GLYCOTECHNOLOGY

In veterinary dermatology

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Glycotechnology

What the heck is it?

- The use of **sugars**
- Exclusive new components
- In Virbac brand of shampoos & ear cleanser.
- **science** of formulation and innovation

Glyco... techno... logy!
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What the heck is it?

- Exploration & exploitation of cutting-edge knowledge
- New method for the management of
  - infections
  - inflammatory diseases
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Sugars ??

Sugar cube

Metabolic

Cell surface landscape

Sugar moieties

Lipid bilayer

Proteins

Structural

Virbac
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Body language

- Cells appear to one another as “sugar-coated”
- Cells must “talk” to each other
- Their language is written in sugar units on cell surface, = “keys” unlocking functions

.desktop-Sugars are the molecules most widely used in cell-to-cell communication
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Sugars & cell-cell interaction

**Contact**
*eg microbial adhesion*

**Distance**
*eg inflammation cascade*
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Exogenous sugars allow to fool the process!

Exogenous sugars

Contact

eg微生物黏附

Distance

eg炎症级联反应

细胞间相互作用

eg微生物黏附

细胞间彼此距离

eg炎症级联反应
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Applications

2-fold

Help fight microbial invasion

- by blocking microbe adherence on host skin

Help reduce inflammatory response to allergen challenge

- by downregulating cell-to-cell alarm cascade in skin
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Inhibition of microbial adherence

How and why microbes stick on skin surface

- Sugar & sugar receptors (lectins) = vital components of cell membranes of both microbes and host cells

- Microbial lectins recognise specific sugars such as D-galactose, D-mannose, exposed on host skin cell membranes in the form of glycoproteins, glycolipids or polysaccharides

- “Anchorage system” for the microbe allowing it to adhere and colonise (virulence factors)
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How microbes stick on skin surface

Staphylococcus adherence on keratinocyte (Scanning electron microscopy)

Staphylococcus adherence on hair (Scanning electron microscopy)

Staphylococcus

Keratinocyte

Host sugar receptor

Lectin
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Microbial proliferation & infections

Tail fold pyoderma (*Pseudomonas aeruginosa*)

Pododermatitis & GS pyoderma (*Staphylococcus intermedius*)

Otitis (*Pseudomonas aeruginosa*)

*Malassezia* dermatitis
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Inhibition of microbial adherence

How to prevent microbes from sticking

- The specific sugars involved in adhesion of bacteria to a number of epithelial tissues were identified some time ago.
- It is possible to block microbial lectin by using the right exogenous sugars.
- Specific sugars (oligo/polysaccharides) effective.
- Interaction between microbes and host cells is multivalent, > need several blocking sugars.
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Inhibition of microbial adherence

How to prevent microbes from sticking

- 3 simple sugars = monosaccharides (MRG)
  - D Mannose
  - L Rhamnose
  - D Galactose

- 1 complex sugar = polysaccharide (APG)
  - Alkyl PolyGlucoside
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Inhibition of microbial adherence

MRG complex against *Pseudomonas aeruginosa*

- *Pseudomonas* (infectious agent)
- Bacterial adhesin (lectin)
- Exogenous Mannose, Galactose, Rhamnose (MRG)
- Glycoproteins
- Corneocyte surface (host skin)
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Inhibition of microbial adherence

APG against *Staphylococcus intermedius*

![Diagram showing inhibition of microbial adherence](image.png)
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Inhibition of microbial adherence

Proofs

- Developed in collaboration with N.A. McEwan & T. Nuttall, senior lecturers in dermatology, Department of Veterinary Science, the University of Liverpool, UK

- Validated in vitro model of bacterial adherence on canine corneocytes (flattened cells of the superficial horny layer)

- Infectious bacterial & yeast strains from clinical cases
Wash and stain

Collect 10 images

Image analysis
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Inhibition of microbial adherence

Proofs

- The 3 monosaccharides MRG decrease *Pseudomonas* adherence to canine corneocytes by 53% (McEwan et al, NAVDF Sarasota, 2005)

![Images showing corneocytes and inhibition effects after incubation with control buffer solution (PBS) and anti-adhesive sugars.](image)
Inhibition of microbial adherence

Proofs

Percentage of *Pseudomonas* adherence

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 sugars</td>
<td>46.6</td>
</tr>
<tr>
<td>L-rhamnose</td>
<td>72.6</td>
</tr>
<tr>
<td>D-galactose</td>
<td>79.2</td>
</tr>
<tr>
<td>D-mannose</td>
<td>80.9</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
</tr>
</tbody>
</table>

(McEwan et al, NAVDF Sarasota, 2005)
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Inhibition of microbial adherence

Proofs

- The polysaccharide APG decreases *Staphylococcus intermedius* adherence to canine corneocytes by 48% (McEwan et al, ESVD Lisbon, 2006)

![Image of corneocyte and cocci](image_url)

**After incubation with control buffer solution (PBS)**

**After incubation with APG**
Inhibition of microbial adherence

**Glycotechnology**

Proofs

<table>
<thead>
<tr>
<th>Percentage of <em>Staphylococcus</em> adherence</th>
</tr>
</thead>
<tbody>
<tr>
<td>APG</td>
</tr>
<tr>
<td>FOS</td>
</tr>
<tr>
<td>Control</td>
</tr>
</tbody>
</table>

(McEwan et al, ESVD Lisbon, 2006)
The polysaccharide APG decreases Malassezia pachydermatis adherence to canine corneocytes by >50% (McEwan et al, NAVDF Hawaii, 2007)

Proofs

- The polysaccharide APG decreases Malassezia pachydermatis adherence to canine corneocytes by >50% (McEwan et al, NAVDF Hawaii, 2007)

After incubation with control buffer solution (PBS)

After incubation with APG

(G x 1000, immersion)
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Inhibition of microbial adherence

Proofs

(McEwan et al, NAVDF Hawaii, 2007)
Downregulation of inflammation

How sugars are involved in inflammation

- Distance communication between cells mediated by cytokines
- Some cytokines act as “alert signals” > produced by skin cells (keratinocytes) or cells of the immune system (leucocytes) in response to physical/chemical aggression (skin damage, allergen stimulation, ..)
- Called pro-inflammatory cytokines > initiate & perpetuate the cascade of inflammation in skin (leucocyte recruitment, ..)
- Cytokines recognise receptors on target cells
- Sugars are components of cytokine receptors on cells of the epidermis (keratinocytes) and immune cells (leucocytes)
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Cytokine based inflammation signaling cascade in skin

EXOGENOUS ALTERING FACTORS
- Ultraviolet radiations
- Cutaneous
- Pathogen
- Allergen
- Humidity
- Irritants
- Etc.

ENDOGENOUS ALTERING FACTORS
- Etc
- Allergen
- Inflammatory factors (IFN-?, etc.)
- Alteration in anti-microbial film secretion or composition

Epidermal barrier function alteration
Keratinocytes

CYTOKINES PRODUCTION

INFLAMMATORY RESPONSE
- Neutrophils
- Lymphocytes
- Mastocytes

Staphylococcus intermedius
Malassezia pachydermatis
MICROBIAL PROLIFERATION

Inflammatory infiltrate
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Downregulation of inflammation

How to reduce inflammation signals with sugars

- Masking of the cell membrane antigen / receptor by exogenous sugars can interfere with signal induction

- In humans, monosaccharides shown to inhibit the secretion of pro-inflammatory cytokines by activated keratinocytes and suppress manifestations of cellular immunity in vivo > used in topical products for atopic/reactive skin patients
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Downregulation of inflammation

Diagram showing the interaction of a cytokine with a receptor binding domain, resulting in the binding of exogenous sugar to the receptor protein. This interaction leads to a decrease in immune signal production, indicating downregulation of inflammation.
Downregulation of inflammation

Proofs

- Developed in collaboration with the team of P. Bourdeau, senior lecturer in dermatology, Unit of Parasitology, Dermatology & Mycology, National Veterinary School, Nantes, Fr

- Canine keratinocytes cultures

- When stimulated by bacterial extracts > release TNF$_\alpha$ (pro-inflammatory cytokine) (Cadiot et al, 4th WCVD San Francisco, 2000)
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Downregulation of inflammation

Proofs

- Rhamnose is as effective as dexamethasone in reducing pro-inflammatory cytokine production by activated canine keratinocytes (Ibisch et al, *Veterinary Research Communications*, 2006)
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So what?

- In almost every skin disease we need somehow:
  - to control microbial proliferation
  - to combat inflammation

- Glycotechnology offers a new complementary way of doing that
  - The combination of 3 monosaccharides (RMG) and 1 polysaccharide (APG) proved effective
  - Included in topical products of the derm range
  - Act in synergy with existing actives
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SKIN DISEASES

MICROBIAL PROLIFERATION

INFLAMMATION

USUAL CONTROL METHODS

ANTIBIOTICS, ANTISEPTICS

STEROIDS

DRAWBACKS

ANTIBIOTIC RESISTANCE

DISTURBANCE OF CUTANEOUS MICROFLORA

IMMUNODEPRESSION

DETREMENTAL MICROBIAL PROLIFERATION

PROMISING NEW APPROACH

GLYCOTECHNOLOGY
International patent pending no. WO2006106220

The use of the specific sugar combination

1. to inhibit microbial pathogen adhesion (*Pseudomonas*, *Staph* and *Malassezia*) on the skin surface of warm-blooded animals

2. to exert immunomodulating effects in skin

Under free and/or encapsulated forms (*Spherulites®*)

*Spherulites®* promote a long-lasting effect and skin penetration

Applications:

- Prevention of external otitis
- Allergic skin conditions
- Keratoseborrheic disorders
- Skin infections
1 further example of Virbac approach to develop topicals

- Formula based on updated scientific knowledge
- Ingredients selection based on results of validation studies
- New antimicrobial and anti-inflammatory solutions
- Products adapted to practitioner needs

Vets have again the unique privilege of prescribing the most advanced derm products!