Inhibition of 5-lipoxygenase activity by The novel wound-healing agent, OPAL A

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Introduction

- OPAL A consists of a paw paw pulp treated by a process involving heating and alkalinisation*.
- OPAL A may improve healing of wounds that are resistant to standard care therapy (Mitchell *et al.,* 2008; 2010), although the mechanism is yet to be determined.
- We hypothesised that OPAL A has an anti-inflammatory effect involving the inhibition of the pathway leading to generation of pro-inflammatory leukotrienes.

* OPAL A has been developed by Phoenix Eagle Company Pty Ltd, a private West Australian company.

Aims

To examine:

1) The wound healing capacity of OPAL A
2) The ability of OPAL A, and fractions of OPAL A to inhibit 5-lipoxygenase (5-LOX) activity
3) The sensitivity of the filtrate to temperature and bicarbonate during filtrate preparation

Methods

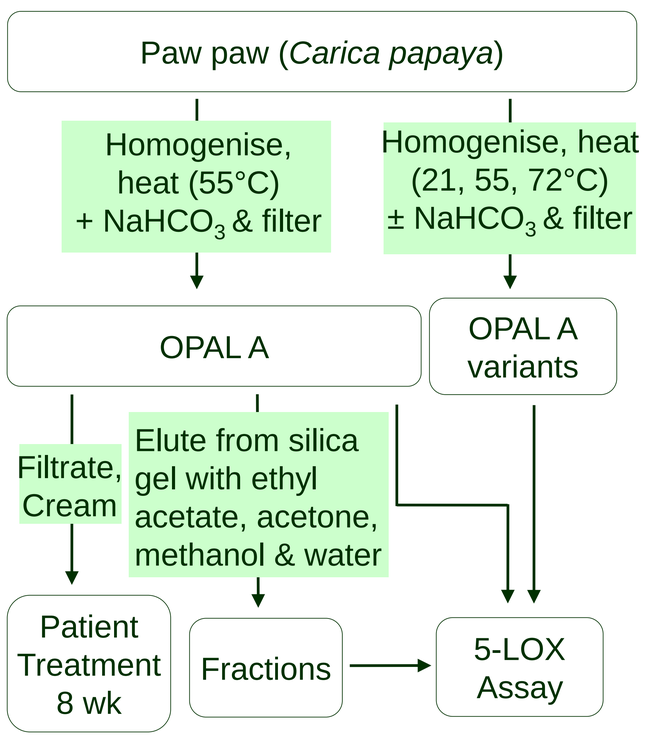

Figure 1 Diagram illustrating the preparation of filtrates, and use of the filtrates, for the treatment of a patient with a non-healing wound and examination of 5-LOX activity.

5-LOX assay

- OPAL A was incubated with 5-LOX and linoleci acid (substrate), in the presence of haemoglobin (catalyst) and DMAB and MBTH (oxidative coupling reactants).

DMAB, 3-(dimethylamino)benzoic acid; MBTH, 3-methyl-2-benzothiazolinone

- 5-LOX catalyses the oxygenation of linoleic acid to yield linoleic hydroperoxide which facilitates production of a purple indamine dye that is detected at A_{598}.

Results

Wound Healing

OPAL A produced a marked improvement in resolution of non-healing wounds in a patient with an infected traumatic leg ulcer.

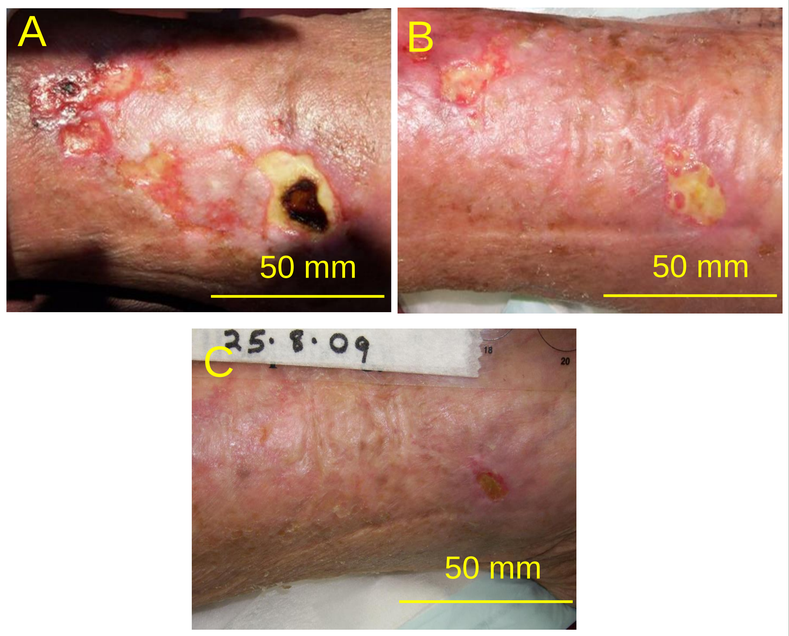

Figure 1 Non-healing infected traumatic ulcer in an 84 yr. man before (A), and after 4 (B) and 8 weeks (C) treatment with OPAL A filtrate and cream. Necrotic material was absent, and slough reduced markedly after 4 weeks. Almost complete healing of the wound was evident after 8 weeks of treatment.

5-LOX activity assay

OPAL A inhibited the 5-LOX activity assay.

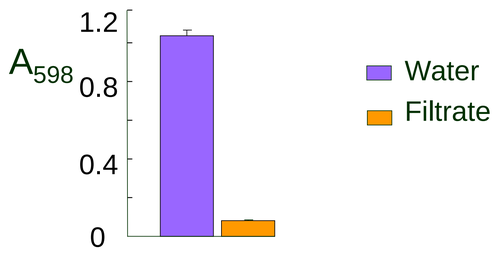

Figure 2 OPAL A markedly inhibited the 5-LOX activity assay (mean±SEM; n=3). This effect may be attributed to either direct inhibition of the 5-LOX enzyme, or a possible antioxidant effect that suppresses the activity of the product, linoleic hydroperoxide.

OPAL A fractionation

The inhibitory activity of OPAL A is attributed to the presence of polar molecules.

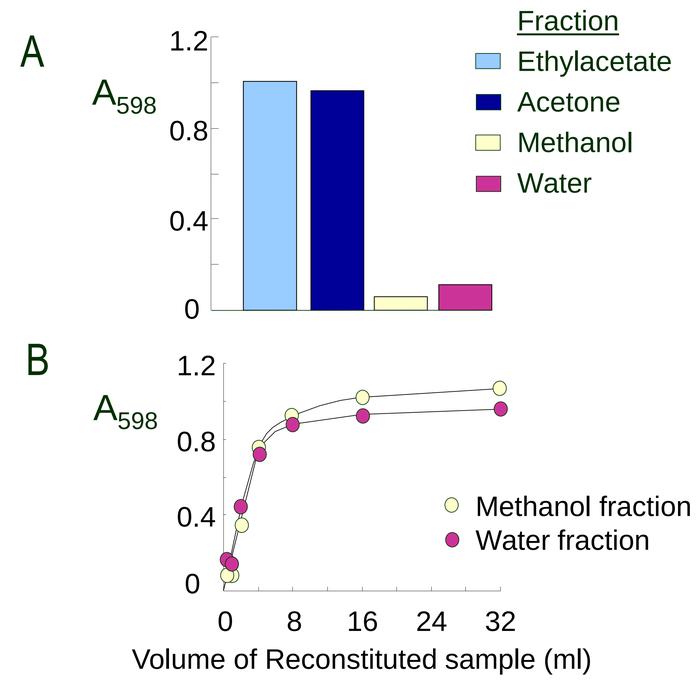

Figure 3 5-LOX activity was not attenuated by the dried and reconstituted ethylacetate or acetone fractions (A), but was attenuated by the dried and reconstituted methanol and water fractions (A, B).

Stability

The active compounds present in OPAL A were stable when the filtrate was prepared under varying temperature and alkalinisation conditions.

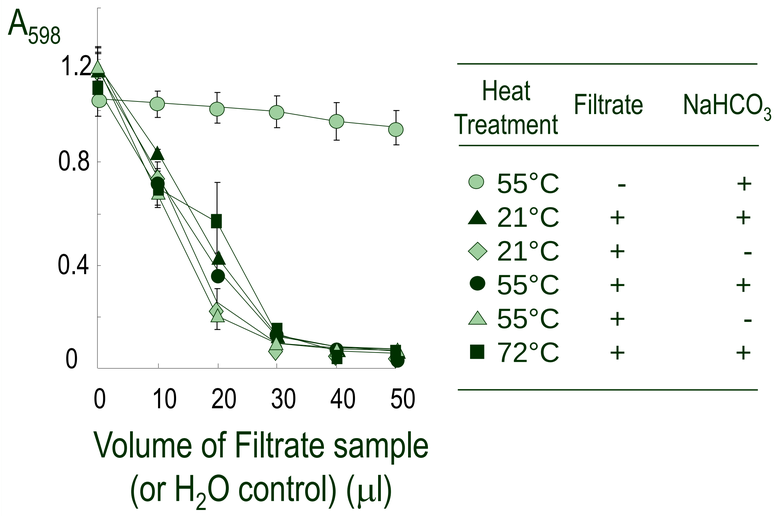

Figure 4 Adjustment in filtrate heat-treatment, and inclusion or exclusion of bicarbonate during filtrate preparation, did not affect the capacity of the filtrate to inhibit the 5-LOX activity assay (n=3).

Conclusion

- OPAL A inhibited a 5-LOX activity assay
- The findings raise the possibility of an anti-inflammatory effect that may serve to improve wound healing.

References

1. Mitchell G. *et al.* AMWA Conference abstract (2008).
2. Mitchell G. *et al.*, AWTRS Conference abstract (2010).