferation or synthesis of other proteins, which suggests a specific effect on the collagen biosynthesis pathway. In vivo
halofuginone administered intraperitoneally eliminated the increase in skin collagen and prevented the thickening of the dermis and the loss of the subdermal fat in both the cGvHD and the Tsk models in a dose-dependent manner attributed to a decrease in collagen type I gene expression [42]. These effects of halofuginone were correlated with a decrease in the number of cells expressing the collagen α1(I) gene that reached the expression level of the matched controls. Moreover, reductions in the levels of autoantibodies specific for human target antigens (anti-topoisomerase I and antifibrillin antibodies) were observed [55]. These results raised an interesting question regarding the association of the ECM biosynthesis pathway and autoimmunity. It has previously been suggested that TGF-β plays a critical role in fibrosis and is the pivotal immunoregulatory cytokine affecting the immune responses by regulating monocyte function, T-cell proliferation, and inflammation [56].

In an attempt to begin developing a local-therapy modality for SSc and cGvHD, the effect of dermal application of halofuginone on skin collagen and collagen α1(I) gene expression in the Tsk mice was determined. Dermal application of halofuginone (0.01% in cream) for 60 days was equal to the systemic administration (1 µg per mouse per day) in reduction of skin collagen α1(I) gene expression and was almost as effective in reducing skin thickness. The effect of halofuginone was time-dependent; 40 days of treatment were needed for a significant reduction in the collagen α1(I) gene expression [43]. Physiologically, skin fibroblasts synthesize low levels of collagen. In the Tsk mouse, the onset of fibrosis coincides with the presence of a large number of fibroblasts expressing elevated levels of collagen type I gene [57], as was demonstrated in SSc [57,58]. The selective growth of the subpopulation of fibroblasts producing elevated levels of collagen has been suggested to be the causes of the fibrotic condition [59]. These fibroblasts are probably the target for halofuginone [43]. The effect of halofuginone was restricted to the dermis fibroblasts and did not affect other proliferating, but collagen-nonproducing cells, such as epidermal keratinocytes.

**HALOFUGINONE AND TGF-β PATHWAY**

As a result of the extremely pleiotropic nature of the effects of TGF-β, therapies targeting the expression of the genes encoding TGF-β or its receptors are likely to be associated with an array of side effects caused by abnormal cell proliferation, inflammation, autoimmunity, and other potentially serious complications [71-73]. Drugs that selectively target individual signaling pathways downstream of the TGF-β receptor are, therefore, likely to be more successful. Halofuginone was found to overcome the TGF-β-induced collagen synthesis in human skin fibroblasts [45] and fibroblasts derived from control and Tsk mice [44]. A similar inhibitory effect was observed on promoter activity in fibroblasts transfected with the collagen α2(I) construct or with a construct with the Smad binding site, which suggests that the target of halofuginone is probably downstream in the TGF-β pathway. Although increased gene expression for TGF-β receptors was observed in SSc fibroblasts [74], no effect of halofuginone was observed on the expression of the TGF-β receptors gene in the Tsk fibroblasts, which further supports the hypothesis that downstream effects were involved. Halofuginone was found to inhibit phosphorylation of TGF-β-dependent Smad3, but not that of Smad2, which resulted in inhibition of Smad3 binding to DNA [44]. These results demonstrate the specificity of halofuginone activity, although the involvement of other members of the Smad family in halofuginone-dependent inhibition of collagen synthesis cannot be ruled out.

In most animal models of fibrosis, regardless of the tissue, halofuginone had a minimal effect on collagen content in the control, nonfibrotic animals, whereas it exhibited a profound inhibitory effect in the fibrotic organs. These results suggest a different regulation of vated levels of TGF-β were found in involved skin lesions of SSc patients [62,63], in murine models of sclerodermatous GvHD [64], and in the pathogenesis of graft vascular disease [65]. There was association between TGF-β expression and severe GvHD in allogeneic bone marrow transplantation (BMT) [66]. TGF-β is co-localized with collagen type I mRNA in involved lesions in the early stages of inflammation before the onset of fibrosis and was implicated in the initiation of fibrosis in SSc [26]. Following binding of TGF-β to its receptor, signaling to the nucleus occurs predominantly by phosphorylation of cytoplasmatic mediators of the Smad family that are divided into 3 groups [60]. The regulation of matrix proteins in general [67] and of collagen type I gene expression in particular by TGF-β involved the Smad3 signaling pathway [68,69], and increased presence of nuclear Smad3 has been observed in SSc fibroblasts [70].
the house-keeping and usually low level of expression of collagen type I genes on one hand, and the over-expression induced by the fibrogenic stimulus, which is usually an aggressive and a rapid process, on the other. Halofuginone mainly affects the stimulated collagen synthesis; therefore, when it was administered systemically, it was actually targeted to the desired fibrotic location without affecting collagen synthesis in other locations. In culture, halofuginone phosphorylated Smad3 and was more effective in reducing collagen synthesis by Tsk fibroblasts after they had been stimulated with a profibrotic agonist such as TGF-β; whereas no effect on Smad3 phosphorylation and reduced effect on collagen synthesis was observed in control cells [44].

**HALOFUGINONE AFFECTS PREEXISTING FIBROSIS**

In cGVHD and SSc, the skin is not the only organ affected by fibrosis; survival studies in SSc and cGVHD patients have indicated a distinct dependency on the involvement of internal organs [17,24]. In cases of SSc, the prognosis was dependent on the involvement of internal organs, particularly the lungs, heart, and kidneys. Although the gastrointestinal tract is the most frequently affected organ, its involvement is less prognostic. As mentioned above, halofuginone has been found to be efficacious in inhibiting collagen synthesis in multiple organs. In most of these studies, halofuginone was used as a preventive agent; it was administered before—or together with—the fibrotic stimulus [37-41]. In the Tsk mouse, halofuginone treatment caused a decrease in the preexisting fibrotic condition as measured by changes in collagen gene expression, collagen content, and skin morphology [42-44]. In a liver fibrosis model using rats with established fibrosis, halofuginone administration resulted in complete resolution of the fibrosis. The levels of collagen, collagen α1(I) gene expression, and α-smooth muscle–positive cells, all of which are characteristic of the fibrotic condition, were reduced almost to the control levels [36]. Furthermore, liver regeneration that was blocked in rats with established fibrosis occurred almost in a normal rate in halofuginone-treated rats [75].

**HUMAN STUDIES FOR THE LOCAL TREATMENT OF SSc AND CGVHD**

A Phase I study with topical application of 0.1% halofuginone was conducted in 14 healthy volunteers. The results of the study showed safety and tolerability following repeated topical applications of halofuginone cream. There was no skin irritation or systemic absorption of halofuginone (Dr. S. Tamin, unpublished data, 1998). As a proof of principle, halofuginone was locally applied on the skin of a 22-year-old male with acute promyelocytic leukemia who underwent successful BMT from his HLA-matched brother. Three-and-a-half months after BMT, he developed severe, extensive, de novo cGVHD with skin, mucosal, gastrointestinal tract, and liver involvement. The skin cGVHD was manifested by scattered lichenoid papules and mottled hyperpigmentation, mainly of the neck and upper chest and back, as well as of both axillae, lower abdomen, and flanks. Oral examination disclosed severe lichenoid lesions with hypertrophy of all the buccal and labial mucosa, atrophy of the papillae of the tongue with oral pain, xerostomia, and loss of taste. The skin cGVHD progressed, and within 8 months, the patient developed severe bilateral scleroderma and tightening of the skin resembling sclerodermoid changes that then progressed to contractures with pain and limitation of movements [76]. The protocol included once-daily topical application of 0.03% halofuginone ointment to the left side of the neck and the posterior aspect of the left shoulder for a period of 6 months, while the right and corresponding side served as control. After 1 month, softness of the left side of the neck and upper back was noted by clinical observation. There was no itching, rash, or erythema. Over the next few weeks, the softness intensified and was accompanied by gradual loosening of the sclerosis and tightness in the left side of the neck. In comparison, no change was observed in the untreated, control right side. No change in the skin pigmentation was noticed on either side. Within 3 months, there was an improvement in the pain and dysphagia, most probably related to the improvement in the severe sclerosis and fibrosis of the neck, and after 6 months of therapy rotation of the neck, only to the treated side, had improved from 30% to 70%. Skin biopsies were taken pretherapy from clinically involved and uninvolved areas at the left and right upper back below the scapulas, from the involved-treated areas after 3 and 6 months of topical halofuginone therapy and 3 months following termination of halofuginone treatment [76]. The level of collagen α1(I) gene expression and the collagen content were much higher in the affected area compared with the macroscopically nonaffected area. Three months of halofuginone treatment caused a marked decrease in collagen α1(I) gene expression and collagen content, and a further decrease in both parameters was observed after an additional 3 months of treatment (Figure 1). However, 3 months after cessation of treatment, the levels of collagen α1(I) gene expression returned almost to baseline levels, indicating that the halofuginone-mediated inhibitory effect of collagen synthesis is reversible [76]. The transient effect of halofuginone was probably the result of the chronic process providing a continuing stimulus to fibrogenesis. Throughout the treatment period, there was no
local or systemic toxicity, and no side effects were noticed. Repeated blood biochemistry tests of liver and kidney functions did not disclose any changes from the pretreatment values, blood counts were normal, and no halofuginone could be detected in the patient’s sera (high-performance liquid chromatography assay sensitivity for halofuginone is 1 ng/mL), all of which suggest that topical application of halofuginone is safe and will not cause any systemic effects in other locations.

A phase II trial was performed to assess the safety and efficacy of halofuginone in diffuse SSc patients with changes in the upper extremities resulting in a modified Rodnan skin score of at least 10/21. The modified Rodnan skin thickness score measured on a 0-3 scale (0, normal; 1, mild thickness; 2, moderate thickness; 3, severe thickening) of 7 regions of the upper extremity was used [77]. Thirteen patients were treated once daily with topical application of 0.01% halofuginone on either left or right upper extremities (hand, forearm, upper arm). Eleven of these patients completed a 6-month treatment period. Safety results showed that there were no clinically relevant changes in vital signs (blood pressure, heart rate, body temperature, and body weight) and laboratory evaluations that included hematology (red blood cell, white blood cell, and platelet counts, hemoglobin, and hematocrit) and clinical chemistry (creatine phosphokinase, aspartate transaminase, γ-glutamyl transferase, lactose dehydrogenase, albumin, total and indirect bilirubin, urea, creatinine, glucose, sodium, and potassium). The most frequently reported adverse event that was attributed to the treatment was dermatitis of varying degree and severity observed macroscopically that did not dictate cessation of the treatment. Efficacy evaluation results (changes in total skin score from baseline by 2 raters) showed a statistically significant reduction in the mean total skin score after 3 months of treatment (Table 1). Of 12 patients, 5 met responder criteria of at least 25% reduction in the total skin score in the treated arm, and the mean time for response was 2.6 months. The results of the human safety and efficacy studies provide a basis for the proof of concept of using halofuginone as the drug for treatment of dermal fibrosis and paved the way for a phase III placebo-controlled, double-blind trial.

Table 1. Total Skin Score of the Treated Arm of Responders and Nonresponders

<table>
<thead>
<tr>
<th>Time of Treatment (months)</th>
<th>Efficacy-Eligible Patients</th>
<th>Nonresponders</th>
<th>Responders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 12)</td>
<td>(n = 7)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>Baseline</td>
<td>12.5 ± 2.4</td>
<td>13.1 ± 2.2</td>
<td>11.7 ± 2.6</td>
</tr>
<tr>
<td>Baseline</td>
<td>11.8 ± 2.0</td>
<td>12.0 ± 1.7</td>
<td>11.5 ± 2.6</td>
</tr>
<tr>
<td>1</td>
<td>10.9 ± 4.5</td>
<td>12.1 ± 1.9</td>
<td>7.2 ± 5.7</td>
</tr>
<tr>
<td>2</td>
<td>10.8 ± 4.0</td>
<td>12.4 ± 2.3</td>
<td>8.7 ± 5.2</td>
</tr>
<tr>
<td>3</td>
<td>9.6 ± 3.6</td>
<td>10.9 ± 2.0</td>
<td>8.2 ± 4.4</td>
</tr>
<tr>
<td>4</td>
<td>10.4 ± 2.9</td>
<td>12.7 ± 1.2</td>
<td>8.6 ± 3.4</td>
</tr>
<tr>
<td>6</td>
<td>10.1 ± 3.7</td>
<td>11.3 ± 1.8</td>
<td>8.6 ± 5.0</td>
</tr>
</tbody>
</table>

Halofuginone caused a significant (P = 0.03) reduction in the mean total skin score of the efficacy-eligible patients after 3 months of treatment compared with baseline.
PHARMACOKINETICS AND SAFETY: ORAL FORMULATION

An ascending, single, oral-dose Phase I study in healthy volunteers was performed to assess the safety, tolerability, and pharmacokinetics of orally administered halofuginone. The study was double-blind in a single center and involved 26 healthy, male volunteers. The doses tested were between 0.07 to 2.5 mg/d, and the drug was given with food during diet-controlled meals. Single, oral doses of 0.07 and 0.5 mg halofuginone were found to be safe and well tolerated. No clinically significant adverse events were recorded in these groups, whereas simulated steady state from the 0.5-mg group surpassed the predicted therapeutic exposure. At 1.5 to 2.5 mg, halofuginone was moderately tolerated, with incidence of gastrointestinal adverse events (nausea and vomiting) associated with dose increments. A daily dose of 1.5 mg halofuginone was designated as the maximal tolerated dose. To improve the tolerability profile of orally administered halofuginone, a 3-way, crossover, split-dose study in 8 male volunteers was initiated in which 3 dose regimens (0.25 mg × 8; 0.5 mg × 4 or 2.0 mg × 1) were assessed. Dividing the dose into several daily portions, thereby reducing the slope increment of plasma levels of halofuginone, allowed the exposure to be increased without increasing gastrointestinal adverse events.

CONCLUSION

Any perturbation in the cellular function of any tissue that causes excessive collagen deposition and results in fibrosis may be a target for halofuginone therapy. Because halofuginone inhibited collagen type I synthesis on the transcriptional level and an increase in the ECM degradation capabilities, it is a promising candidate for treatment of diseases caused by, or associated with, excessive ECM, such as SSc and cGvHD. The local application of halofuginone is safe, without indications of systemic absorption. Systemically, therapeutically effective plasma levels can be reached at a dosage that is tolerated. Thus, halofuginone meets the criteria as a potential antifibrotic drug for further evaluation in the treatment of cGvHD.

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