Cysteine Proteases May Play a Role in the Wound Healing Properties of OPAL A

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BACKGROUND

Chronic wounds
• Wounds that do not heal satisfactorily in the manner and time frame expected.\(^1\)
• Multiple causes and three major types: venous, diabetic, and pressure ulcers.\(^2\)
• Treatment often difficult, slow, and expensive; estimated annual chronic wound management costs exceed $25 billion in the US.\(^3\)

Cysteine proteases
• Enzymes that degrade protein.
• Play an important role in the biology of the skin, under healthy conditions, and during disease, disease recovery, and wound healing; particularly important in the healing of chronic wounds.
• Papain, an important cysteine protease, is only found in nature in the paw paw plant (Carica papaya) and is only extracted commercially from the latex of unripe (green) paw paw fruit (referred to as ‘latex papain’).
• Papain, and its associated enzymatic activity, has not previously been detected in the flesh of the paw paw fruit itself, ripe or otherwise.\(^4\)\(^6\)

OPAL A
• A filtrate derivative of ripe paw paw flesh.
• Shows preliminary evidence of wound healing properties (Figure 1).
• Mechanism of action is unknown.

Figure 1. Preliminary evidence of wound healing properties of OPAL A

TEMPORAL SCALP

Following 7 weeks treatment with OPAL A

To investigate the potential debriding activity of OPAL A filtrate, we used a novel debriding assay that involves the synthesis of an artificial eschar composed of collagen, fibrin, and elastin.\(^7\)

OBJECTIVES AND METHODS

Objectives
• Mass spectrometry data from Proteomics International (Murdoch University / University of Western Australia) indicated that OPAL A contains a suite of closely related cysteine proteases including, but not limited to, papain; some of these proteases may be able to mediate the wound healing activity of OPAL A.
• Therefore, the aim of this study was to characterise the activity and potential role of cysteine proteases in OPAL A during wound healing.

Methods

OPAL A filtrate
• OPAL A filtrate is produced from paw paw through the patented process of heating the pulped flesh to 55°C, adding 10% w/w sodium bicarbonate, and subsequently filtering the resultant mixture.

Cysteine protease assay
• Colorimetric assays were performed to ascertain whether active cysteine proteases could be identified in 4 separate batches of OPAL A filtrate.
• Catalysed hydrolysis of the chromogenic substrate L-BAPNA, which is commonly used to study protease activity, was monitored.
• The release of the hydrolysis product, 4-nitroaniline, was measured by UV spectroscopy, specifically at an absorbance of 410 nm (A\(_{410}\)).
• To demonstrate that the catalysis was thiol-dependent (i.e. was due to cysteine protease activity and not that of other proteases), the thiol-specific inhibitor 2PDS was used to stop catalysis.
• As a further control, excess L-cysteine was subsequently added to the inhibited reactions to sequester 2PDS, thereby reactivating the thiol-dependent enzyme(s).

In vitro debriding assay
• To investigate the potential debriding activity of OPAL A filtrate, we used a novel debriding assay that involves the synthesis of an artificial eschar composed of collagen, fibrin, and elastin.\(^7\)
**RESULTS**

**Figure 2. Active proteases are present in OPAL A**

Rate of protease activity for 4 batches of OPAL A; each batch is indicated by a different colour. Protease activity is also presented after inhibition (OPAL A + 2PDS) and after reactivation (OPAL A + 2PDS + L-cysteine).

**Protease activity of OPAL A**

- Activity detected in each batch of OPAL A, with variability between batches.
- Activity completely inhibited by the addition of 2PDS, indicating that the activity was only due to cysteine proteases.
- For most batches of OPAL A, activity was greater after reactivation, suggesting that a fraction of the cysteine proteases in OPAL A exists in a reversibly inactive (oxidised) form.

**Debriding activity of OPAL A**

- OPAL A is more effective than latex papain at digesting the major eschar component collagen. Digestion of collagen by latex papain has not previously been reported in the literature.8
- OPAL A is more effective than latex papain at digesting fibrin.
- OPAL A is less effective than latex papain at digesting elastin.
- Although OPAL A appears to be more effective than latex papain at digesting two of the three major eschar components, the cysteine protease proportion of the OPAL A protein content is currently unknown. This suggests that optimisation of protein content may further enhance the debriding ability of OPAL A, particularly with respect to digestion of collagen, elastin, and fibrin.

**CONCLUSIONS**

**Active cysteine proteases are present in OPAL A, which is a derivative of ripe paw paw flesh.**

This is in contrast to expectations that active cysteine proteases are only found in the latex of unripe paw paw fruit.

**OPAL A was more effective than latex papain at debriding major eschar components.**

OPAL A, and its suite of closely related cysteine proteases, including but not limited to, papain, was able to effectively debride the major eschar components collagen and fibrin.

To enhance debriding activity, optimisation of the OPAL A filtrate is ongoing.

Debridement of major eschar components by cysteine proteases may be one mechanism of action by which OPAL A is able to effectively heal chronic wounds.

**REFERENCES**


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