Successful reduction of matrix metalloproteinases (MMPs) and elastase activity by a novel thermo-reversible antimicrobial gel*

Jeyachchandran Visvalingam, Ph.D; Anna Muzaleva, BSc; Miloslav Sailer, Ph.D; Robert B Huizinga, Ph.D, RN.

[1]Kane Biotech Inc, Winnipeg, Manitoba, Canada
jvisvalingam@kanebiotech.com

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Abstract

This study aimed to determine the effectiveness of a novel thermo-reversible antimicrobial gel (TRG) against gelatin-degrading matrix metalloproteinases (MMPs) and elastase using *in vitro* models. The effect of TRG on MMP-2 and MMP-9 activity was assessed using agarose-gelatin plates. Commercial protease assay kits for elastase, TNF-α converting enzyme (TACE), and collagenase were used to evaluate the impact of TRG on their activity. TRG treatment significantly reduced the zone of clearance for the two MMPs tested, compared to the untreated control, indicating a reduction in MMP-2 and MMP-9 activity. Elastase activity decreased by over 80% with TRG treatment, while a 100% reduction in activity was observed for TACE and collagenase.

In Memory of Dr. Gregory Schultz, PhD
Chief Scientific Officer, Kane Biotech

It is with profound sadness we acknowledge the unexpected passing of Greg Schultz, a world-renowned expert on wound care and biofilms. Greg helped lead the way at Kane in advancing the development and commercialization of our coactiv+™ and DispersinB® technologies as part of his on-going search for the solution to the biofilm problem in healthcare. His contributions were significant and will be realized for decades to come. His loss will simply be immeasurable.

Introduction

Chronic wounds can be characterized by elevated levels of harmful matrix metalloproteinases (MMPs), elastase, and presence of biofilms, which often cause prolonged inflammatory response [1,2]. The most common chronic wound pathogen, *Pseudomonas aeruginosa*, is also known to secrete MMPs [3]. Elevated levels of MMPs and elastase disrupt normal tissue re-epithelization and delay wound healing [1]. Tumor Necrosis Factor-alpha (TNF-α) converting enzyme (TACE) converts the inactive TNF-α precursor into its active form [4]. TNF-α is a cytokine that plays a crucial role in cellular immunity and the inflammatory response. A novel thermo-reversible antimicrobial gel (TRG) containing metal chelating agents, poloxamer 407, and antimicrobial preservative polyhexanide with mildly acidic pH, has been shown to have lasting antimicrobial and antibiofilm activity against wound related pathogens. The TRG's ability to chelate divalent cations required for MMP activity may potentially inhibit these proteases. Additionally, a mildly acidic pH and antimicrobial agent of TRG may reduce the activity of elastase, which requires mildly alkaline pH [1]. This study was aimed at determining the effectiveness of TRG against gelatin-degrading MMPs, and elastase using *in vitro* models.

Materials and Methods

Effect of TRG on MMPs using gelatin plates: Agarose plates containing porcine and bovine gelatin were inoculated with MMP-2 or MMP-9 alone, or with MMP+TRG and incubated for 24 hours at 37°C and zone of clearance that indicates MMP activity was visualized using Coomassie Blue G250 staining (Fig 1).

Effect of TRG on elastase, TACE and collagenase activity: Assays were performed using commercial assay kits. Enzyme control, TRG treatment, TRG without enzyme and buffer controls were maintained, Absorbance values recorded and percent activity reduction for each enzyme was calculated.

Effect of *P. aeruginosa* biofilm proteases on a porcine skin explant model: An approximately 1.5 in. piece of porcine skin was sterilized in bleach, and denatured alcohol. *P. aeruginosa* biofilm was developed on a pig skin placed on a soft tryptic soy agar (TSA) for 72 hours at 37 °C. TRG treatment was applied to a set of skins and incubated for 72 hours at 37 °C. The explants of TRG-treated and an untreated control were rinsed in sterile PBS. Tissue disintegration by bacterial protease was tested by visually assessing skin elasticity change.

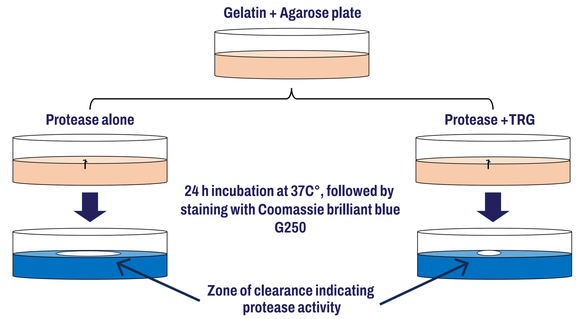

Figure 1. Agarose and gelatin plate assay for assessing protease activity.

Results

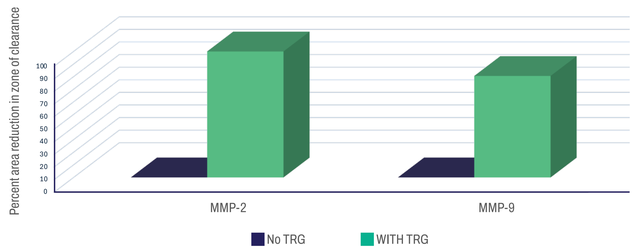

Figure 2. The effect of TRG on MMP-9 and MMP-9 activity determined by using changes on zone of clearance.

Zone of clearance assay with MMP-2 and MMP-9: TRG was able to effectively reduce MMP-2 and MMP-9 activity by 100% and 81%, respectively as measured by change in zone of clearance (Fig 2).

Anti-protease activity: The effect of TRG on elastases, TACE, and collagenase was using commercial protease activity assay kits. TRG inhibited elastase activity by >80% and other two enzyme by 100% (Fig 3).

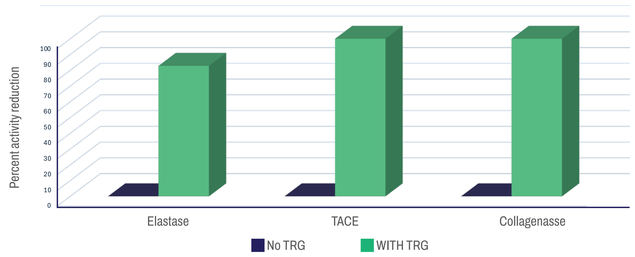

Figure 3. The effect of TRG on Elastase, TACE and collagenase as assessed by commercial assay kits .

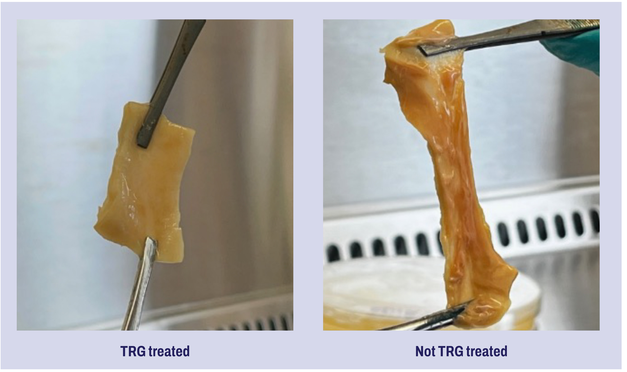

Figure 4. Effect of TRG on *P. aeruginosa* proteases affecting skin integrity during biofilm growth. Left – mature biofilm after treatment with TRG for 72 hours; Right – untreated mature biofilm.

Discussion

Levels of TNF-α in chronic wounds are approximately 100 times higher than in acute wounds. TNF-α can affect connective tissue regeneration during the wound-healing process, as it influences the synthesis of collagen, collagenase, and matrix metalloproteinases (MMPs) [1]. Therefore, TACE inhibitors could be valuable therapeutic options for chronic wounds. Based on the results obtained, evidence suggests that TRG is a potent inhibitor of TACE. Additionally, TRG also inhibits the activity of neutrophil elastase, MMP-2, MMP-9, and collagenase. It is also evident that TRG prevented deterioration of porcine skin by *P. aeruginosa* biofilm, suggesting TRG could control bacterial proteases. The ability of TRG to inhibit multiple enzymes associated with chronic wounds suggests that it may be a promising therapeutic option.

Conclusions

Since elevated levels of TACE, MMPs and elastases in wounds interrupt normal healing of chronic wounds, reduction of their activity is a key for better wound healing outcomes. This novel TRG is formulated with metal-chelating agents, poloxamer 407 and polyhexanide, is mildly acidic and was effective against detrimental proteases. Taken together, this novel TRG would be a beneficial tool for combating chronic wounds given the effective antimicrobial/antibiofilm properties, as well as anti-protease activities. Further studies would help confirm its anti-protease activity *in vivo.*

References

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