Clinical observations and physiological data supporting a vascular response as a mechanism of the novel wound-healing agent, OPAL A

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Principles of standard care

- Debridement
- Infection control
- Moist clean environment
- Pressure bandaging for venous ulceration
The OPAL Process

- Pulped pawpaw
- Filtrates extracted from pulps prepared according to the OPAL Process
  - Involves heat and alkalinisation. Filtrate extracted.
- Filtrates mixed together and preservative added
- Product named OPAL A
Ulcer treatment

- Ulcer treatment evolved to:
  - Filtrate - applied to wound
  - Filtrate in ointment base - applied to skin around and proximal to wound
  - Daily application of both
Requirements for wound healing

Adequate oxygen, 
Adequate Nutrients
Intact immune system

Good Arterial blood supply
Good venous blood supply

Inhibitors of wound healing

Infection
Tissue hypoxia
  Macrovascular
disease - PVD
  Microvascular
disease – esp. diabetes,
  raised, constant tissue
  pressure

Inadequate nutrition
Impaired venous circulation
Impaired systemic immune
response
Left first and second toes  15 May 2008
85 yr woman. Generalised arteriopathy.
Sudden breakdown of skin from poor arterial supply made worse
with cold weather.
OPAL 001 filtrate applied to wound, cream to foot and leg to knee.
Date 29 May 2009 Two weeks of treatment
Rapid improvement.
24 June 2008. Six weeks
Second toe almost healed. First toe. Odd lesion is exogenous nail.
Left temple marginally viable skin graft.
Graft repair of removal of major skin cancer.

OPAL 001 used starting 24 April 2008
29 May 2008
Five weeks.
Graft now fully viable. Ulcers at margins healing
24 June 2008
Eight weeks
Central ulcer closing.
Graft skin now normal.

5 August 2008
Three months
Graft fully healed
Diabetic ulceration of toes

55 Yr male. Date 20th April. Diabetic, alcoholic. Poor nutrition. The toe at the commencement of treatment. The tip is necrotic and probably infected. The nail is grossly abnormal.
Five weeks  Date 22 May 2003
Toe virtually healed. Toenail removed and new nail forming.
Improved microcirculation
Left outer mid-shin
16 Nov08

Elderly man.
Chronic extensive DVTs,
Psoriasis.

Healing ulcer

Compromised skin
Skin quality vastly improved
Hypothesis

That a major contributor to OPAL A’s apparent wound healing property is vasodilation.
Does OPAL A mediate NO-dependent relaxation of blood vessels?

Endothelial cell

L-NAME

NOS (inactive)

Ca\(^{2+}\)-calmodulin

↑[Ca\(^{2+}\)]_i

NOS (active)

L-Arginine

NO + Citrulline

Smooth muscle cell

NO

GC (basal) → GC (activ.)

cGMP

GTP

Relaxation
OPAL A mediates vasorelaxation in human umbilical vein by production of NO

No Alkalinisation of OPAL A (Bath pH = 7.6)

Alkalinised OPAL A (Bath pH adjusted to 7.4 to 7.8)

% KCl

Volume (ml)

Time control (water)  OPAL A + L-NAME  OPAL A
Endothelial cells remain intact in the bath, and OPAL A is non-toxic to the cells

Immunohistochemistry showing intact endothelium

vWF

Human umbilical vein (post-organ bath expt.)

MTT cell viability assay

\[ A_{(570-690)} \]

- Cell free control
- Water control
- OPAL A
Noradrenaline (NA) maintains basal vascular tone

Sympathetic varicosities

\( \alpha_1 \text{-Adr} \) → Contraction

Smooth muscle cell
OPAL A initially attenuates, then facilitates oxidation of NA
Oxidation of NA: requirement for $\text{O}_2$, metal ions and a protein

- OPAL A
- $\text{NaHCO}_3$
- $\text{NaHCO}_3 + \text{N}_2$
- OPAL A + $\text{N}_2$

$A_{(490)}$

Time (min)

$A_{(490)}$

Time (min)

$A_{(490)}$

Time (min)
Does OPAL A metabolise NA to reduce basal tone?
Isolated Rabbit Aorta: OPALA causes a small reduction in efficacy of NA. No change in potency.

Developed tension (% 60 mM KCl)

\[ pEC_{50} \ (n=6) \]
- NA + PBS: 6.55 ± 0.14
- NA + OPALA: 6.30 ± 0.12
Conclusions

- OPAL A provides effective wound resolution where hypoperfusion at the arteriolar level contributes to wound aetiology.

- OPAL A produced nitric oxide-dependent vasorelaxation in a human blood vessel preparation. Improved perfusion at the wound site may contribute to the observed clinical improvements.

- Whilst OPAL A appeared to oxidise NA, this caused only a small reduction in efficacy of NA, and no significant change in potency.
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