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Article:

Lee, VE orcid.org/0000-0002-0167-8775 and O'Neill, AJ orcid.org/0000-0003-3222-5493
(2017) Batumin does not exert its antistaphylococcal effect through inhibition of aminoacyl-tRNA synthetase enzymes. *International Journal of Antimicrobial Agents*, 49 (1). pp. 121-122. ISSN 0924-8579

<https://doi.org/10.1016/j.ijantimicag.2016.10.005>

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1 **Batumin does not exert its antistaphylococcal effect through inhibition of**
2 **aminoacyl-tRNA synthetase enzymes**

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Victoria E. Lee and Alex J. O'Neill*

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10 Antimicrobial Research Centre, School of Molecular and Cellular Biology, University
11 of Leeds, Leeds, United Kingdom.

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19 *Corresponding author. Antimicrobial Research Centre and School of Molecular and
20 Cellular Biology, University of Leeds, Leeds, LS2 9JT, United Kingdom Tel: +44 (0)113
21 3435600; Fax: +44-(0)113-343-5638; E-mail: a.j.oneill@leeds.ac.uk

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26 Sir,

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28 We write in response to an article regarding the antibiotic batumin by Klochko *et al.*
29 that appeared earlier this year in the journal [1]. The stated aim of this study was “to
30 identify possible molecular targets for batumin as well as mechanisms of its
31 antistaphylococcal activity”. Apparently on the basis that batumin and the clinically
32 deployed antibacterial drug mupirocin share a 9-hydroxynonanoic acid moiety, and
33 that the biosynthesis of both antibiotics is directed by operons that exhibit some
34 degree of sequence similarity, the authors formulated the hypothesis that batumin
35 shares the same molecular target as mupirocin: the isoleucyl-tRNA synthetase (IleRS)
36 enzyme that plays an essential role in protein synthesis. With a view to providing
37 support for this hypothesis, Klochko *et al.* undertook two *in silico* investigations. The
38 first of these involved molecular docking of batumin into X-ray crystal structures of
39 IleRS, the results of which implied that batumin and mupirocin might bind with
40 similar affinity to this enzyme. The second entailed analysis of the genome sequence
41 of a batumin producer organism (*P. batumici*), which led to the identification of
42 three genes encoding paralogues of leucyl-tRNA synthetase (LeuRS), and prompted
43 the authors to suggest that batumin might also/ predominantly target LeuRS.

44

45 Whilst *in silico* analyses such as these may have a place in generating or refining a
46 hypothesis as to the mode of action (MOA) of batumin, the results they provide are
47 predictions at best, and do not constitute evidence to support the hypothesis that
48 batumin acts by inhibiting aminoacyl-tRNA synthetase (aaRS) enzymes. In
49 consequence, and lacking a direct experiment to test their hypothesis, the study by
50 Klochko *et al.* did not progress beyond pure speculation. Though the authors made
51 mention of the fact that their ideas would ultimately require experimental
52 corroboration, this did not prevent them from presenting firm conclusions regarding
53 the MOA of batumin that went well beyond what their results could justify (e.g. “It
54 was found that batumin acted very similarly to mupirocin by inhibiting aminoacyl
55 tRNA synthetases.”). Here we present experimental evidence to show that their
56 conclusions regarding the MOA of batumin are in fact wrong.

57

58 Antibiotics that mediate their antibacterial effect through inhibition of aaRS enzymes
59 will act to rapidly deplete the bacterial cell of charged tRNA species, an early
60 consequence of which will be inhibition of protein synthesis. For example, when
61 mupirocin at 4XMIC is added to logarithmic phase cultures of *Staphylococcus aureus*
62 strain SH1000, a dramatic (~65%) reduction in protein synthesis is observed within
63 10 minutes relative to an untreated control, as determined [2] by measuring
64 incorporation of the radiolabeled amino acid L-[3,4-³H(N)]-glutamine into
65 polypeptides (Figure 1). By contrast, in an otherwise identical experiment using
66 batumin (Enzo Life Sciences, Exeter, UK) at 4XMIC in place of mupirocin, no
67 inhibition of protein synthesis was observed (Figure 1). It is therefore apparent that
68 the antibacterial action of batumin is distinct from that of mupirocin, and does not
69 involve inhibition of one or more aaRS enzymes.

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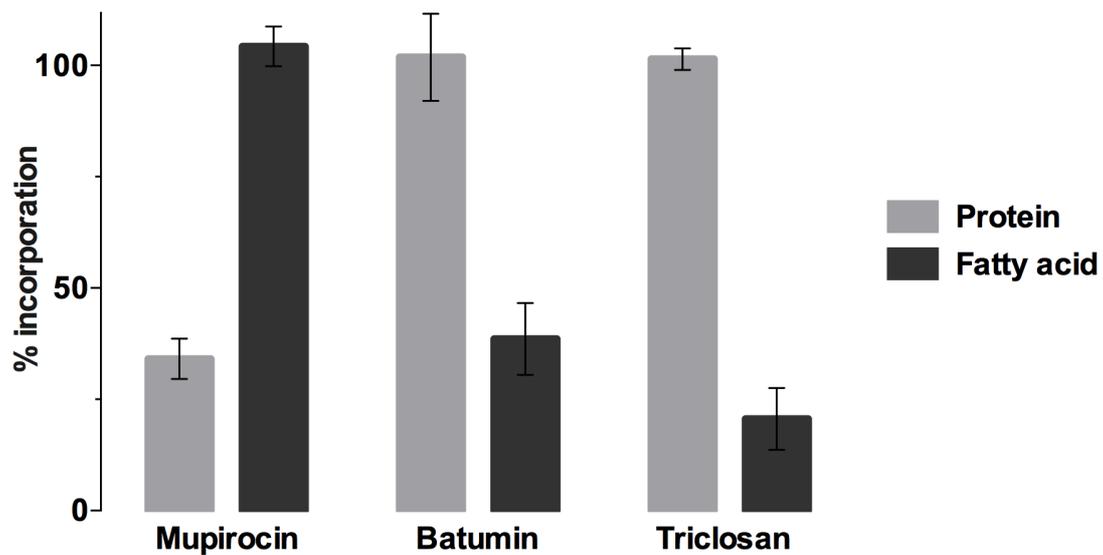
71 A prior hypothesis regarding the MOA of batumin considered that this compound
72 exerts its antibacterial effect through direct inhibition of fatty acid biosynthesis [3].
73 This proposal stemmed from the observation that an isoform (BatG) of the fatty acid
74 synthesis enzyme, FabI, is encoded within the batumin biosynthesis cluster in some
75 producer strains, and that heterologous expression of *batG* in *Escherichia coli* and *S.*
76 *aureus* confers a substantial reduction in batumin susceptibility [3]. With a view to
77 reconciling their speculations with these observations, Klochko *et al.* proposed that
78 both batumin and mupirocin mediate their antibacterial effect by indirectly
79 impairing fatty acid synthesis as a secondary consequence of inhibiting aaRS
80 enzymes and inducing the stringent response. We examined the effect of 4X MIC
81 batumin on fatty acid synthesis in *S. aureus* SH1000 by measuring incorporation of
82 the radiolabeled precursor, [1,2-¹⁴C]-acetic acid. Batumin and a known inhibitor of
83 this pathway, triclosan, both caused a dramatic reduction (>60%) in fatty acid
84 synthesis relative to the untreated control in 10 minutes (Figure 1). That batumin
85 achieved such rapid and substantial inhibition of fatty acid synthesis, and before any
86 detectable impact on the synthesis of other cellular macromolecules (protein),
87 corroborates the original hypothesis that batumin directly inhibits fatty acid
88 synthesis. The proposal that aaRS inhibitors ultimately mediate their antibacterial
89 effects through indirect inhibition of fatty acid synthesis is not supported by the

90 observation that mupirocin, though demonstrating considerable inhibition of protein
91 synthesis, exerted no inhibitory effect on fatty acid synthesis (Figure 1).

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93 Thus, whilst further experimental studies will be required to more precisely
94 delineate the MOA of batumin, the available evidence discounts inhibition of one or
95 more aaRS enzymes, and implies that batumin mediates its antibacterial effect
96 directly through inhibition of fatty acid biosynthesis.

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100 **Figure 1. Effect of batumin and control agents at 4XMIC on protein and fatty acid**
101 **synthesis in *S. aureus* SH1000, as measured by incorporation of radiolabeled**
102 **precursors over a 10 minute period.** Incorporation is shown as a percentage of that
103 in the untreated control. Datum points represent the means of at least three
104 experimental determinations, and error bars show standard deviations.

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112 **References**

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