An Investigation into Multifaceted Mechanisms of Action of Allantoin in Wound Healing

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Conflict of Interest:
AP: Scioderm consultant, honoraria, investigator, grants.
RN: Consultant to Amicus. HD, AR, CV, JC, JB: Amicus employee
Background

• Allantoin is a heterocyclic organic compound produced by animals, bacteria, and plants
• While different allantoin-containing preparations have been used clinically to study its therapeutic effects in wound healing, its mechanisms of action are not known with certainty

Methods

• Systematic literature search for preclinical and clinical studies to further understand the mechanism of action (MOA) of allantoin in the context of wound healing
• The following search terms were used: wound, burn, scar, pruritus, anti-inflammatory, antioxidant, fibroblast, collagen, antifungal, antimicrobial, necrotic, and keratolytic
• ~100 preclinical studies (in vitro and animal models) and ~30 clinical studies were found focusing on MOA impacting the wound healing process
Anti-Inflammatory Effects

- Allantoin 5% emulsion resulted in fewer inflammatory cells in wounds compared with no treatment at all time points (Wistar rat model).
- Allantoin 1% treatment resulted in nearly complete inhibition of edema caused by chemical burn with phorbol myristate acetate (PMA) applied to mouse ear.

Number of Inflammatory Cells in a Rat Wound Model by Treatment

% Reduction of Edema Caused by PMA

\[ \text{Inhibition of Edema Caused by PMA} \]

\[ \text{96.11} \pm 5.41 \quad 68.4 \pm 7.04 \quad 92.15 \pm 0.32 \]

\[ \text{Allantoin (1\%)} \quad \text{α-Bisabolol (0.1\%)} \quad \text{18 β-glycyrrhetinic acid (1\%)} \]

\[ ^a P < 0.05 \text{ for emulsion containing 5\% allantoin compared with control group.} \]


Anti-Inflammatory Effects

• In cell cultures, allantoin 200 µM led to downregulation of maturation surface molecule CD40 and dendritic cell maturation marker CD83, and a slight increase in the secretion of tumor growth factor (TGF)-β.

Allantoin Reduced Expression of Pro-inflammatory Markers and Cytokines in Cell Culture Assays

Red Box: Control
Blue Box: Allantoin (200 µM) Treatment

CD40 Expression
CD83 Expression
IL-12 p70 Secretion
PBL Proliferation

Concentration (pg/mL)

% Proliferation

TGF-β, tumor growth factor beta; FACS, fluorescence-activated cell sorting; DC, dendritic cells; DC + B-met, DCs incubated with β-methasone (50 ng/mL); DC + All, DCs incubated with allantoin (200 µM); DC + Enox, DCs incubated with enoxolon (50 µm); DC + Bisa, DCs incubated with bisabolol (0.01%); DC + BZ4, DCs incubated with benzophenone-4 (0.01%); PHA, phytohemagglutinin; ELISA, enzyme-lined immunosorbent assay.

Keratolytic Activity

- In patients with psoriasis, 14-days open-label treatment with allantoin decreased disease manifestations including a reduction in hyperkeratosis.

### Effects of Allantoin (2% w/w) in Psoriasis

#### Example Study Results: Effect of Allantoin Preparation With Lanacolic Vehicle on Hyperkeratosis

<table>
<thead>
<tr>
<th>Degree(^a) of hyperkeratosis</th>
<th>+++</th>
<th>++</th>
<th>+</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients prior to therapy, n</td>
<td>9</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7 days post-treatment</td>
<td>3</td>
<td>7</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>14 days post-treatment</td>
<td>0</td>
<td>6</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

\(^a\)Degree expressed as: + + + (very expressed), + + (moderately expressed degree), + (poorly expressed), 0 (not expressed).

**Anti-microbial Effects**

- Allantoin exhibited antibacterial activity against many bacterial strains\(^1\) and exhibited antifungal and antiviral activity against several strains of microorganisms in vitro\(^2\)
- Allantoin in a solid lipid nanoparticle containing copaiba oil resulted in potent antifungal activity\(^3\)

### Antibacterial Activity Tested Against Standard and Isolated Strains of Microorganisms\(^1\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Escherichia coli MIC (µg/mL)</th>
<th>Pseudomonas aeruginosa MIC (µg/mL)</th>
<th>Proteus mirabilis MIC (µg/mL)</th>
<th>Klebsiella pneumoniae MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allantoin</td>
<td>ATCC 35218 Isolated strain ESβL+</td>
<td>ATCC 10145 Isolated strain</td>
<td>ATCC 7002 Isolated strain ESβL+</td>
<td>RSKK 574 Isolated strain ESβL+</td>
</tr>
<tr>
<td>Allantoin</td>
<td>8</td>
<td>4</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Ampicillin (reference)</td>
<td>2</td>
<td>&gt;128</td>
<td>2</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Ofloxacin (reference)</td>
<td>0.12</td>
<td>0.5</td>
<td>&lt;0.12</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>Acinetobacter baumannii MIC (µg/mL)</th>
<th>Staphylococcus aureus MIC (µg/mL)</th>
<th>Enterococcus faecalis MIC (µg/mL)</th>
<th>Bacillus subtilis MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allantoin</td>
<td>RSKK 02026 Isolated strain</td>
<td>ATCC 25923 Isolated strain MRSA</td>
<td>ATCC 29212 Isolated strain</td>
<td>ATCC 6633 Isolated strain</td>
</tr>
<tr>
<td>Allantoin</td>
<td>2</td>
<td>16</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Ampicillin (reference)</td>
<td>2</td>
<td>&gt;128</td>
<td>0.5</td>
<td>0.12</td>
</tr>
<tr>
<td>Ofloxacin (reference)</td>
<td>0.12</td>
<td>64</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

MIC, minimum inhibitory concentration.

Fibroblasts

- Allantoin and crude extracts of allantoin-containing *Arrabidaea chica* added to human fibroblast cell cultures demonstrated a concentration-dependent increase in fibroblast proliferation
- Allantoin and *A. chica* extract significantly improved surgically induced wound healing in Wistar rats by days 5 and 10 compared with saline control

**Concentration-Dependent Fibroblast Growth Percentage Induced by Allantoin and A. chica Extract**

**Wound Healing Observed as Percent of Contraction for Each Treatment Group**

![Graph showing concentration-dependent fibroblast growth percentage induced by Allantoin and A. chica extract.](image)

![Bar chart showing wound healing observed as percent of contraction for each treatment group.](image)

*Different from saline; †different from allantoin (100 mg/mL). *P*<0.001 †P*<0.01.

Collagen Synthesis

- Allantoin and *A. chica* extract improved collagen synthesis in the wounds of Wistar rats analyzed 11 days after injury.
- Allantoin 5% emulsion and the emulsion alone increased collagen stimulation compared with control (without treatment) at all times.

**Effects of Allantoin and A. chica Extract on Collagen Synthesis in Wistar Rat Wounds**

- Hydroxyproline (µg/mL)
- Days From Wound Initiation

**Effects of Allantoin on Collagen Synthesis in Wistar Rat Wounds**

- Control (without treatment)
- Emulsion
- Emulsion with 5% allantoin

\(^{a}P<0.001\) compared with fibroblast control group.


• Studies used different formulations of allantoin and there was a lack of information on the stability or dermal penetration of allantoin
• Additional research is warranted to further characterize clinically relevant mechanisms of action in human skin and to determine the relative role of these various mechanisms in wound healing