AmpC-inducible Enterobacteriaceae are infrequent pathogens in the community-acquired pneumonia and complicated skin and skin structure infection indications sought for ceftaroline itself; and, secondly, for indications where AmpC-inducible Enterobacteriaceae are likely, e.g. nosocomial pneumonia, ceftaroline is being developed in combination with NXL104, which inhibits these (and many other) β-lactamases.

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Transparency declarations

D. M. L. has shareholdings, or acts as enduring attorney for a shareholder, in AstraZeneca, Dechra, EcoAnimal Health, GlaxoSmithKline, Merck (following takeover of Schering-Plough) and Pfizer, and has had research contracts or conference finance in the past 3 years from AstraZeneca, Coixa, Cerexa, Johnson & Johnson, Merck, Novartis, Novexel, Pfizer, Phico, Theravance and Wyeth. Both authors are employed by the Health Protection Agency and are influenced by their views on antibiotic usage.

References

Sir,

Acanthamoeba spp. are free-living protozoa that are widely spread in the environment and can infect the human eye, causing a sight-threatening keratitis. Acanthamoeba keratitis is a potentially devastating ocular infection with an increasing number of cases, where the use of soft contact lenses, especially under inappropriate care conditions, and corneal abrasion are considered the two most important risk factors. The current treatment of Acanthamoeba keratitis involves a combination of an amoebicidal drug, such as hexamidine (Desomedine™) or propamidine isethionate (Brolene™), with chlorhexidine gluconate or biguanides possessing cysticidal properties. In spite of adequate treatment, the final visual acuity is severely compromised and secondary ocular complications can occur, worsening the prognosis. Drug resistance has also been reported. The severity of this pathology makes research for new effective drugs necessary.

Nitric oxide (NO) plays a key role in a myriad of physiological systems and S-nitrosothiols (RSNOs) are NO donor molecules possibly involved in NO-dependent biological signalling processes. S-nitrosoglutathione (GSNO) is an endogenous RSNO that displays antimicrobial activity in vitro and S-nitroso-N-acetylcysteine (SNAC) is a non-endogenous RSNO that has biochemical properties similar to GSNO, including in vitro antimicrobial properties against Leishmania. Sodium nitroprusside (SNP) is an inorganic NO donor complex where NO is coordinated as a nitrosonium ligand (NO⁺) to the iron (Fe²⁺) centre, and has been clinically used for decades to reduce blood pressure in hypertensive emergencies and during surgery.

In this work, we show for the first time that GSNO and SNAC have potent microbicidal activities against Acanthamoeba castellanii trophozoites and are able to kill >95% of the protozoa after 24 h of incubation at 1 mM; thus, creating a new perspective for the topical treatment of this disease.

GSNO and SNAC were synthesized as described previously, and their solutions were used immediately after preparation. Trophozoites of A. castellanii strain ATCC 30011 were grown axenically in sterile tissue culture flasks (25 cm²) at 25°C in Neff medium and incubated at 25°C in 96-well sterile tissue culture plates with GSNO, SNAC or SNP solutions at three different final concentrations: 100, 500 and 1000 μM. Control wells received 50 μL of sterile distilled water. Cellular viability was assessed after 3, 8 and 24 h of incubation, in a single blinded 0.4% Trypan Blue exclusion assay in three independent experiments run in duplicate. Statistical differences were evaluated by the Wilcoxon test and a P value of <0.05 was considered significant.

Figure 1 shows representative pictures of Fuchs–Rosenthal haemocytometer measurements of cellular viability using the Trypan Blue exclusion assay, after 24 h of incubation of Acanthamoeba castellanii trophozoites with (a) GSNO, (b) SNAC and (c) SNP at a concentration of 1000 μM. (d) Control group.

![Figure 1. Representative pictures of Fuchs–Rosenthal haemocytometer measurements of cellular viability using the Trypan Blue exclusion assay, after 24 h of incubation of Acanthamoeba castellanii trophozoites with (a) GSNO, (b) SNAC and (c) SNP at a concentration of 1000 μM. (d) Control group.](https://academic.oup.com/jac/article-abstract/65/3/588/747996/589)

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while the efficacy of SNAC increased from 80% to 98% after 3 and 24 h, respectively. SNAC showed no statistically significant difference relative to GSNO after 24 h. Figure 2 shows no amoebicidal activity for SNP at any of the concentrations tested. There was no statistically significant difference among the three independent experiments or between the duplicate experiments (P > 0.05), in all cases.

The strong amoebicidal effect of GSNO and SNAC at the 500 and 1000 μM concentrations, obtained in this work, indicates that these two primary S-nitrosothiols must exert microbicidal action against trophozoites of *A. castellanii* due to their ability to act as NO donors, underscoring the possibility of clinical treatment of *A. castellanii* infection through the topical application of GSNO or SNAC solutions to the eye. The dose-dependence results show that the effective microbicidal concentrations are lower for GSNO than for SNAC. Considering that both GSNO and SNAC have the SNO moiety located in a cysteine residue with similar chemical stabilities and NO lability, this result shows that the stronger amoebicidal action of GSNO may be associated with some specific interaction of this molecule with the cell wall of the parasites or with a more effective mechanism of penetration into the parasites through membrane channels. This proposal is reinforced by previous reports of the antimicrobial action of GSNO in *in vitro* against *Leishmania*. In this respect, it is important to consider that GSNO is found endogenously in mammals and may mediate the cytotoxic actions of NO in vivo. Thus, the differentiated amoebicidal action of GSNO might have been acquired by the immune system of mammals through evolutionary selective pressure and/or biochemical adaptation to the acting environments.

It is also significant that SNP, a hydrosoluble NO donor, applied at the same concentrations as GSNO and SNAC had no relevant amoebicidal effect, even at 1000 μM. This fact shows that the chemical nature of the NO donor plays a specific role in promoting the actions of NO in vivo. It must be recalled that in RSNOs, NO is bound through a covalent S-N bond, while in SNP, NO is coordinated to an iron centre as a nitrosonium ligand (NO⁺). Although both compounds are able to spontaneously release NO in aqueous medium through thermal reactions, the labilities of NO when these compounds are present in the biological medium are different. This difference in biological reactivity is probably associated with the primary reactions that these NO donors undergo in the presence of other hydrosoluble biological molecules. In the case of the microbicidal actions of NO, it has already been shown that RSNOs are capable of inactivating several enzymes through transnitrosation reactions with the sulphydryl groups of cysteine residues, one of the main targets being the cysteine proteases, which are essential to the survival of many parasites. The microbicidal actions of SNAC and GSNO, via cysteine protease inactivation, have already been demonstrated *in vitro* in the killing of *Leishmania* promastigotes. Such reactions involve the exchange of NO⁺ from the RS-NO moiety by a proton (H⁺) from the cysteine residue of the target enzyme, according to the equation:

\[
RS⁻NO + RS⁺H → RS⁺H + RS⁻NO
\]

where RS⁻ and RS⁺ are the sulphur radicals of the nitrosothiol and of the target sulphydrylated molecule, respectively.

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**Figure 2. In vitro microbicidal activity of GSNO, SNAC and SNP against Acanthamoeba castellanii trophozoites after incubation for 3, 8 and 24 h at three different concentrations:** (a) 100 μM; (b) 500 μM; and (c) 1000 μM.

![Graph showing microbicidal activity](https://example.com график.png)
It must be noted that NO in SNP also has a nitrosonium (NO⁺) character⁹ and is capable of reacting with cysteine residues according to:

\[ \text{Fe}^{II}(\text{CN})_5\text{NO}^+ \text{CN}^- + \text{RS}^- \rightarrow \text{Fe}^{II}(\text{CN})_5\text{N}^-\text{OSR} \text{CN}^- \] (2)

followed by the release of free NO and the formation of a dimer (RSSR) according to:

\[ \text{Fe}^{II}(\text{CN})_5\text{N}^-\text{OSR} \text{CN}^- + \text{H}_2\text{O} \rightarrow \text{Fe}^{II}(\text{CN})_5\text{H}_2\text{O} \text{CN}^- \text{CN}^- + \text{NO}^- + 1/2 \text{RSSR} \] (3)

However, these reactions are very slow at a physiological pH, where the thiol groups are mainly protonated. Evidently, the direct exchange of NO from SNP by a proton of an SH group is impossible in such a coordination complex. Therefore, SNP can be considered unable to promote the direct inactivation of a cysteine residue through a transnitrosation reaction and this characteristic may be the cause for its non-significant amoebicidal action. It is, thus, possible that GSNO and SNAC may react directly with Acanthamoeba cysteine proteases, like caspases, leading to their effective inactivation. Of course, other molecular targets can also be involved in the actions of GSNO and SNAC.

These results suggest that S-nitrosothiols are potential therapeutic drugs for the treatment of Acanthamoeba infections. Further studies aimed at understanding the molecular mechanisms involved in the amoebicidal actions of GSNO and SNAC, their pharmacokinetic and pharmacodynamic parameters, and their ocular toxicity in animal models of Acanthamoeba keratitis are necessary.

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**Transparency declarations**

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**References**


**In vitro bactericidal activity of ceftobiprole against hospital- and community-associated methicillin-resistant Staphylococcus aureus**

Sonia Borbone, Floriania Campanile, Dafne Bongiorno and Stefania Stefani*

Department of Microbiology, University of Catania (I), Via Androne 81, 95124 Catania, Italy

*Corresponding author. Tel: +39-095-2504714; Fax: +39-095-2504734; E-mail: stefanis@unict.it

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Sir, Ceftobiprole, formerly designated BAL9141/Ro63-9141, is a pyrrolidinone-3-ylidene-methyl cephalosporin with activity against methicillin-resistant Staphylococcus aureus (MRSA), Enterobacteriaceae and Pseudomonas aeruginosa. This anti-MRSA characteristic represents a remarkable evolution of the cephalosporin class of antimicrobial agents that have good coverage of Gram-negative bacteria but have hitherto lacked activity against MRSA.¹ ² The efficacy of ceftobiprole was assessed in clinical trials of treatment of complicated skin and skin structure infections (cSSSIs) and it was recently approved in Canada for this indication including non-limb-threatening diabetic foot infections without osteomyelitis.³ Further Phase III clinical trials of cSSSIs have recently been completed and are under review by the US FDA and the European Medicines Agency.

The objective of this investigation was to evaluate the antibacterial and bactericidal activity of ceftobiprole, compared with those of other drugs, against a group of clinically relevant and molecularly characterized healthcare-associated (HA) and community-associated (CA) MRSA strains isolated in Italy. The strains are representative of MRSA currently circulating in hospitals and the community in Italy.⁴ ⁵