Mechanism of Action of aCT1

Key Take-Aways

- aCT1 is a mimetic peptide of Connexin43 (Cx43), a transmembrane gap junction protein, which interacts with Cx43's binding partners, including tight junction protein zonula occludens 1 (ZO-1).
- aCT1 modulates junctional activity to reduce inflammation, protect cell barrier integrity, prevent edema, and promote re-epithelialization.
- aCT1 has been safely administered as an active pharmaceutical ingredient (API) to over 450 patients under INDs #074836 and #154394.

aCT1 Composition

Alpha-connexin Carboxyl-Terminus 1 (aCT1) is a synthetic peptide designed to mimic a portion of the transmembrane gap junction protein Connexin43 (Cx43). It is a patented New Chemical Entity with a low molecular weight of approximately 3.6 kDa, comprised of 25 amino acids. Specifically, aCT1 peptide has a compact two-domain design based on linkage of an antennapedia cell internalization domain (aa 1-16; RQPKIWFPNRRKPWKK) to the C-terminal, PDZ2 binding, cytoplasmic regulatory domain of Cx43 (aa 17-25; RPRPDDLEI) [2].

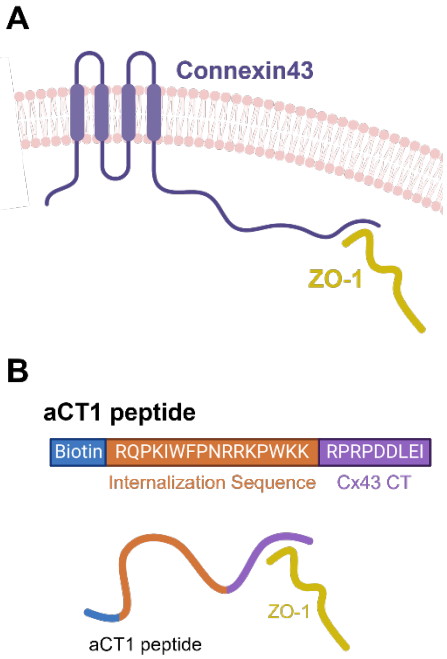

Figure 1. aCT1 peptide. (**A**) aCT1 is a peptide mimetic of the C-terminal domain of Connexin43 (Cx43) that binds zonula occludens 1 (ZO-1). (**B**) aCT1 contains a cell internalization sequence and the nine C-terminal most amino acids of Cx43. [1]

aCT1 Targets

aCT1 interacts with known binding partners of Cx43, including the tight junction scaffolding protein zonula occludens 1 (ZO-1) [3, 4]. aCT1 was developed as a molecular tool to inhibit ZO-1 binding to the Cx43 C-terminus by binding to the PDZ2 motif on ZO-1 itself [4]. aCT1 has also been shown to interact directly with Cx43's C-terminal domain [5] and may have other binding partners such as CCN3 [6], 14-3-3 proteins [7], SH3-mediated interactions [8], and various protein kinases [9].

Impact on Cx43 Hemichannels and Gap Junction Channels

Cx43, as other connexins, is a transmembrane protein that assembles into Cx43 hexamers to form hemichannels at the plasma membrane (connecting cytoplasm to extracellular space). Two hemichannels on adjacent cells can dock together to form gap junctions that physically connect the cytoplasm of neighboring cells, protected from the extracellular environment. In an uninjured environment, hemichannels have a low probability of opening, which is markedly increased in the injured state [10] – a phenomenon that has been targeted in various injury models for therapeutic benefit [11-13]. Gap junctions regulate the direct transfer of ions and small molecules via gap junction intercellular communication (GJIC), which is important in maintaining healthy cell and tissue function.

Cx43 is the most ubiquitously connexin expressed in the skin [14], is expressed in a number of ocular tissues including the lens and retina [15], and is abundantly expressed in a variety of lung cells [16]. Abnormal Cx43 expression is associated with dysregulated cell proliferation, migration, and wound healing [17, 18].

Cx43 binds the PDZ2 domain of ZO-1, which regulates the size and stability of gap junctions by altering the relative proportion of hemichannels to gap junction channels. When aCT1 disrupts Cx43/ZO-1 interactions, this causes a shift in Cx43 from nonjunctional hemichannels to gap junctional complexes. Collectively, this ZO-1 mediated translocation of hemichannels into gap junctions simultaneously enhances GJIC while reducing hemichannel activity, such as release of ATP or other small molecules into the extracellular space that can drive an inflammatory response [3]. aCT1 has been shown to affect the connexin life cycle in several ways, from increasing gap junction plaque size to influencing kinase activity to regulating localization of Cx43 [4, 9, 19-21].

Xequel Bio has demonstrated in multiple injury models across organ systems that aCT1 treatment reduces inflammation at the tissue level. For example, in ocular corneal injury models, aCT1 treatment has reduced inflammatory signaling molecules such as IL-6, IL-1β, TNFα, Cox-2, MMP-9, and VEGF. Activated neutrophils have been shown to release ATP via Cx43 hemichannels, and Cx43-dependent release of ATP has been shown to recruit macrophages [22, 23]. Notably, inhibiting Cx43 hemichannel mediated ATP release has been shown to reduce the early inflammatory response [24], and aCT1 has been shown to recruit more hemichannels into gap junctions, thereby depleting the hemichannel pool. These data support a role for reduction in hemichannel-mediated ATP release as a trigger of downstream events that culminate in the observed reduction in inflammatory cell infiltration with aCT1 treatment.

Impact on Tight Junctions

Tight junctions form the intercellular barrier between epithelial and endothelial cells, controlling paracellular permeability of water, ions, and macromolecules. Tight junction assembly is dependent upon the oligomerization of integral membrane proteins known as claudins into tight junction strands, to form a barrier between cells [25]. Claudin assembly at the cell membrane is directed by ZO-1, a cytosolic scaffold protein that anchors tight junctional transmembrane proteins to the cellular cytoskeleton. ZO-1 binds claudin C-termini and promotes claudin oligomerization into tight junction strands, forming a seal between neighboring cells [26]. In addition, ZO-1 interacts with transmembrane protein occludin, which contributes to tight junction stability and optimal barrier function [27]. ZO-1 also regulates the assembly of adherens and gap junctions, supporting a general role for ZO-1 in intercellular adhesion and junctional stability [3, 28, 29].

When aCT1 binds ZO-1, ZO-1's claudin selective PDZ1 domain may be exposed and therefore able to interact with claudins [30]. aCT1 stabilizes ZO-1 at the plasma membrane, allowing claudins to oligomerize into tight junction strands, thus preventing tight junction degradation in response to injury and supporting accelerated re-establishment of cell barriers [31]. Xequel Bio has demonstrated aCT1's ability to promote cell barrier integrity and reduce edema across several organ systems, supporting a role for aCT1 in the regulation of tight junctions' response to injury.

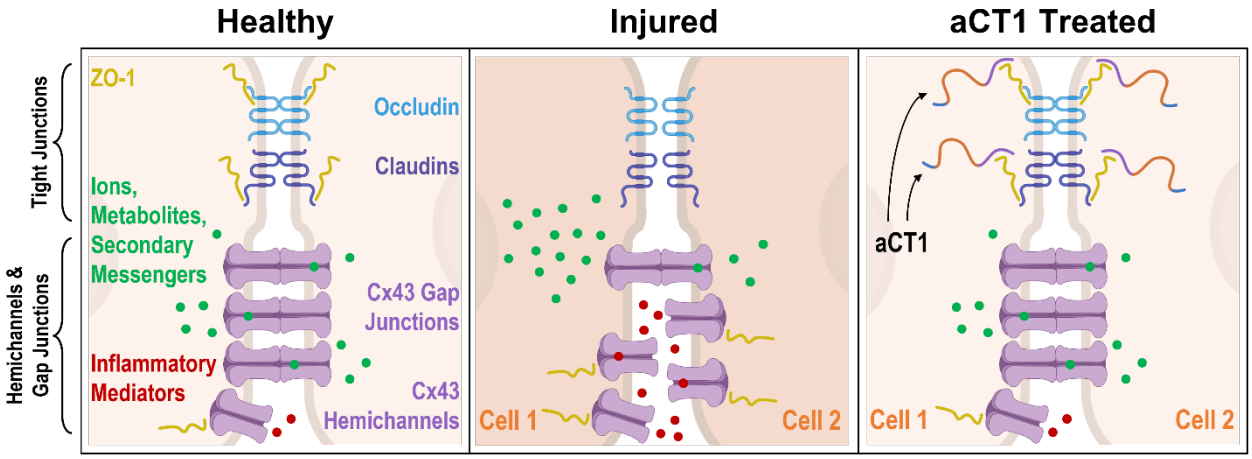

Figure 2. aCT1's mechanism of action. When aCT1 is introduced to injured cells, it disrupts ZO-1 and Cx43 binding, causing a shift from hemichannels toward gap junctions. This improves GJIC and reduces excessive inflammation, as well as likely allowing ZO-1 to relocate to tight junctions and thereby stabilize barrier function. [1]

Clinical Impact

In addition to effects on inflammation, cell barrier integrity, and edema, aCT1 has demonstrated an ability to promote re-epithelialization and accelerate wound healing. aCT1 has been shown to alter fibroblast migration, collagen fiber type, and collagen fiber deposition pattern in dermal and surgical models [32, 33]. In addition, blocking ATP release via Cx43 has been shown to ameliorate fibrosis [34]. These data support a potential beneficial effect of aCT1 on fibrosis that warrants further exploration in future studies.

In numerous preclinical studies and seven clinical trials, aCT1 has demonstrated efficacy and tolerability in the treatment of acute and chronic dermal and ocular wounds [35-38]. Preclinical animal studies have supported aCT1's ability to improve the wound healing response by tempering inflammation, coordinating cellular communication, and promoting a regenerative state [21, 31-33, 35, 39-42]. Clinical studies have validated these outcomes, demonstrating decreased neutrophil infiltration and increased wound closure rate. Importantly, the low molecular weight of the 25 amino acid peptide is expected to reduce the risk of an immunogenic response. In addition, no treatment-related serious adverse events (SAEs) have occurred throughout administration of aCT1 to over 450 patients, highlighting aCT1's safety profile.

This multifactorial mechanism – reducing hemichannel activity, promoting GJIC, reinforcing tight junction integrity – tempers aberrant inflammation at the site of injury, protects cell barriers, and promotes a healthy regenerative response.

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