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Local imipenem activity against decreases in vivo in the presence of siliconized latex

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Title: Local imipenem activity against *Pseudomonas aeruginosa* decreases in vivo in the presence of siliconized latex.

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Abstract: Zinc eluted from siliconized latex (SL) increases resistance of *Pseudomonas aeruginosa* to imipenem in vitro. A foreign body peritonitis model was used to evaluate the activity of imipenem using SL or silicone (S) implants. No differences were observed in mortality, positive blood cultures and tissue bacterial counts between SL and S implants. Implant-associated counts, however, were significantly higher in the SL group. It is concluded that SL decreases the activity of imipenem against *P. aeruginosa*.

September 22, 2010

Dr. Alex van Belkum
Editor-in-Chief
European Journal of Clinical Microbiology & Infectious Diseases

Dear Dr. van Belkum,

Please find enclosed the revised version of the manuscript entitled “Local imipenem activity against *Pseudomonas aeruginosa* decreases in vivo in the presence of siliconized latex” (Ref.: Ms. No. EJCMIID-D-10-00315R1). Following your suggestion we have introduced the changes suggested by reviewer # 1. These changes are detailed below:

Reviewer #1: The revised manuscript addresses the issue raised.

One clarification should be made in the text as well. The authors should clearly state that the experiment was repeated 3 times with 5 animal per group and not just state that 15 animals were studied.

As suggested by reviewer 1, the following paragraph has been added in the revised manuscript in order to clarify it (Pg 4 line 74).

“The *in vivo* studies were performed three times using 5 mice per assay for a total of 15 mice per group. Also, the experiments were performed on different days, thus avoiding bias that might vary from one day to another. The size of the groups (n=15) are sufficient for detecting a statistical difference, between the groups, of 1.5 cfu/g, with an alpha error of 0.05 and a beta error of 0.20. Animals were randomized into four groups on the basis of implant type and the treatment received for 72 h”

Pg 3 line 35-the author should state that this was In-Vitro data (ref 1)

Following the suggestion of reviewer 1, this comment has been included in the new version of the manuscript (Pg 3 line 33).

“In the *in vitro* study, we have observed that eluates from SL urinary catheters decrease the activity of carbapenems against *P. aeruginosa* [1]”

Yours sincerely,

Cristina Pichardo

1 TITLE:

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2 2 Local imipenem activity against *Pseudomonas aeruginosa* decreases in vivo in the presence of siliconized
3 latex.
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7 5 Pichardo C¹, Conejo MC², Docobo-Pérez F¹, Velasco C², López-Rojas R¹, García I², Pachón-Ibáñez
8 ME¹, Rodríguez JM², Pachón J¹, Pascual A².
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11 7**14 FROM:**

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27 17

28 18 **RUNNING TITLE:** Experimental foreign body peritonitis by *P. aeruginosa*
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32 20 **KEY WORDS:** *Pseudomonas aeruginosa*, zinc, latex, experimental foreign body peritonitis model.
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23 **ABSTRACT**

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25 Zinc eluted from siliconized latex (SL) increases resistance of *Pseudomonas aeruginosa* to imipenem in
26 vitro. A foreign body peritonitis model was used to evaluate the activity of imipenem using SL or silicone
27 (S) implants. No differences were observed in mortality, positive blood cultures and tissue bacterial
28 counts between SL and S implants. Implant-associated counts, however, were significantly higher in the
29 SL group. It is concluded that SL decreases the activity of imipenem against *P. aeruginosa*.

30

31 **Introduction**

32

33 *Pseudomonas aeruginosa* is a significant cause of urinary tract infections in patients with urinary
34 catheters. Siliconized latex (SL) is the biomaterial most commonly used to make urinary catheters. In the
35 *in vitro* study, we have observed that eluates from SL urinary catheters decrease the activity of
36 carbapenems against *P. aeruginosa* [1]. The effect is related to changes in the outer membrane protein
37 profile of *P. aeruginosa* in the presence of zinc eluted from SL [2]. This reversible phenomenon is
38 mediated by the expression of the heavy metal efflux pump CzcCBA in the presence of high zinc
39 concentrations [3,4]. Since the clinical relevance of this phenomenon is unknown, an experimental model
40 of foreign body infection in mice was used to evaluate the treatment of *P. aeruginosa* infection with
41 imipenem in the presence of SL *versus* silicone (S) implants.

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43

44 **Material and methods**

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46 *Bacterial strain.*

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48 *P. aeruginosa* PAO1, for which the MIC of imipenem is 1 µg/ml, was used throughout the study
49 [1].

50

51 *Antimicrobial agents and Catheter.*

52

53 Imipenem (laboratory standard powder) and Imipenem plus cilastatin was obtained from Merck
54 and Sharp and Dohme (Madrid, Spain).

55 S catheters (10 argyle all silicone Foley catheter 8FR/CH; Sherwood Medical, Tullamore,
56 Ireland) and SL catheters (two-way pediatric silicone-coated latex Foley catheter 8FR/CH; Kendall Co.,
57 Kangar, Malaysia) with an internal diameter of 0.8 mm and an external diameter of 2.8 mm were cut into
58 segments of 10 mm and used as implants.

59

60 *Experimental animal model.*

61

62 Immunocompetent pathogen free C57BL/6 female mice weighing 14 to 16 g were use in our
63 model. Animals were housed in regulation cages and given free access to food and water. The studies
64 were approved by the Ethics Committee of the University Hospitals Virgen del Rocío (Seville, Spain).
65 Plasma levels of imipenem were determined after administration of a single intramuscular (im) dose of 60
66 mg/kg. Total plasmatic drug concentrations were measured using the bioassay method (*Micrococcus*
67 *luteus* ATCC 9341 as the indicator strain), pharmacokinetics and pharmacodinamics parameters were
68 determined.

69 The murine model of foreign-body peritonitis described by Espersen et al. was used [3].
70 Experimental infection was produced by intraperitoneal injection with 1 ml of bacterial suspension in
71 saline (10^6 - 10^7 CFU/ml) in the lateral abdominal wall opposite of the operation wound fifteen minutes
72 after insertion of the implant.

73 Preliminary experiments were carried out to evaluate survival of animals after placement of
74 catheter compared to animals that underwent the operation without catheter placement. In both groups the
75 survival rate was 100%.

76 The *in vivo* studies were performed three times using 5 mice per assay for a total of 15 mice per
77 group. Also, the experiments were performed on different days, thus avoiding bias that might vary from
78 one day to another. The size of the groups (n=15) are sufficient for detecting a statistical difference,
79 between the groups, of 1.5 cfu/g, with an alpha error of 0.05 and a beta error of 0.20. Animals were
80 randomized into four groups on the basis of implant type and the treatment received for 72 h: SL implant
81 not treated (control SL); S implant not treated (control S); SL implant imipenem treated (imipenem SL),
82 240 mg/kg/d, (four doses of 60 mg/kg) im; and S implant imipenem treated (imipenem S), 240 mg/kg/d,
83 (four doses of 60 mg/kg) im; the dosing regimen of imipenem chosen resulted in a $\Delta T/MIC$ for the PAO1
84 strain which was 48.8% of the dosing interval. The animals were observed for 72 h for mortality, and
85 surviving mice were killed 4 h after the last dose. All mice were analyzed immediately after death. Once
86 the animals were dead, the thorax and abdomen were opened. Blood was obtained by cardiac puncture
87 and qualitative culture was carried out by inoculating sterile tubes with 2 mL of MHB and incubating for

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88 24 h at 37°C. Then, 100 µl of each sample was plated on Columbia sheep-blood agar and incubated for 24
89 h at 37°C. The results of the blood cultures were expressed as positive or negative. On the other hand, the
90 peritoneal cavity was inspected, and the foreign body (catheter), the liver and the spleen, were aseptically
91 removed for culture. One ml of sterile saline was slowly flushed through the catheter to remove blood and
92 non-adherent organisms. Later, the catheter was placed in 1 ml of saline in a 2-ml tube. The tube was
93 sonicated for 8 min at 40-60 kHz (Ultrasons 513 water bath sonicator [P Selecta]) and quantitative
94 cultures of the saline were performed (catheter washings). The liver and the spleen were extracted and
95 separated onto a sterile Petri plate and weighed. The tissues were processed for quantitative culture, after
96 being homogenized in 2 ml of sterile saline solution (Stomacher 80 Tekmar CO., Cincinnati, OH, USA).
97 After ten-fold dilution, aliquots of 100 µL were plated onto Columbia sheep-blood agar and incubated for
98 24 h at 37°C; results were expressed as the mean ± SD of the log CFU/g of the tissues and the mean ± SD
99 of the log CFU/ml of catheter washings. Sterile cultures were considered to have ≤1 log CFU/ml of
100 catheter washings. Statistical comparisons between groups were made using the variance analysis
101 (ANOVA) and multiple comparisons post-hoc tests (Dunnnett or Tukey-Kramer). To compare the number
102 of survivors and the number of sterile tissues, the Fisher exact test was used. A value of p<0.05 was
103 considered significant.

104

105 **Results and Discussion**

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107 *Pharmacokinetics studies.*

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109 The intraday and interday variation of the assays were 2.62%±2.44% and 3.22%±1.91%; the
110 linearity (r^2) of the assay 0.91±0.02 and the lower limit of detection was 0.01 µg/ml. The maximum
111 plasma concentration (C_{max}) of imipenem and the terminal half-life ($t_{1/2}$), were 30.56 µg/ml and 0.5 h,
112 respectively. The time during which the plasma concentration remained above the MIC ($\Delta T/MIC$) was
113 2.93 h.

114

115 *Experimental murine foreign-body peritonitis.*

116

117 In the experimental model, the mortality in control groups was 80% (table 1). In imipenem-
118 treated groups the mortality was reduced to 0% and 6% for SL and S implants, respectively ($p < 0.01$). No
119 difference in mortality was observed among treated groups.

120 A significant decrease in bacterial counts obtained from livers, spleens and catheters was observed
121 in the imipenem-treated groups compared to control groups ($p < 0.01$). Moreover, catheter clearance was
122 greater in the imipenem S compared to the imipenem SL treated group ($p = 0.021$).

123 The percentage of positive blood cultures in the treated groups was lower than that of the
124 controls ($p < 0.01$), without differences between treated groups. However, there were no significant
125 differences in the percentage of positive cultures of peritoneal washings between control and treated
126 groups.

127 Our results show differences in the clearance of bacteria between SL and S catheter groups
128 treated with imipenem. The lower clearance obtained in the catheter culture from the infected animals
129 carrying SL catheters could be related to the loss of OprD2 induced by zinc, as has been demonstrated in
130 vitro [5]. However, there were no differences in the bacterial counts in the liver and the spleen, as well as
131 in the percentage of positive blood cultures, which suggests that this might be a local rather than a
132 systemic effect.

133 The clinical implications of these results are difficult to ascertain. Zn eluted from siliconized
134 latex favours the survival of *P. aeruginosa* at a local level. Whether or not this local phenomenon has an
135 effect on the prognosis of these infections in clinical practice remains to be demonstrated [6, 7]. However,
136 these data suggest that latex urinary catheters should not be used in humans. In our opinion, latex urinary
137 catheter should be changed to other materials that are more biocompatible, such as silicone. In fact, this
138 change has already been made in many hospitals [7]. However, siliconized latex urinary catheters are still
139 used in many countries because they are very cheap.

140 In summary, imipenem activity against *P. aeruginosa* might locally decrease in to the presence
141 of siliconized latex devices, with this effect probably due to the zinc eluted from the biomaterial.

142

143 **Acknowledgements**

144

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148 ERDF, Spanish Network for the Research in Infectious Diseases (REIPI RD06/0008).

149 There are no conflicts of interest regarding this work.

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178 Table 1: Effect of imipenem therapy on the clearance of *Pseudomonas aeruginosa* PAO1 from catheter, liver, spleen, blood and peritoneal fluid from mice implanted
179 with siliconized latex or silicone catheters.

Catheter/treatment	Inoculum Log CFU/ml	Liver Log CFU/g	Spleen Log CFU/g	Catheter Log CFU/ml	Percentage of positive blood culture	Percentage of positive peritoneal washing Culture	<i>Exitus</i>
SL Control	6.98±0.23	8.24±1.21	8.23±1.61	6.90±0.87	100%	100%	80%
S Control	6.98±0.23	8.52±0.88	8.24±1.63	7.33±0.46	100%	100%	80%
SL imipenem	6.89±0.35	3.04±0.80*	4.01±0.91*	3.82±2.02*	57.1%*	100%	0%*
S imipenem	6.98±0.23	3.11±1.03*	4.06±1.04*	2.27±1.71*#	53.3%*	93.3%	6%*

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182 * p<0.01 with respect to SL Control and S Control; #p=0.021 with respect to SL imipenem.

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