Monosaccharide inhibition of adherence by *Pseudomonas aeruginosa* to canine corneocytes

Neil A. McEwan*, Christophe A. Rème†, Hugo Gatto† and Timothy J. Nuttall*

*Faculty of Veterinary Science, University of Liverpool, Liverpool, UK
†Medical Department, Virbac SA, Carros, France
Correspondence: Dr Neil McEwan, Faculty of Veterinary Science, Small Animal Teaching Hospital, The University of Liverpool, Leahurst, Chester High Road, Neston, Wirral CH64 7TE, UK.
E-mail: n.a.mcewan@liverpool.ac.uk

**What is known about the topic of this paper**
- Antibiotic resistance by *Pseudomonas aeruginosa* is a recognized and growing problem.
- Microbial adhesion is an acknowledged important process in colonization and infection.
- *P. aeruginosa* is known to bind to carbohydrate molecules.

**What this paper adds to the field of veterinary dermatology**
- This is the first study to demonstrate blocking of adhesion by *P. aeruginosa* to canine corneocytes.
- Monosaccharides have potential for the treatment of *Pseudomonas* infections.

**Abstract**

The effect of D-galactose, D-mannose, L-rhamnose and dextrose on the adherence to canine corneocytes by three strains of *Pseudomonas aeruginosa* was studied in six healthy dogs. Canine corneocytes were collected from the inner aspect of the pinna using adhesive discs (D-Squame®). Half millimetre of bacterial suspension in phosphate-buffered saline (PBS) with or without the addition of a monosaccharide was placed over the corneocyte layer and incubated in moist chambers. Image analysis was used to quantify bacterial adherence to corneocytes. The three strains of *Pseudomonas* adhered well to canine corneocytes. All monosaccharides tested inhibited the adherence of *Pseudomonas* to canine corneocytes. The mean reduction in adherence for individual sugars at a concentration of 0.1% was 40.2% (dextrose), 30.8% (D-mannose), 25.6% (D-galactose) and 19.4% (L-rhamnose). When D-galactose, D-mannose and L-rhamnose were used in combination at 0.1% concentration, the mean reduction in adherence was 52.9%. The monosaccharides studied may have a potential role in the management of *Pseudomonas* infections in dogs.

Accepted 22 April 2008

**Sources of Funding**
This study was supported by a research grant from Virbac SA.

**Conflict of Interest**
No conflict of interest declared.

**Introduction**

Adherence is an established prerequisite for microbial colonization and subsequent invasion.1,2 *Pseudomonas aeruginosa* is known to adhere to various epithelial surfaces using lectins as adhesins.3 The receptors for these lectins include simple sugars.3-6 Because of the growing concern of the development of widespread and multiple antibiotic resistances6 by *P. aeruginosa*, novel therapies that do not depend on antibiotics, such as inhibition of microbial adhesion, would be a welcome addition to the armoury for the treatment of *Pseudomonas* infections. The aim of this study was to determine the antiadhesive properties of three monosaccharides (D-galactose, D-mannose and L-rhamnose) by three strains of *Pseudomonas* to canine corneocytes. Dextrose was included in the study as a control monosaccharide.

**Materials and methods**

Six healthy dogs, belonging to staff at the University of Liverpool, were used in the study. None of the dogs had evidence of skin disease on clinical examination or a history of skin disease. The animals had not received either systemic or topical treatments (including shampoos) for at least 3 weeks prior to collection of corneocytes. The dogs had a mean age of 4.7 years with a range of 2-8 years. There were three neutered females, two neutered males and one entire male. Two dogs were cross-breeds and the remaining four dogs were: German shepherd dog, Border collie, Cairn terrier and greyhound.

Three strains of *P. aeruginosa*, identified by conventional microbiological techniques and isolated from samples obtained from clinical cases of canine otitis, were used. Bacteria were cultured on sheep blood agar, subcultured into liquid medium (Oxoid nutrient broth no. 2, Unipath Ltd, Basingstoke, Hampshire, England) and frozen at −70 °C in 1 mL aliquots. When required, a frozen aliquot of bacteria was thawed and plated on sheep blood agar and incubated at 38 °C for 24 h. Colonies were harvested and the bacteria were washed twice in phosphate-buffered saline (PBS) by centrifugation with the...
The study of individual sugars used at concentrations of 0.05% and 0.1% on adherence by three strains of *Pseudomonas aeruginosa* to canine corneocytes

<table>
<thead>
<tr>
<th>Monosaccharides</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Values for all dogs (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>63.6</td>
<td>46.7</td>
<td>37.9</td>
<td>51.2</td>
<td>68.6</td>
<td>72.8</td>
<td>56.8 13.7 43.2</td>
</tr>
<tr>
<td>D-galactose</td>
<td>58.9</td>
<td>55.9</td>
<td>55.1</td>
<td>58.2</td>
<td>42.6</td>
<td>88.0</td>
<td>59.8 15.0 40.2</td>
</tr>
<tr>
<td>D-mannose</td>
<td>76.7</td>
<td>62.0</td>
<td>99.8</td>
<td>83.1</td>
<td>78.0</td>
<td>104.6</td>
<td>84.0 15.8 16.0</td>
</tr>
<tr>
<td>L-rhamnose</td>
<td>42.5</td>
<td>71.5</td>
<td>95.7</td>
<td>75.8</td>
<td>88.3</td>
<td>72.9</td>
<td>74.5 18.3 25.6</td>
</tr>
<tr>
<td>0.10%</td>
<td>79.3</td>
<td>73.5</td>
<td>88.1</td>
<td>81.1</td>
<td>87.5</td>
<td>97.5</td>
<td>81.2 13.4 18.8</td>
</tr>
<tr>
<td>0.10%</td>
<td>40.5</td>
<td>84.7</td>
<td>87.5</td>
<td>100.6</td>
<td>85.4</td>
<td>85.0</td>
<td>80.6 20.5 19.4</td>
</tr>
<tr>
<td>L-rhamnose</td>
<td>0.10%</td>
<td>78.4</td>
<td>74.5</td>
<td>78.3</td>
<td>88.9</td>
<td>89.6</td>
<td>48.5 52.4 24.0</td>
</tr>
<tr>
<td></td>
<td>48.6</td>
<td>70.9</td>
<td>71.9</td>
<td>68.1</td>
<td>68.5</td>
<td>87.1</td>
<td>69.2 12.3 30.8</td>
</tr>
</tbody>
</table>

Each cell represents the percentage of bacterial adherence compared to control (*Pseudomonas aeruginosa* added without sugar).

The effects of combined D-galactose, D-mannose and L-rhamnose used at concentration of 0.1% on adherence by three strains of *Pseudomonas aeruginosa* to canine corneocytes

<table>
<thead>
<tr>
<th>Strain of <em>Pseudomonas aeruginosa</em></th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Mean</th>
<th>SD</th>
<th>Mean reduction in adherence</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>28.3</td>
<td>37.0</td>
<td>73.3</td>
<td>28.3</td>
<td>37.0</td>
<td>73.3</td>
<td>46.2</td>
<td>21.3</td>
<td>53.8</td>
</tr>
<tr>
<td>P2</td>
<td>38.6</td>
<td>50.1</td>
<td>53.5</td>
<td>35.9</td>
<td>48.4</td>
<td>52.4</td>
<td>46.5</td>
<td>7.4</td>
<td>53.5</td>
</tr>
<tr>
<td>P3</td>
<td>56.3</td>
<td>40.1</td>
<td>50.8</td>
<td>61.8</td>
<td>30.4</td>
<td>52.3</td>
<td>48.6</td>
<td>11.5</td>
<td>51.4</td>
</tr>
<tr>
<td>Combined results</td>
<td>47.1</td>
<td>13.8</td>
<td>52.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each cell represents the percentage of bacterial adherence compared to control (*Pseudomonas aeruginosa* added without sugar).

The resulting suspension adjusted to an optical density of approximately 0.15 at 570 nm (OD570).

A modification of an adhesion assay previously developed and validated was used to quantify adhesion by *Pseudomonas aeruginosa* to corneocytes. Prior to the study, repeatability of the counting method and the optimal bacterial concentration was determined. Briefly, corneocytes were collected from the inner aspect of the pinna by using a 22-mm-diameter adhesive disc (D-Squame®, CuDerm Corporation, Dallas, TX, USA). Prior to sampling, surface debris was removed from the collection site by applying five successive adhesive tape strips (Sellotape® Original, Henkel Consumer Adhesives, Winsford, Cheshire, UK). The adhesive disc was placed over the corneocyte layer and incubated for 45 min in moist chambers. All monosaccharides were required from the same animal, discs were applied sequentially and the counting process.

In the first part of the study, three *P. aeruginosa* strains were each tested in two dogs at sugar concentrations of 0.05% and 0.1%. In the second part of the study, a combination of D-galactose, D-mannose and L-rhamnose in 0.1% solution was tested in the same six dogs using the same three strains of *P. aeruginosa*. Bacteria incubated with PBS without sugar was used as a positive control and PBS alone was used as a negative control. Dextrose was included as a control monosaccharide. The antiadhesive effect of the monosaccharide under test was calculated as a percentage of the adherence shown by the positive control.

Statistical analysis

Data were tested for normality before statistical analysis. One-way analysis of variance (ANOVA) with Tukey post-test was used to analyse the appropriate data. Significance was set at *P* < 0.05. All analyses were performed using Graphpad Prism version 4.00 (Graphpad Inc., San Diego, CA, USA [www.graphpad.com]).

Results

All three test sugars and dextrose inhibited adherence by *Pseudomonas aeruginosa* to corneocytes. The mean adherence compared to the positive control for individual sugars at 0.05% was 56.8% (dextrose), 76.0% (L-rhamnose), 81.2% (D-mannose) and 94.0% (D-galactose). Incubation with dextrose significantly reduced adherence compared to D-galactose and D-mannose (Tukey post-test, *P* < 0.05). The mean adherence using individual sugars at 0.1% was 59.8% (dextrose), 69.2% (L-rhamnose), 74.5% (D-galactose) and 80.6% (D-mannose). There were no significant differences among the sugars at this concentration. When the three sugars were used in combination at 0.1% concentration, the mean adherence was 47.1%, which gave a reduction in adherence of 52.9%. The mixture of the three sugars gave a significantly lower adherence compared to D-galactose and D-mannose (Tukey post-test, *P* < 0.01), L-rhamnose (Tukey post-test, *P* < 0.05) but not dextrose. Tables 1 and 2 and Figs 1 and 2 summarize all data.

Discussion

All three strains of *P. aeruginosa* were shown to adhere strongly to canine corneocytes which supports a similar finding by Forsythe *et al.* This study is the first to document sugar inhibition of adherence by *P. aeruginosa* to...
Sugars have been known to have the potential to block bacterial adhesion to animal cells for over two decades and a major goal of many current studies has been the inhibition of bacterial adherence. Few studies have been conducted in the veterinary field. One study demonstrated that mannose and N-acetyl-D-galactosamine played a role in inhibiting the adhesion of *Streptococcus zooepidemicus*, *P. aeruginosa* and *Escherichia coli* to equine endometrial epithelial cells. Other studies have shown that *Pseudomonas* adherence to various substrates could be blocked by the following sugars: D-galactose (human buccal epithelial cells),10 D-galactose and D-mannose (collagen type I molecules),11 D-galactose (erythrocytes)12 and D-galactose (rodent tracheal epithelial cells).13 In a human study of *P. aeruginosa* otitis, patients treated with a combination of galactose, mannose and N-acetyleneuraminic acid recovered more rapidly than a control group. As bacteria typically employ several different adhesins, it has been proposed that using mixtures of sugars which block more than one bacterial adhesin are likely to be more effective than using a single sugar. Results of our study support this hypothesis.

*Pseudomonas aeruginosa* produces a number of adhesive molecules, including sialic acid-binding, ganglioside-binding and hydrophobic adhesins. Lectins are commonly used by bacteria as adhesins and are proteins capable of binding saccharide structures with high specificity and affinity (reviewed in14–16). Two soluble lectins, PA-IL17 and PA-III18 have been identified from *P. aeruginosa* and these adhesive molecules are considered to be virulence factors playing important roles in human infection and tissue damage.19 Sugar inhibition experiments demonstrate that D-galactose and L-fucose inhibit both lectins,21 although clear preferences of D-galactose for PA-IL and of L-fucose for PA-III exist. The PA-III lectin will also recognize other monosaccharides including D-mannose.22 The ability of D-galactose and D-mannose to inhibit *P. aeruginosa* adherence in this study may be due to blocking of the PA-IL and PA-III lectins. L-rhamnose can inhibit bacterial adherence23 but no studies have been specifically conducted for *P. aeruginosa*. L-rhamnose is chemically similar to L-fucose both being deoxy sugars which may suggest that L-rhamnose may also block the PA-III lectin.

Microbial adherence can be thought of a multistep process with initial nonspecific or reversible binding followed by specific and irreversible binding. Biofilms, produced by some bacteria including *P. aeruginosa*, may also contribute to the adhesive process. EPS (extracellular polymer substances) is used as a collective term for the sugar components of microbial biofilms. Monosaccharides, including those used in this study, often form part of microbial biofilms. Competitive inhibition of the PA-IL lectin results in reduced biofilm production.24 Although biofilm development is typically a later phenomenon, it is conceivable that the inhibitory effects of the sugars in this study are related to biofilm activity. Lastly, sugars may influence adhesion by binding to keratinocytes. Human keratinocytes are known to have specific sugar receptors on their cell surface25 some of which recognize L-fucose and L-rhamnose.

**Figure 1.** The effect of monosaccharides on adhesion by *Pseudomonas aeruginosa* to canine corneocytes. All sugars at 0.1% concentration. Combined sugars consist of D-galactose, D-mannose and L-rhamnose. Bars show mean percentage adherence with standard error bars compared with control (bacteria without sugar added). *P < 0.01 compared to combined sugars.

© 2008 The Authors. Journal compilation © 2008 ESVD and ACVD.
McEwan et al.

Antiadhesive therapy of bacterial disease is an attractive prospect particularly as drug-resistant bacteria are increasing. As bacteria utilize several adhesins, treatments that target multiple adhesins are likely to be more efficient therapeutic agents.  

In conclusion, this study demonstrates that P. aeruginosa adheres strongly to canine corneocytes. The monosaccharides tested resulted in reduced adherence by P. aeruginosa to canine corneocytes. The combination of the three monosaccharides, D-galactose, D-mannose and L-rhamnose, was more effective than individual monosaccharides.

Acknowledgements

The authors would like to acknowledge the help and support freely given by staff of the Connective Tissue, and Microbiology and Infectious Disease Research Groups at The University of Liverpool Faculty of Veterinary Science.

References


Résumé Cette étude a évalué les effets du D-galactose, du D-mannose, du L-rhamnose et du dextrose sur l’adhésion aux cornéocytes canins de trois souches de Pseudomonas aeruginosa chez 6 chiens sains. Les cornéocytes ont été récoltés sur la face interne des pavillons auriculaires en utilisant des disques adhésifs (D-Square®). Un demi millimètre d’une suspension bactérienne dans du phosphate buffered saline (PBS) avec ou sans addition de monosaccharides a été placé sur la couche de cornéocytes et mis à l’incubation. Une analyse d’image a été réalisée pour quantifier l’adhésion bactérienne. Les trois souches de Pseudomonas adhéraient bien aux cornéocytes. Tous les monosaccharides testés ont inhibé l’adhésion de Pseudomonas aux cornéocytes. La réduction moyenne de l’adhésion pour une concentration de 0.1 % était de 40.2 % (dextrose), 30.8 % (L-rhamnose), 25.6 % (D-galactose), et 19.4 % (D-mannose). Lorsque le D-galactose, le D-mannose et le L-rhamnose ont été combinés à la concentration de 0.1 % la réduction moyenne d’adhésion était de 52.9 %. Les monosaccharides étudiés ici pourraient avoir un potentiel pour le traitement des infections à Pseudomonas chez le chien.
Resumen  Se estudiaron en seis perros sanos los efectos de la D-galactosa, D-manosa, L-ramnosa y dextrosa en la adherencia a corneocitos caninos de cepas de Pseudomonas aeruginosa. Los corneocitos se tomaron de la parte interna del oído externo usando discos adhesivos (D-Squame®). Se colocaron sobre los cultivos de corneocitos medio millilitro de suspensión bacteriana en solución tamponada de fosfatos con o sin la adición de monosacáridos y se incubaron en cámaras húmedas. Se utilizó análisis de imagen para cuantificar la adherencia bacteriana a los corneocitos. Las tres cepas de Pseudomonas se adhirieron bien a los corneocitos caninos. Todos los monosacáridos probados inhibieron la adherencia de Pseudomonas a los corneocitos. La reducción media de la adherencia para azúcares individuales a la concentración de 0.1% fue de 40.2% (dextrosa), 30.8% (L-ramnosa), 25.6% (D-galactosa) y 19.4% (D-manosa). Cuando se utilizaron en combinación al 0.1% D-galactosa, D-manosa y L-ramnosa la reducción en la adherencia fue del 52.9%. Los monosacáridos estudiados pueden tener potencial en el control de la infección por Pseudomonas en perros.

Zusammenfassung  Die Wirkung von D-Galaktose, D-Mannose, L-Rhamnose und Dextrose auf die Adhäsion von drei Pseudomonas aeruginosa Stämmen an canine Corneozyten wurde bei sechs gesunden Hunden untersucht. Canine Corneozyten wurden von der Innenseite der Ohrenschel mit Klebe-Disks (D-Squame®) entnommen. Ein halber Millimeter bakterieller Suspension in Phosphat buffer (PBS) wurde mit oder ohne die Zugabe eines Monosaccharids über eine Lage von Corneozyten gegeben und diese in feuchten Kammern inkubiert. Die Bildanalyse wurde verwendet, um die bakterielle Anhaftung an den Corneozyten quantitativ zu bestimmen. Die drei Pseudomonas Stämme hafteten gut an den caninen Corneozyten. Alle getesteten Monosaccharide verhinderten die Anhaftung der Pseudomonas an die caninen Corneozyten. Die durchschnittliche Vermindeung der Adhäsion für die einzelnen Zucker in einer Konzentration von 0.1% lag bei 40.2% (Dextrose), bei 30.8% (L-Rhamnose), bei 25.6% (D-Galaktose) und bei 19.4% (D-Mannose). Wenn D-Galaktose, D-Mannose und L-Rhamnose in einer Konzentration von 0.1% kombiniert wurden, lag die durchschnittliche Verminderung der Adhäsion bei 52.9%. Die untersuchten Monosaccharide könnten eine mögliche Rolle beim Management von Pseudomonas Infektionen des Hundes spielen.