

# Profile of crofelemer for the symptomatic treatment of diarrhea in HIV-infected persons

Christina Leonard<sup>1</sup>  
Poorvi Chordia<sup>1</sup>  
Rodger D MacArthur<sup>1,2</sup>

<sup>1</sup>Department of Infectious Diseases, Wayne State University, Detroit, MI, USA; <sup>2</sup>Newland Immunology Center of Excellence, Southfield, MI, USA

**Abstract:** Diarrhea due to noninfectious causes is a major problem in human immunodeficiency virus (HIV)-infected persons, and is frequently related to antiretroviral therapy and HIV-associated enteropathy. Crofelemer is a first-in-class antidiarrheal agent that is United States Food and Drug Administration approved for noninfectious diarrhea in persons with HIV on antiretroviral therapy. Crofelemer is derived from the blood-red sap of *Croton lechleri*, a South American plant whose latex is associated with various healing attributes. In fact, it has a unique effect on chloride channels in the gastrointestinal lumen, and leads to decreased efflux of sodium molecules and water, thereby decreasing the frequency of stools. Crofelemer – a plant-based compound, discovered and investigated as the result of the increased prevalence of ethnobotany – is a novel and effective agent with a good safety profile. It could potentially improve the quality of life for HIV-infected patients and hopefully, in turn, will improve antiretroviral therapy compliance.

**Keywords:** chloride channels, secretory diarrhea, botanical, sangre de grado, intra-luminal

## Introduction

Crofelemer is the first botanical agent approved for oral administration by the United States Food and Drug Administration (FDA). It was approved in late 2012 for the treatment of noninfectious diarrhea in human immunodeficiency virus (HIV)-infected persons on antiretroviral therapy. Diarrhea is a common and important side effect in persons with HIV infection. When severe, diarrhea can lead to electrolyte abnormalities; but, most importantly, it is a physical and psychological burden. Chronic, secretory diarrhea is more than an inconvenient problem as it causes significant adverse effects on quality of life from the workplace to social and recreational activities. Noninfectious diarrhea in HIV-infected persons is usually secretory in nature and caused by either antiretroviral therapy (ART), HIV-associated enteropathy, or HIV-associated malignancies and pancreatitis.<sup>1</sup> ART is the most common cause of noninfectious diarrhea, and although all classes of ART can contribute to diarrhea, it is particularly seen with ritonavir-boosted protease inhibitor regimens.<sup>2</sup>

Previously, supportive therapy with hydration, dietary adjustments, and commercially available antidiarrheal and stool-bulking agents was the mainstay of treatment, although not always effective. These therapeutic methods are generalized practices that do not specifically target the physiologic etiology of the diarrhea. Crofelemer, a novel antidiarrheal agent, is an intestinal luminal chloride-channel-directed agent that is transitioning the treatment of noninfectious HIV-associated diarrhea from objective and supportive to scientific and evidence-based. The Antidiarrhea Therapy in HIV Disease–Emerging Treatment Concepts (ADVENT) trial – a Phase III randomized

Correspondence: Rodger D MacArthur  
22301 Foster Winter Drive, 2nd Floor  
Southfield, MI 48075, USA  
Tel +1 248 763 2099  
Fax +1 248 203 1245  
Email macarthur@wayne.edu

double-blind placebo-controlled study – played a pivotal role in the FDA approval of crofelemer for the treatment of noninfectious diarrhea in HIV-infected persons.<sup>3</sup> While the scientific data behind and success of crofelemer make it an exciting agent, the fact that it is a plant-derived extract, discovered and investigated based on the increasing practice of ethnobotany, makes it even more interesting. The source plant is *Croton lechleri*, a South American tree that produces a latex that comprises numerous bioactive agents, many of which are currently undergoing evaluation for treatment of several conditions from skin inflammation to cancer.

## Botanicals as drugs

The FDA defines a botanical drug as one that consists of vegetable materials and is intended for the purpose of diagnosing, mitigating, treating, or curing disease.<sup>4</sup> Frequently, these botanicals are complex materials without a clearly identifiable singular active ingredient. It is likely that the biologic activity of these materials is a result of complex and synergistic interactions between multiple ingredients as a result of years of coevolution within a living organism. As such, the FDA also specifies that botanicals consist of rather virgin components; no substance that has been fermented, chemically modified, or excessively purified can be considered a botanical.<sup>4</sup> While many dietary supplements also consist of vegetable material and are considered botanicals, they are contained “in a special category under the general umbrella of foods, not drugs,” and, thus, do not require FDA approval.<sup>4,5</sup> Any drug must be scrutinized by the Center for Drug Evaluation and Research prior to marketing and selling. Botanical drugs carry the burden of stringent regulation, but also the benefit of comprehensive research, definitive data, and official FDA approval to support their use for a distinct illness. There are very few botanical drugs on the market, and most are topically-administered agents; crofelemer is the only oral botanical drug currently approved for use by the FDA.

## The history of sangre de drago

“Sangre de drago”, or “dragon’s blood” is the Spanish name for the sap from the *C. lechleri* tree that is native to parts of the Amazon in South America, specifically in Colombia, Ecuador, and Peru.<sup>6</sup> *C. lechleri* is one of many species that fall under the family *Euphorbiaceae*. Many of the plants in this family have similar properties and, therefore, have had similar medicinal uses worldwide and throughout the centuries. *C. lechleri*, specifically, is one of this family that is known for the thick dark-red sap that oozes out of the tree after an oblique cut is made through its bark. For centuries,

this healing sap has been used for sealing and healing minor wounds, as well as for treatment of stomach ulcers, herpes ulcers, hemorrhoids, insect bites and stings, and many other ailments for which the sap can be applied topically or ingested. In developing countries where this family of tree exists, sangre de drago is about as much of a staple in any medicine cabinet as adhesive bandages or hydrogen peroxide. In fact, a 2000 survey found that 57% of a randomly interviewed sample of Peruvians admitted to using sangre de drago for the treatment of diarrhea.<sup>7</sup>

In the past, the classical method of natural product screening consisted of previously established taxonomic findings and immunopharmacologic studies, as well as hundreds of random screenings. Utilization of the practice of ethnobotany – the study of the relationship between humans and plants – is increasingly common.<sup>6</sup> The practice of medicine using holistic and naturopathic methods is far older than the practice of Western medicine. With the rising interest in many of these methods, with their roots deep in the evolution of our species, we have also seen a rise in the amount of scientific data and literature supporting the benefits of these methods. With the medical community’s increasing acceptance of these practices, now based on quantifiable benefits as opposed to anecdotal evidence, there is an increased recognition of the potential of “old remedies” to bear scientific fruit. As such, investigators are turning to a more organized and more intentional method of finding potentially medicinal compounds in nature. From traditional texts and local herbal medicine usage to interviews with traditional indigenous healers, investigators are leaving no stone unturned in their efforts to find effective agents.<sup>6</sup>

Unfortunately, with this increase in study of natural plants for bioactivity, the problem of over farming has arisen. With *C. lechleri* specifically, its wealth of active chemical components has led to extensive scientific study for a number of different conditions, which in turn has led to an over harvesting of the plant. The yield from any particular tree reaches its peak by the time the tree is approximately 6 years old. Unfortunately, the process of slowly and consistently tapping a living tree is associated with increased susceptibility of the tree to fungal infections and an overall high rate of tree death.<sup>7</sup> As a result, the preferred method of gathering latex for industrial production is by cutting down the tree, in which case the latex is extracted much faster.<sup>7</sup> The result has been near-endangerment of the tree. As a result, Salix Pharmaceuticals (Raleigh, NC, USA), the company that sells the drug crofelemer in North America and parts of Europe, has committed to planting two

to three new trees for every one cut down in the harvesting of sap.

## Chemical composition of sangre de drago

Sangre de drago contains chemicals of three different categories: terpenoids, alkaloids, and phenolic compounds.<sup>7</sup> Terpenoid compounds found in the sap of *C. lechleri* include a number of diterpenes, and beta-sitosterol-beta-D-glucopyranoside and beta-sitosterol, among other steroids. The primary alkaloid component of the sap is taspine, while the leaves and bark have a number of additional alkaloid compounds. Taspine is one of the two components within sangre de drago that is credited with having the greatest bioactivity; the other is proanthocyanidin. Proanthocyanidin is one of two phenolic compounds within the sap (flavonol being the other). Both taspine and proanthocyanidin have a potent antioxidant capacity, and the latter is comprised of procyanidin oligomers built of random sequences of (+)-gallo catechin, (-)-galloepicatechin, (+)-catechin, and (-)-epicatechin monomers. Its approximate weight is 2,200 Da, which limits systemic absorption when given orally.<sup>8</sup>

## Production of crofelemer

The latex of the tree is chilled, which induces phase separation of the gross product into solid residue and supernatant. The aqueous phase, which contains the crofelemer, is filtered and exposed to low-pressure liquid chromatography on an ion-exchange column. The concentrated result of this process is crofelemer rich and is next purified and eluted, then dried using a vacuum.<sup>9</sup> The final dried powder is packaged into enteric-coated beads.<sup>10</sup>

## Mechanisms associated with secretory diarrhea

Secretory diarrhea is simply the result of an imbalance between the absorption and secretion of electrolytes in the gastrointestinal lumen.<sup>11</sup> Intestinal fluid secretion involves the influx of chloride ions via the  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  symporter on the basolateral membrane enterocytes. These chloride ions are, in turn, actively secreted into the gastrointestinal lumen on the apical side of the enterocytes. This luminal secretion is achieved by dedicated chloride ion channels, and the chloride ion efflux results in an electrical gradient that is responsible for drawing sodium and water into the gastrointestinal lumen. To date, three chloride channels have been implicated in this process: cystic fibrosis transmembrane conductance regulator (CFTR); the calcium-activated chloride channel (CaCC); and the chloride channel type-2 channel.<sup>12</sup>

The CFTR channel is activated by cyclic adenosine monophosphate (cAMP)-dependent phosphorylation and is one of the most important chloride channels in the human body; it is present in the epithelial cells in a number of other places including the airways, pancreas, sweat ducts, and testes.<sup>13</sup> The CFTR chloride channel is also associated with enterotoxin-mediated diarrhea due to *Vibrio cholera* or enterotoxigenic *Escherichia coli*. *V. cholera* toxin results in increased intracellular cAMP, while enterotoxigenic *E. coli* toxin results in increased cAMP and cyclic guanine monophosphate. Both cAMP and cyclic guanine monophosphate then cause activation of the CFTR chloride channel, which results in a massive efflux of sodium and water. Currently, research is being focused on production of CFTR inhibitors.<sup>13</sup>

The CaCCs are found on the apical membranes of enterocytes and play a major role in ART-associated diarrhea. In 2003, Rufo et al studied the effects of nelfinavir – a protease inhibitor – on CaCCs. They found that nelfinavir increases activation of CaCCs, which, in turn, leads to secretory diarrhea.<sup>14</sup> Studies conducted by Tradtrantip et al demonstrated that both the CaCC and CFTR chloride channels are inhibited by crofelemer. Thus, the antisecretory properties of crofelemer are based on a dual mechanism of action.<sup>15</sup>

Finally, the chloride channel type-2 channels are located on the lateral membranes of enterocytes, and their role in chloride-ion secretion is not well understood.<sup>11</sup>

## Pharmacodynamics

Tradtrantip et al have thoroughly investigated crofelemer's mechanism of action on the gastrointestinal luminal membrane, ie, the dual inhibition of two structurally different chloride channels. The two chloride channels are cAMP-stimulated cystic fibrosis transmembrane conductance regulator (CFTR) and CaCC.<sup>15</sup> Inhibition of chloride-ion secretion results in decreased efflux of sodium and water and, therefore, improvement in diarrheal symptoms.<sup>15</sup>

In the study performed by Tradtrantip et al,<sup>15</sup> crofelemer demonstrated a concentration-dependent inhibition on both the luminal CFTR channels, as well as an even stronger inhibition of the CaCCs. Regarding the CFTR channels, increasing concentrations of crofelemer produced more rapid, although partial, inhibition of CFTR, with maximal inhibition being 60% and a half maximal inhibitory concentration ( $\text{IC}_{50}$ ) value of 7  $\mu\text{M}$ . Crofelemer also demonstrated concentration-dependent inhibition of the CaCCs via voltage-independent inhibition mechanism, with maximum inhibition >90% and an  $\text{IC}_{50}$  of 6.5  $\mu\text{M}$ . Finally, given that crofelemer

is a locally acting minimally absorbed molecule, the study also investigated the reversibility of crofelemer as a result of washout. Washout is the product of dilution and excretion of the antidiarrheal as a result of the sheer volume of fluid moving through the gastrointestinal lumen during secretory diarrhea. Investigators found that crofelemer resisted washout, and there was less than 50% reversal of CFTR inhibition after 4 hours. Crofelemer's inhibitory effect on the intestinal chloride channels persists even in the presence of severe diarrhea.

## Pharmacokinetics and metabolism

Plasma concentrations of crofelemer are typically undetectable after oral ingestion, regardless of dose and temporal relation to food.<sup>16</sup> As a result, standard pharmacokinetic parameters have not been determined.<sup>10</sup> In addition, while crofelemer may have some *in vitro* effect on cytochrome P450 isozyme 3A4, the only documented *in vivo* effect to date is a 20% decreased exposure to lamivudine when crofelemer was dosed at 500 mg every 6 hours.<sup>16</sup> While in clinical trials, doses of crofelemer have ranged from 125 to 500 mg every 6–12 hours; the ADVENT trial determined the optimal dose of crofelemer to be 125 mg twice daily.<sup>3</sup> The current dose of crofelemer approved by the FDA for secretory diarrhea in HIV-infected persons is 125 mg as a delayed-release tablet taken twice daily. As such, appreciable drug interactions have, as yet, been inconsequential.

## FDA approval

Before it was known as crofelemer, SP-303 was an investigational drug derived as described in the Introduction. In one of the first preclinical studies in the 1990s, Gabriel et al used a mouse model to administer cholera toxin in order to stimulate diarrhea. The postulation that SP-303 would be successful was based on the fact that it was known to have activity on the cyclic-AMP chloride secreting channels *in vitro*. When SP-303 was administered to the mice, the effectiveness of therapy was measured based on the amount of fluid accumulation in the small intestine after the mice were killed and dissected, and their intestines weighed (before and after excess fluid removal).<sup>10</sup> The investigators confirmed that the drug had significant effect on chloride channels, and, thus, fluid secretion, *in vivo*.<sup>10</sup> A subsequent trial in humans in India showed that crofelemer, compared to placebo, decreased stool volume in persons diagnosed with cholera.<sup>16</sup>

Another study published in the late 1990s was performed by Holodniy et al. This time, humans were the subject of the double-blind randomized placebo-controlled Phase II study. The investigators evaluated the efficacy and safety of SP-303 in

AIDS patients in multiple centers in northern California. They enrolled 51 patients with chronic diarrhea, 26 of whom received SP-303, while the other 25 received placebo. The results showed statistically significant reductions in both mean baseline stool weight (451 g/24 hours versus 150 g/24 hours over the first 4 days;  $P=0.008$ ) and abnormal stool frequency (three/24 hours versus two/24 hours;  $P=0.04$ ). No serious adverse effects of the drugs were noted in the study population.<sup>17</sup>

The ADVENT trial, however, was the pivotal study whose results led to FDA approval of crofelemer for treatment of noninfectious diarrhea in HIV-infected individuals.<sup>3</sup> The ADVENT trial was a multicenter randomized double-blind study with a two-stage design as well as a placebo controlled period followed by an open-label extension phase. The study population included 376 HIV-infected persons on ART with CD4 cell counts of  $>100$  cells/mm<sup>3</sup> with diarrhea for  $>1$  month and no evidence of intraluminal pathogens of diarrhea. The primary endpoint was proportion of subjects with a monthly response (two or less watery stools per week for  $\geq 2$  of 4 weeks). During stage I, the primary goal was to determine the optimal dose of crofelemer. Subjects were randomized 1:1:1:1 into four groups: one that received placebo twice daily and three that received varying doses of crofelemer (125 mg, 250 mg, or 500 mg) twice daily. Based on the efficacy and safety data from stage I, crofelemer 125 mg twice daily was selected as the optimal dose for stage II. During stage II, subjects were randomized 1:1 into either the treatment group using optimal dose crofelemer or control group using placebo twice daily. After 4 weeks, clinical response was achieved by a significantly greater proportion of subjects in the crofelemer group versus placebo (17.6% versus 8.0%,  $P=0.01$ ). Finally, a 5-month extension phase was then executed, during which subjects who had previously received placebo were given crofelemer. This group experienced significant benefit after only 1 month when compared to their previous 1 month of placebo (36% versus 9%; odds ratio =5.85,  $P<0.0001$ ). This group also had significantly ( $P<0.0001$ ) greater odds of achieving clinical response in each of the remaining 4 months.

## Safety and tolerability

Crofelemer is a large molecule with significant polarity, and is thus poorly absorbed from the gastrointestinal tract. As a result, it produces minimal side effects. Due to crofelemer's minimal oral absorption, standard pharmacokinetic parameters such as area under the curve, maximum concentration, and half-life cannot be estimated. In the randomized placebo-controlled phase of the ADVENT trial, the most common adverse effects were gastrointestinal (dyspepsia, flatulence, abdominal pain,

and hemorrhoids) or infections (upper respiratory tract or urinary). Overall, the number of adverse effects experienced in the treatment group was similar to the number in those who received placebo (34.6% versus 32.8%).<sup>3</sup> In fact, 3% of patients in the placebo group discontinued the treatment due to side effects versus none in the crofelemer group. Perhaps most important is the lack of drug–drug interactions. While in vitro studies have shown that crofelemer may inhibit cytochrome P450 isozyme 3A4, its lack of significant systemic absorption renders the in vivo effect on other drug levels unlikely.<sup>18</sup>

## Conclusion

Crofelemer has come a long way from its origin in the Amazon as sangre de drago to pharmacy shelves. As the first in its class FDA-approved antidiarrheal for treatment of noninfectious diarrhea in HIV-infected persons, it reduces the frequency and improves the consistency of stool with essentially no side effects. In turn, it should improve quality of life and, possibly, ART compliance in the HIV-infected population. In addition, crofelemer is now in clinical trials to assess efficacy against cholera infection in the developing world. A Phase II clinical trial was recently completed in India, and more than 140 other countries are potential sites for further study.<sup>19</sup> The increasing prevalence of ethnobotany as a method of finding biologically active plants and plant-extracts will likely continue to yield useful human medicine.

## Disclosure

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