Journal of Biotechnology 239 (2016) 115–125

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Dusquetide: A novel innate defense regulator demonstrating a significant and consistent reduction in the duration of oral mucositis in preclinical data and a randomized, placebo-controlled phase 2a clinical study

Mahesh Kudrimoti[a], Amarintha Curtis[b], Samar Azawi[c], Francis Worden[d], Sanford Katz[e], Douglas Adkins[f], Marcelo Bonomi[g], Jenna Elder[h], Stephen T. Sonis[i,j], Richard Straube[k], Oreola Donini[k,*]

[a] Radiation Oncology, University of Kentucky, 800 Rose Street, Lexington, KY, 40536, USA
[b] Gibbs Cancer Center, Spartanburg Regional Hospital, 101 E Wood, Spartanburg, SC, 29303, USA
[c] Veteran's Affairs Long Beach Hospital, 5901 E 7th Street, Mail Code 114A, Long Beach, CA, 98022, USA
[d] Department of Medicine, University of Michigan Health System, 1500 E Medical Center Drive, Ann Arbor, MI, 48109, USA
[e] Department of Radiation Oncology, Willis-Knighton Cancer Center, 2600 Kings Highway, Shreveport, LA,71103, USA
[f] Division of Hematology and Oncology, Washington University, 660 South Euclid Avenue, Saint Louis, MO, 63110, USA
[g] Department of Hematology and Oncology, Wake Forest Health Sciences Medical Center, 1 Medical Center Blvd., Winston-Salem, NC, 27157, USA
[h] PharPoint Research, 5003S Miami Blvd. #100, Durham, NC, 27703, USA
[i] Division of Oral Medicine, Brigham and Women's Hospital and the Dana-Farber Cancer Institute, Boston MA, USA
[j] Biomodels LLC, 313 Pleasant Street, Watertown, MA 02472, USA
[k] Soligenix Inc., 29 Emmons Drive, Suite C-10, Princeton, NJ, 08540, USA

ARTICLE INFO

Article history:
Received 16 May 2016
Received in revised form 7 October 2016
Accepted 12 October 2016
Available online 13 October 2016

Chemical compounds studied in this article:
Dusquetide (PubChem CID: 71722017)
Dextran sulfate sodium (PubChem CID: 7849109)
Paclitaxel (PubChem CID: 36314)
5-Fluorouracil (PubChem CID: 3385)

Keywords:
Innate
Immune
Oral mucositis
Head and neck cancer
Cancer supportive care
Dusquetide

ABSTRACT

Dusquetide, a novel Innate Defense Regulator, modulates the innate immune system at a key convergence point in intracellular signaling pathways and has demonstrated activity in both reducing inflammation and increasing clearance of bacterial infection. Innate immunity has also been implicated in the pathogenesis of oral mucositis (OM), a universal toxicity of chemoradiation therapy (CRT). Testing the hypothesis that dusquetide can mitigate the development and duration of OM, preclinical studies have been completed and correlated with interim results from a Phase 2 clinical study in patients undergoing CRT for head and neck cancer. Dusquetide reduced the duration of OM in mouse and hamster models by approximately 50%, which was recapitulated by the 50% reduction of severe OM (SOM) in the Phase 2 trial. A reduction in the clinical rate of infection was also observed, consistent with previously reported preclinical studies. In aggregate, these results not only demonstrate the safety and efficacy of dusquetide in addressing this unmet medical need, but also provide proof of concept for the translation of dusquetide action between animal models and the human clinical setting, and further support the contention that innate immunity is an important driver for the initiation and continued impact of OM.

1. Introduction

Oral mucositis (OM) is a universal toxicity of chemoradiation therapy (CRT) used for cancers of the oral cavity, oropharynx and larynx, whose incidence continues to increase despite aggressive intervention (Ryerson et al., 2016). Severe OM (SOM) occurs in almost 75% of these patients and is among the most debilitating and painful side effects related to treatment (Elting et al., 2009). SOM is associated with increased opioid use, weight loss,

Abbreviations: AUC, area under the curve; CRT, chemoradiation therapy; DAMP, damage-associated molecular patterns; DRC, data review committee; IDR, innate defense regulator; IV, intravenous; OM, oral mucositis; PAMP, pathogen-associated molecular pattern; SOM, severe oral mucositis; UOM, ulcerative oral mucositis.

* Corresponding author.
E-mail addresses: odonini@shaw.ca, ODonini@Soligenix.com (O. Donini).

http://dx.doi.org/10.1016/j.jbiotec.2016.10.010

reliance on supplemental feeding and hydration, breaks in treatment, unplanned office, or emergency room visits and frequent hospitalizations resulting in an incremental cost of $18,000 per patient (Nonzee et al., 2008). OM, including SOM, is a common feature of many other treatment regimens for cancers other than head and neck, albeit generally occurring with lesser frequency and duration (Peterson et al., 2011; Kudrimoti et al., 2008).

There are no Food and Drug Administration (FDA)-approved drugs to ameliorate SOM in patients with head and neck squamous cell carcinoma. The only approved therapy for oral mucositis in any context is palifermin (Nguyen et al., 2015; Lucchese et al., 2016), a tissue growth factor that is approved for use in patients with hematologic malignancies receiving myelotoxic therapy requiring hematopoietic stem cell support. However, palifermin is associated with a potential risk of stimulating/encouraging solid tumor proliferation (McDonnell and Lenz, 2007) and is therefore used in hematologic cancers only. Other approaches, such as photobiomodulation, have also been evaluated, including in head and neck cancer patients (Fekrazad and Chiniforush, 2014); although, concerns have been raised here as well about the potential negative impact on tumor control (Sonis et al., 2016)

As the biological complexity of OM has become increasingly clear, preclinical and clinical data have accumulated supporting a role for an exaggerated innate immune response as a key initiating event and a continuing contributor in its pathogenesis (Sonis, 2004). The response of the innate defense system to the resulting CRT-induced damage-associated molecular patterns (DAMPs) results in an inflammatory cascade that culminates in apoptosis of epithelial stem cells, mucosal atrophy and ultimately ulceration (Sonis, 2004, 2007; Sonis et al., 2007; Logan et al., 2007). The resulting ulceration continues to send DAMP signals, as well as potential pathogen-associated molecular pattern (PAMP) signals due to infection, further exacerbating the inflammatory response. Therefore, the innate immune response is a potential target to attenuate both the incidence and duration of CRT-induced OM (Sonis, 2004, 2007).

Dusquetide (SGX942) is a first-in-class Innate Defense Regulator (IDR) that modulates the innate immune response to both PAMPs and DAMPs by binding to p62, a key adaptor protein that functions downstream to the key sensing receptors (e.g., toll-like receptors [TLRs], etc.) that trigger innate immune activation (Yu et al., 2009). There are no other drug candidates which target the p62 protein. When the innate immune sentinel cell is activated, the presence of dusquetide modulates the cellular signaling from a pro-inflammatory, pro-macrophage response to an anti-inflammatory, heightened pro-macrophage response. This leads to decreased inflammation with increased bacterial clearance and tissue healing (North et al., 2016; Scott et al., 2007; Yu et al., 2009). Importantly, both circulating and tissue resident innate immune cells respond to dusquetide treatment, making it useful even in the context of immunosuppression (North et al., 2016). Dusquetide therefore has the potential to address each of the stages of the pathogenesis of oral mucositis, decreasing the innate immune amplification of the damage signaling (and subsequent exacerbation of tissue damage), decreasing the incidence and increasing the clearance of any secondary infections and aiding in the tissue healing and resolution of mucositis. As such, dusquetide would be expected to decrease the duration of OM. Importantly, dusquetide does not mitigate the direct damage done by CRT to the tumor (or the surrounding normal tissue).

The safety of single and multiple ascending doses of intravenous (IV) dusquetide was demonstrated in a placebo-controlled study of 84 healthy human volunteers, in which dusquetide was found to be safe and well tolerated (North et al., 2016). In the groups receiving multiple doses, most adverse events (AEs) were related to minor infusion/venipuncture reactions. Other reported AEs were somnolence (4/20 dusquetide patients, 2/10 placebo patients), alanine aminotransferase (ALT) elevation (3/20 dusquetide patients, 1/10 placebo patients), and back pain (3/20 dusquetide patients, 1/10 placebo patients). *In vitro* exposure to endotoxin of peripheral blood cells from the same cohort, demonstrated an increase in anti-inflammatory markers and a decrease in inflammatory markers following administration of low doses (0.15–2.0 mg/kg) of dusquetide relative to those subjects exposed to placebo (North et al., 2016). Blood from patients exposed to high doses (3.0–8.0 mg/kg) of dusquetide, on the other hand, had responses similar to those receiving placebo (North et al., 2016).

Given the proposed role of innate immunity in mucositis, dusquetide was tested first in preclinical models of mucositis. Subsequently, dusquetide was assessed in a well-characterized clinical setting in a Phase 2a study specifically focusing on oral mucositis in order to evaluate dusquetide's safety, efficacy and consistency of the response with preclinical results.

2. Materials and methods

2.1. Animal models

All experimental procedures using animals were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and performed in IACUC-approved research facilities with the approval of the facility's Animal Care and Use Committee (approval numbers 09-1215-03, 09-1215-04 and 10-0527-1 from the Biomodels LLC IACUC).

2.1.1. Mouse model of chemotherapy-induced mucositis

5-fluorouracil (60 mg/kg IP) was administered to male C3H/HeN mice on Days −4 and −2. On Day 0, a chemical burn was applied to the underside of the mouse tongue, inducing mucositis which generally peaked on Day 2 (Sonis et al., 1990). Mouse tongues were scored from 0 to 5 for mucositis daily from Days 1 to 14 by 2 blinded observers. Body weights were also measured daily and colitis severity was determined by video endoscopy on Days 4 and 7. Dusquetide (25 mg/kg IV) was administered after chemotherapy on Days −1, 2 and 5 in 3 independent experiments and the average duration as a percent of the placebo (saline) response was averaged across the 3 experiments. The dusquetide response was statistically significant ($p \leq 0.01$) in each experiment.

2.1.2. Hamster model of fractionated radiation-induced OM

Canulated male Golden Syrian hamsters were treated with 7.5 Gy of radiation, directed at the everted left cheek pouch, on Days 0, 1, 2, 3, 6, 7, 8 and 9 (Ara et al., 2008). Mucositis was evaluated every second day by 2 blinded observers between Days 7 and 35, with peak mucositis severity generally occurring around Day 19. Dusquetide (25 mg/kg IV) was administered either every third day between Days 0 and 33 (Q3d D0-33; evaluated in 2 experiments), every 3rd day between Days 6 and 33 (Q3d D6-33; evaluated in 1 experiment), on days of radiation (evaluated in 2 experiments), or every third day during radiation treatment (i.e., Days 0, 3, 6 and 9; evaluated in 1 experiment). Results are presented as the percent duration relative to the placebo (saline) response in each experiment, and are averaged over 2 experiments where possible.

2.1.3. Mouse model of colitis

Dextran sulfate sodium (DSS) was administered as a 3% DSS solution in the drinking water of male C57BL/6 mice from Days 0 to 5 of the study (Hamilton et al., 2011). Colitis was monitored by video endoscopy on Days 7, 14 and 21. Dusquetide (25 mg/kg IV) was administered every third day from Days 0 to 18 (Q3d d0-18), from Days 3 to 18 (Q3d d3-18) or from Days 6 to 18 (Q3d d6-18).