

Potential clinical significance of statins on methicillin resistance reversion in *Staphylococcus aureus*



Potencial significado clínico de las estatinas en la reversión de la resistencia a meticilina en *Staphylococcus aureus*

Dear Editor,

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of hospital-acquired and healthcare-associated infections worldwide.¹ MRSA bacteremia is associated with higher mortality than methicillin-susceptible *S. aureus* bacteremia.² For MRSA bacteremia, the IDSA guidelines recommend treatment with vancomycin or daptomycin.³ However, strains showing higher vancomycin MICs have been associated with increased risk of treatment failure and death,⁴ and the emergence of non-susceptible mutants with daptomycin treatment is concerning.⁵ In view of these therapeutic difficulties, one possibility is to search for adjuvant substances that allow the continued use of conventional antimicrobials. Some studies have found that statins treatment has a protective effect against both mortality and the development of

persistent bacteremia in patients with *S. aureus* bacteremia.^{6,7} Statins have no intrinsic bactericidal activity against *S. aureus*,⁸ but synergize with different antimicrobials to increase their bactericidal effect.⁹ In addition, it has been shown that several statins can reverse methicillin resistance in the community-associated MRSA strain USA300.⁸ Despite this phenomenon was confirmed in an experimental murine model,⁸ significant questions remain unresolved, and no studies have evaluated the impact of statins as a strategy to reverse methicillin resistance against clinical isolates of MRSA under human therapeutic concentrations. We tested statins against a set of clinical isolates of MRSA belonging to the most prevalent high-risk clones in human infections to address this question.

Nineteen MRSA isolates recovered from patients with nosocomial or healthcare-associated bacteremia were selected for this study. Isolates belonged to the following high-risk clones: ST5 (thirteen isolates), ST8 (four isolates), ST22 (one isolate) and ST45 (one isolate). Thirteen isolates belonged to *agr* group II and the remainder to *agr* group I. All isolates harboured *SCCmec* type IV, and none of the isolates carried Pantone-Valentine leukocidin genes.¹⁰ *S. aureus* ATCC 29213 and MRSA USA300 strains were included as controls.

Table 1

Oxacillin MIC of MRSA clinical isolates in the presence of the lactone form of simvastatin.

Strain	Sequence type	SSCmec type	Agr group	Oxacillin MIC (mg/L) ^a							
				400 μmol/L SIM ^b	200 μmol/L SIM	100 μmol/L SIM	50 μmol/L SIM	25 μmol/L SIM	12.5 μmol/L SIM	6.25 μmol/L SIM	0 μmol/L SIM
ATCC 29213	–	–	–	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
MRSA USA300	ST8	SSCmec IV	agrI	0.06	0.06	8	16	16	16	16	16
MRSA 14	ST5	SSCmec IV	agrII	2	4	8	64	64	64	64	64
MRSA 15	ST5	SSCmec IV	agrII	8	8	64	64	64	64	64	64
MRSA 16	ST5	SSCmec IV	agrII	2	2	4	8	32	32	32	32
MRSA 17	ST5	SSCmec IV	agrII	0.06	0.06	16	64	64	64	64	64
MRSA 18	ST5	SSCmec IV	agrII	0.06	0.06	32	32	32	32	32	32
MRSA 19	ST5	SSCmec IV	agrII	0.06	0.06	4	16	16	16	16	16
MRSA 20	ST5	SSCmec IV	agrII	0.06	0.06	8	64	64	64	64	64
MRSA 21	ST5	SSCmec IV	agrII	0.06	0.06	8	64	64	64	64	64
MRSA 22	ST5	SSCmec IV	agrII	0.06	0.06	4	32	32	32	32	32
MRSA 23	ST5	SSCmec IV	agrII	0.06	0.06	32	32	32	32	32	32
MRSA 24	ST5	SSCmec IV	agrII	0.06	0.06	16	16	16	16	16	16
MRSA 25	ST5	SSCmec IV	agrII	0.06	0.06	32	64	64	64	64	64
MRSA 26	ST5	SSCmec IV	agrII	0.06	0.06	16	64	64	64	64	64
MRSA 27	ST8	SSCmec IV	agrI	0.06	0.06	8	32	32	32	32	32
MRSA 28	ST8	SSCmec IV	agrI	0.06	0.06	32	32	32	32	32	32
MRSA 29	ST8	SSCmec IV	agrI	0.06	0.06	16	32	32	32	32	32
MRSA 30	ST8	SSCmec IV	agrI	0.06	0.06	0,06	64	64	64	64	64
MRSA 32	ST22	SSCmec IV	agrI	0.06	0.06	16	128	128	128	128	128
MRSA 33	ST45	SSCmec IV	agrI	0.06	0.06	4	16	32	32	32	32

^a According to EUCAST breakpoints, *S. aureus* isolates with oxacillin MIC values >2 mg/L are mostly methicillin-resistant due to the presence of the *mecA* or *mecC* genes.

^b SIM: lactone form of simvastatin (inactive form).

The active forms of five statins were tested: fluvastatin, rosuvastatin, atorvastatin, simvastatin and lovastatin (Axxora, Brussels, Belgium). In the case of simvastatin and lovastatin, the prodrug form (lactone) was also included (Axxora). Susceptibility to statins and oxacillin (Sigma-Aldrich, Madrid, Spain) was determined by broth microdilution. All isolates showed MIC values of all statins tested above 200 $\mu\text{mol/L}$ and oxacillin MICs $\geq 16 \text{ mg/L}$ (Table 1). Statin–oxacillin synergy was evaluated using the checkerboard method. None of the statins evaluated showed synergistic effects with oxacillin, except for the prodrug form of simvastatin. The lactone form of simvastatin reversed methicillin resistance (defined as the reduction of oxacillin MIC to $\leq 2 \text{ mg/L}$) in eighteen isolates (95%) at 400 $\mu\text{mol/L}$, and in seventeen isolates (89%) at 200 $\mu\text{mol/L}$. However, methicillin resistance reversion was not observed in any isolate at lower concentrations of simvastatin (Table 1). The MIC of the lactone form of simvastatin was $>800 \mu\text{mol/L}$, which means that this effect is observed at sub-MIC concentrations of simvastatin.

Several studies have shown the pleiotropic effects of statins, including bactericidal activity against gram-positive bacteria, such as *S. pneumoniae*, and reversion of methicillin resistance in *S. aureus*.^{8,11} These effects were detected at statin concentrations of 1–100 $\mu\text{mol/L}$. Nevertheless, the mean concentration of statins in human serum is only 1–15 nmol/L, and the peak concentration is 6–50 nmol/L, with this concentration being reached in serum for a very short period of time.¹²

According to our results, the inactive form of simvastatin is the only statin tested which is able to reverse methicillin resistance in *S. aureus* clinical isolates, although this effect was observed at sub-MIC simvastatin concentrations above 100 $\mu\text{mol/L}$. Moreover, this concentration of statin is much higher than that reached in serum with therapeutic doses, so that it cannot be used as a potential adjuvant to reverse methicillin resistance. Why this effect is not observed with the prodrug form of simvastatin remains unknown.

These results contrast with those previously found in a murine model of pulmonary infection.⁸ This discrepancy might be partially explained by the differences in dosage. In humans, the dosage of statins is approximately 0.1–1 mg/kg, while in the murine model, doses of 20 and 50 mg/kg were used. However, these doses were similar to those used in previous *in vitro* studies.¹¹ The metabolism, pharmacodynamics and action of statins differ in humans and mice so that the results observed in murine models should be taken with caution before being applied to humans.

This study shows that simvastatin maybe a potential strategy to reverse methicillin resistance in *S. aureus*, although, unfortunately, this effect is not observed at therapeutic human concentrations.

Funding

This work was supported by the Plan Nacional de I+D+i 2017–2020 and the Instituto de Salud Carlos III (project PI18/00715), Subdirección General de Redes y Centros de Investigación Cooperativa, Ministerio de Economía, Industria y Competitividad, the Spanish Network for Research in Infectious Diseases (REIPI; RD16/0016/0001 and REIPI RD16/0016/0009), co-financed by the European Development Regional Fund “A Way to Achieve Europe,” operative programme Intelligent Growth 2014–2020.

Conflicts of interest

None to declare.

Acknowledgement

The authors would like to thank Dr. Carmen Velasco for giving us the *S. aureus* clinical isolates used in this study.

Bibliografía

1. Limbago B, Fosheim GE, Schoonover V, Crane CE, Nadle J, Petit S, et al. Characterization of methicillin-resistant *Staphylococcus aureus* isolates collected in 2005 and 2006 from patients with invasive disease: a population-based analysis. *J Clin Microbiol*. 2009;47:1344–51.
2. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. *Clin Infect Dis*. 2003;36:53–9.
3. Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. *Clin Infect Dis*. 2011;52:e18–55.
4. Soriano A, Marco F, Martínez JA, Pisos E, Almela M, Dimova VP, et al. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *Clin Infect Dis*. 2008;46:193–200.
5. Sharma M, Riederer K, Chase P, Khatib R. High rate of decreasing daptomycin susceptibility during the treatment of persistent *Staphylococcus aureus* bacteremia. *Eur J Clin Microbiol Infect Dis*. 2008;27:433–7.
6. López-Cortés LE, Gálvez-Acebal J, Del Toro MD, Velasco C, de Cueto M, Caballero FJ, et al. Effect of statin therapy in the outcome of bloodstream infections due to *Staphylococcus aureus*: a prospective cohort study. *PLoS One*. 2013;8:e82958.
7. Smit J, López-Cortés LE, Thomsen RW, Schønheyder HC, Nielsen H, Frøsløv T, et al. Statin use and risk of community-acquired *Staphylococcus aureus* bacteremia: a population-based case–control study. *Mayo Clin Proc*. 2017;92:1469–78.
8. García-Fernández E, Koch G, Wagner RM, Fekete A, Stengel ST, Schneider J, et al. Membrane microdomain disassembly inhibits MRSA antibiotic resistance. *Cell*. 2017;171:1354–67, e20.
9. Pauchard L-A, Blot M, Bruyere R, Barbar S-D, Croisier D, Piroth L, et al. Linezolid and atorvastatin impact on pneumonia caused by *Staphylococcus aureus* in rabbits with or without mechanical ventilation. *PLoS One*. 2017;12:e0187187.
10. Velasco C, López-Cortés LE, Caballero FJ, Lepe JA, de Cueto M, Molina J, et al. Clinical and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* causing bacteraemia in Southern Spain. *J Hosp Infect*. 2012;81:257–63.
11. Bergman P, Linde C, Pütsep K, Pohanka A, Normark S, Henriques-Normark B, et al. Studies on the antibacterial effects of statins – *in vitro* and *in vivo*. *PLoS One*. 2011;6:e24394.
12. Keskitalo JE, Pasanen MK, Neuvonen PJ, Niemi M. Different effects of the ABCG2 c.421C>A SNP on the pharmacokinetics of fluvastatin, pravastatin and simvastatin. *Pharmacogenomics*. 2009;10:1617–24.

Jesús Machuca^{a,*}, María Carmen Conejo^b, Álvaro Pascual^{a,b,c,d}, José Manuel Rodríguez-Martínez^{b,c,d}

^a Unidad Clínica de Enfermedades Infecciosas, Microbiología y Medicina Preventiva, Hospital Universitario Virgen Macarena, Seville, Spain

^b Departamento de Microbiología, Facultad de Medicina, Universidad de Sevilla, Seville, Spain

^c Red Española de Investigación en Patología Infecciosa (REIPI), Instituto de Salud Carlos III, Madrid, Spain

^d Instituto de Biomedicina de Sevilla IBIS, Hospital Universitario Virgen Macarena/CSIC/Departamento de Microbiología, Universidad de Sevilla, Seville, Spain

* Corresponding author.

E-mail address: jesus.machuca.sspa@juntadeandalucia.es (J. Machuca).

<https://doi.org/10.1016/j.eimc.2021.10.001>

0213-005X/ © 2021 Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica. Published by Elsevier España, S.L.U. All rights reserved.