Effects of SN514-066b on Eschar Protein Digestion, Burn Wound Debridement and Healthy Skin Irritation

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Abstract

SN514 is a thermolysin-like enzyme under development as a debrider. The enzyme showed excellent digestion of fibrin, elastin and collagen *in vitro*. Burn wound studies in Yorkshire pigs showed efficient eschar debridement with minimal periwound erythema. Direct treatment on intact porcine skin for 5 days produced no to limited erythema.

A human 21-day cumulative irritation study using Webril patches taped to the backs of 38 healthy adult volunteers compared four enzyme concentrations (0.10%, 0.20%, 0.40%, and 0.80% w/w) to the hydrogel vehicle, saline and 0.2% sodium lauryl sulfate using randomized placements and blinded evaluation.

Irritation was observed to increase stepwise by concentration, confirming formulation accuracy. Each enzyme concentration was found to be "possibly mild in use" (Berger and Bowman method). No treatment emergent adverse events were observed during the study.

The overall findings support clinical dose range testing for tolerance and preliminary efficacy.

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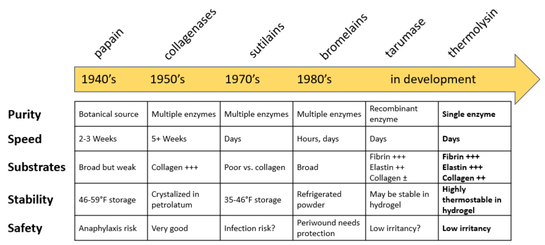
Introduction



Purity	Botanical source	Multiple enzymes	Multiple enzymes	Multiple enzymes	Recombinant enzyme	**Single enzyme**
Speed	2-3 Weeks	5+ Weeks	Days	Hours, days	Days	**Days**
Substrates	Broad but weak	Collagen +++	Poor vs. collagen	Broad	Fibrin +++ Elastin ++ Collagen ±	**Fibrin +++ Elastin +++ Collagen ++**
Stability	46-59°F storage	Crystallized in petrolatum	35-46°F storage	Refrigerated powder	May be stable in hydrogel	**Highly thermostable in hydrogel**
Safety	Anaphylaxis risk	Very good	Infection risk?	Periwound needs protection	Low irritancy?	**Low irritancy**

Enzymatic debridement has long been considered a potentially ideal approach to making burn or chronic cutaneous wounds ready to heal without damaging healthy tissue. Experience over decades with enzymatic debriders has been disappointing, with shortcomings including variable purity, stability, quality and selectivity, excessively aggressive formulations, application site pain, Ficus-fruit latex protein hypersensitivity reactions, or slow speed of debridement. SN514 is a single, purified, thermostable protease made by Anoxybacillus caldiproteolyticus, formulated in a ready to use hydrogel stable at room temperature for years.

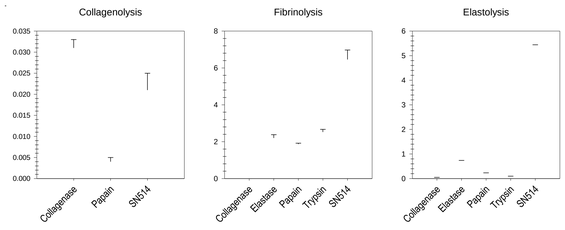
SN514 and the hydrogel formulation SN514-066b have been extensively evaluated in relevant pre-clinical models. We report here key findings from studies of in vitro enzyme activity and animal burn wound debridement, followed by the results of a first in human 21-day Cumulative Irritation Test (CIT) designed to evaluate tolerability on healthy intact skin.

Methodology & Results

METHODS – In Vitro

SN514 in tris buffer solution (pH 7.4) was tested against type I collagen (calf skin, Elastin Products Company (EPC), Owensville MO) at 1.0 mg/mL (0.1% w/v), against fibrin at 0.01 mg/mL (0.001%, generated using fibrinogen and thrombin (Sigma, St. Louis MO), and against elastin (elastin-remazol E194S, EPC) at 0.1 mg/mL (0.01%) 10. Enzyme solutions were prepared using 50 mM Tris Buffer at pH 7.4 (Tris[Hydroxyl]Aminomethane). Reactions were carried out at 37 ° C for 180 m, 40 m, and 60 m respectively in 96 well microplates using 200 µL each of enzyme solution and substrate solution. A microplate absorbance reader running SOFTmax PRO software was used for kinetic analyses.

RESULTS

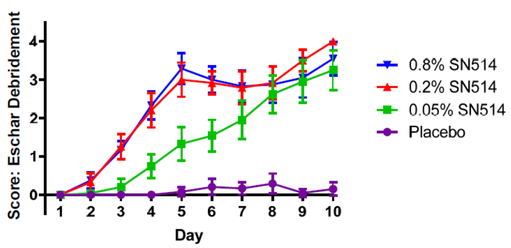


Compared with high potency papain, SN514 showed more specific proteolytic action on wound insoluble proteins of collagen (4xhigher), fibrin (3x higher) and elastin (10x higher). SN514 displayed the highest fibrinolytic activity compared with the other proteases in the test, with high potency papain, trypsin and elastase showing equivalent activity against fibrin. SN514 was more potent than all other enzymes tested against elastin, including elastase. Type I collagenase showed the highest collagenolytic activity.

METHODS – Pig burn eschar

Animal studies were approved by the Institutional Animal Care and Use Committee at the test facility (University of North Texas Health Sciences Center in Fort Worth, TX). Commercially raised Yorkshire-cross pigs (20-25 kg) each had multiple 2 cm diameter dorsal burn wounds created under tiletamine/zolazepam / xylazine / buprenorphine and anesthesia maintained using inhaled isoflurane. Wounds were made using brass rods heated to 100° C in a sand bath then held on the skin for 45 seconds. In Study A treatments were applied daily for 10 days after allowing the eschar to harden for 4 days. In Study B, areas of shaved intact skin were also treated with enzyme for 5 days.

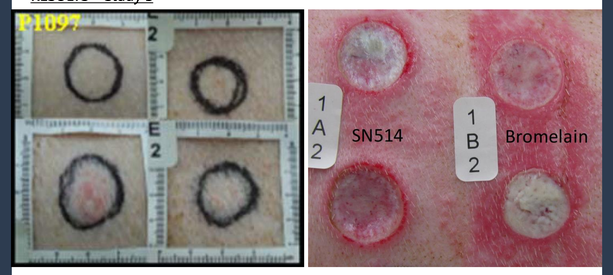
RESULTS – Study A



Debridement showed clear ranks between the test articles: 0.8% and 0.2% SN514 performed the same, each better than 0.05% SN514; and 0.8%, 0.2%, and 0.05% SN514 performed better than Placebo. 0.8% and 0.2% SN514 were significantly superior to Placebo from Day 2 to Day10/EOS, while 0.05% SN514 was significantly superior to Placebo from Day 4 to Day 10/EOS.

Results

RESULTS – Study B

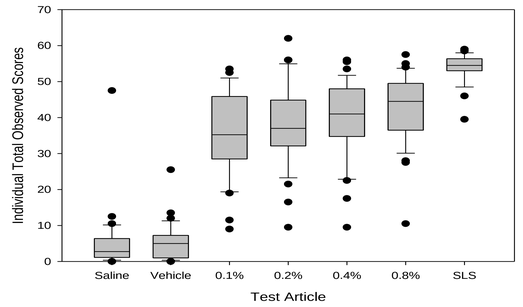


Left - representative photos from Day 1 (upper) and Day 5 (lower) showing minimal erythema with SN514; Right – showing vasodilation around the burn with minor erythema between burns (left) compared with bromelain (right).

METHODS – 21-Day Human Cumulative Irritation Study

Consenting subjects were adult volunteers aged 18 to 70 years. Test articles were low irritant control (0.9% sodium chloride solution), SN514-066b hydrogel vehicle, 0.10%, 0.20%, 0.40%, and 0.80% SN514-066b, and a positive irritant control (0.2% sodium lauryl sulphate (SLS), applied using an occlusive square patch with rounded edges (Strukmyer Medical, Mesquite, Texas) of approximately 3.8 cm x 3.8 cm, held to the skin using a clear, non-porous polyethylene hypoallergenic adhesive tape (3M Plastic Medical Tape®). Adhered in the center of the patch was an approximately 2.0 cm x 2.0 cm square of Webril® non-woven cotton (GWJ Company, La Quinta, CA) with an approximate thickness of 1 mm, onto which was placed a volume of 0.2 mL of the test article (giving 0.05, 0.10, 0.20, and 0.40 mg/cm2). Patches were applied to the left side of the back in a randomized fashion. Subjects were instructed to keep the patches as dry as possible and return the following day for evaluations and fresh patch applications. Subjects returning on Days 2-21 had their patches removed and sites scored for irritation 15 minutes (±5 minutes) after patch removal. Once a patch evoked a strong reaction (score of 3 or greater) the patch was discontinued and the terminating score (level 3 or greater) carried forward for all remaining visits.

RESULTS



No pain or test article related adverse events were reported. other than the anticipated irritation of skin. Cumulative irritation scores showed the vehicle to be barely different from saline and probably mild in use, while each concentration of SN514 ranked as possibly mild in use. After the first 7 days, average observed erythema scores were 1.7 ± 0.5 for SLS versus 0.6 ± 0.6, 0.6 ± 0.5, 0.5 ± .07, 0.4 ± 0.5, and 0.2 ± 0.2 for the 0.80%, 0.40%, 0.20%, 0.10% and vehicle preparations. The positive control produced edema, vesicle formation and crusting. The enzyme showed less edema but dose dependent erosions, dose independent papule formation and some weeping.

Conclusion

SN514 is a fast acting enzyme with a favorable irritation profile, prepared as a stable, ready to use hydrogel formulation which overcomes many recognized shortcomings of enzyme debriders.

Enzymes used for debridement should ideally be broadly reactive ("nonspecific"), rapidly acting, stable under ambient storage conditions in a ready to use formulation, and tolerable in clinical use. Preclinical testing of SN514 in various formulations and concentrations has shown that when properly formulated it fulfills the desired attributes for broad reactivity, rapid action and shelf stability. The animal studies found rapid debridement with minimal periwound irritation, consistent with the findings of Stone et al. and supporting advancement to the first in human clinical study.

The testing of immediate and cumulative skin irritancy potential remains a standard component of the safety evaluation of topically applied products.. The progressive increase seen in cumulative irritation scores followed with increasing concentration of enzyme, while the vehicle gel showed no apparent potential for worsening wounded tissue.

The development of papules and erosions shows that the enzyme gel is not entirely benign when kept in contact with healthy skin continuously over several weeks. Such breaks in the protective outer surface of the skin can increase the risk of bacterial or fungal invasion. While the magnitude of risk compared with an open burn wound or chronic cutaneous ulcer may be negligible, dosing regimens must be explored which limit contact time to only what is necessary for effective debridement.

A limitation of the clinical study with respect to clinical use of SN514 is that the intent of the CIT study is to categorize the test article, rather than fully characterize it. Once a maximum erythema score was reached, application of the patch stopped and the score and letter grade were carried forward. Cessation of application limited further potential skin damage (e.g. erosion).

Studies in patients with wounds will help to determine the timeframes necessary for debridement, and whether any special measures must be taken to protect the periwound during debridement. A Phase 1 exploration of tolerability in burn wounds (NCT06628037) will examine tolerability in wounds and also inform the ability to use this enzyme on large body surface areas. Additional aspects of use will need to be addressed in future efficacy studies.

References

- Rosenberg L. Enzymatic debridement of burn wounds. In: Total Burn Care. (Herndon DN. ed.); 2012; pp. 131-135e1.
- Shi L, Ermis R, Lam K, et al. Study on the debridement efficacy of formulated enzymatic wound debriding agents by in vitro assessment using artificial wound eschar and by an in vivo pig model. Wound Repair Regen 2009;17(6):853-862
- Stone R, Jockheck-Clark AR, Natesan S, et al. Enzymatic Debridement of Porcine Burn Wounds via a Novel Protease, SN514. J Burn Care Res 2020;41(5):1015-1028