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Short Communication

Repurposing the anticancer drug mitomycin C for the treatment of persistent *Acinetobacter baumannii* infections

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ABSTRACT

Acinetobacter baumannii is an emergent opportunistic bacterial pathogen responsible for recalcitrant infections owing to its high intrinsic tolerance to most antibiotics; therefore, suitable strategies to treat these infections are needed. One plausible approach is the repurposing of drugs that are already in use. Among them, anticancer drugs may be especially useful due their cytotoxic activities and ample similarities between bacterial infections and growing tumours. In this work, the effectiveness of four anticancer drugs on the growth of *A. baumannii* ATTC BAA-747 was evaluated, including the antimetabolite 5-fluorouracil and three DNA crosslinkers, namely cisplatin, mitomycin C (MMC) and merphalan. MMC was the most effective drug, having a minimum inhibitory concentration for 50% of growth in Luria–Bertani medium at ca. 7 μg/mL and completely inhibiting growth at 25 μg/mL. Hence, MMC was tested against a panel of 21 clinical isolates, including 18 multidrug-resistant (MDR) isolates, 3 of which were sensitive only to colistin. The minimum inhibitory concentrations and minimum bactericidal concentrations of MMC in all tested strains were found to be similar to those of *A. baumannii* ATCC BAA-747, and MMC also effectively killed stationary-phase, persister and biofilm cells. Moreover, MMC was able to increase survival of the insect larvae *Galleria mellonella* against an otherwise lethal *A. baumannii* infection from 0% to ≥53% for the antibiotic-sensitive *A. baumannii* ATCC BAA-747 strain and the MDR strains A560 and A578. Therefore, MMC is highly effective at killing the emergent opportunistic pathogen *A. baumannii*.

1. Introduction

As we are on the verge of a post-antimicrobial era, the implementation of novel useful antibacterial therapies is urgent. Among such possible therapies, those that involve the repurposing of drugs are especially attractive since they offer the possibility of employing treatments with compounds that are already in use in the clinical setting, with known pharmacological properties, doses, secondary effects and administration routes. Hence, this approach should save time and resources compared with the development of novel compounds and therapies [1].

In particular, anticancer drugs are promising due to the ample similarities between bacterial infections and growing tumours, such as high replication rates, high tendency to disseminate, high resistance to the immune system and the tendency to become insensitive to treatments [2]. In fact, several anticancer drugs have important antibacterial properties [1], among them the antimetabolite 5-fluorouracil (5-FU) that is currently used for the treatment of several kinds of cancers, including colorectal, oesophageal, breast, pancreatic and skin cancers [3]. 5-FU has broad antimicrobial and antivirulence activities against several bacterial pathogens, including *Pseudomonas aeruginosa*, *Escherichia coli* and some Gram-positive

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bacteria such as *Staphylococcus aureus* [4–6]. Also, it was recently discovered that the potent DNA crosslinker mitomycin C (MMC), used in the clinic for the treatment of bladder, gastric and pancreatic cancers among others [7], has remarkable antibacterial properties against diverse bacterial pathogens including *E. coli*, *S. aureus* and *P. aeruginosa* [8]. In addition, MMC kills exponential-phase, stationary-phase, biofilm and persister cells, i.e. those cells that are notoriously impervious to the action of common antibiotics and that are linked with recurrent infections [8]. Moreover, MMC is also able to kill persister cells of *Borrelia burgdorferi*, the causative agent of Lyme disease [9]. Similarly, cisplatin was recently proven effective against persister cells of *E. coli*, *P. aeruginosa* and *S. aureus*, including clinical strains [10]. However, the antibacterial properties of anticancer drugs have not been evaluated against *Acinetobacter baumannii*, which is an emergent opportunistic pathogen responsible for severe infections, primarily hospital-acquired, that are often very difficult to eradicate owing to their intrinsically high antibiotic resistance [11].

In this work, the antibacterial effects of 5-FU, cisplatin, MMC and merphalan against the reference strain *A. baumannii* ATCC BAA-747 were determined and it was found that MMC had the best antimicrobial properties. Hence, MMC was further tested against a second *A. baumannii* reference strain as well as a set of clinical isolates from burned patients, some of which were multidrug-resistant (MDR) (sensitive only to colistin and amikacin). The ability of MMC to protect *Galleria mellonella* larvae against an otherwise lethal *A. baumannii* infection was also studied.

2. Materials and methods

Full details of the materials and methods used are given the Supplementary data.

2.1. Antibacterial properties of anticancer compounds against A. baumannii *ATCC BAA-747*

To determine the antibacterial properties of the anticancer compounds against *A. baumannii* ATCC BAA-747, cells were inoculated from overnight pre-cultures to an initial optical density at 600 nm of ca. 0.05, without and with different concentrations (0–600 μg/mL) of the four drugs (5-FU, cisplatin, MMC and merphalan) and the cells were then cultured aerobically for 16 h.

2.2. Antibacterial properties of mitomycin C against various A. baumannii *strains*

Owing to the more potent antibacterial activity of MMC compared with the other tested drugs, MMC was further tested against another reference strain (*A. baumannii* ATTC 17978) as well as a panel of 21 *A. baumannii* clinical strains isolated from burned patients; 18 of the burn isolates were MDR and 3 of them were resistant at all of the commonly used antibiotics except for colistin (see Supplementary Table S1 for the antibiotic resistance profile of the strains).

To further evaluate the anti-*A. baumannii* properties of MMC, five clinical isolates were selected and their MIC_{100} and MIC_{50} values (minimum inhibitory concentrations for 100% and 50% of growth, respectively) were evaluated in Luria–Bertani (LB) medium [12].

2.3. In vivo activity of mitomycin C in a G. mellonella model of A. baumannii *infection*

After the promising in vitro properties of MMC against *A. baumannii* strains were demonstrated, its effect in vivo was tested using larvae of *G. mellonella*, an animal model that has previously been used to evaluate *A. baumannii* virulence and its susceptibility to novel antimicrobials such as gallium nitrate [13–15]. The effects of MMC administered at 13–16 mg/kg of weight on the survival of *G. mellonella* infected with an otherwise lethal *A. baumannii* infection with strain ATTC BAA-747, the MDR strain A560 (sensitive only to amikacin and colistin) and the MDR A578 strain (sensitive only to colistin, imipenem and meropenem) were determined. The antibiotics ciprofloxacin and ceftazidime were used for comparison.

CFU counts in the haemolymph of the worms were also determined.

3. Results

3.1. Antibacterial properties of anticancer compounds against A. baumannii *ATCC BAA-747*

Following aerobic culture of *A. baumannii* ATCC BAA-747 with different concentrations of the drugs for 16 h, no growth was observed at 25 μg/mL MMC in LB medium. In contrast, growth was still appreciable up to 600 μg/mL cisplatin, 200 μg/mL merphalan and 200 μg/mL 5-FU (data not shown).

3.2. Antibacterial properties of mitomycin C against various A. baumannii *strains*

MMC was tested against another reference strain as well as a panel of 21 *A. baumannii* clinical strains isolated from burned patients (Supplementary Table S1). Critically, MMC at ≤30 μg/mL was able to inhibit 100% of the growth of all isolates in Mueller–Hinton broth (MHB) [16], and the minimum bactericidal concentration (MBC), i.e. the concentration able to kill all cells, including persister cells, was ≤100 μg/mL (Table 1).

To further evaluate the anti-*A. baumannii* properties of MMC, five clinical isolates were selected and their MIC_{100} and MIC_{50} were evaluated in LB medium [12]. In agreement, no growth of the ATCC strains was observed at 10 μg/mL MMC and no growth of five of the clinical isolates was observed at 10–50 μg/mL MMC (Supplementary Fig. S1). Corroborating the results in MHB, MMC was able to kill 99% of *A. baumannii* ATTC BAA-747 stationary-phase cells at 100 μg/mL and 100% of cells at 200 μg/mL; hence, persister cells were eradicated

Table 1
MIC_{100} and MBC values of mitomycin C against 2 *Acinetobacter baumannii* laboratory strains and 21 clinical strains.

Strain	MIC_{100} in MHB (μg/mL)	MBC (μg/mL)
ATCC BAA-747	2.5	20
ATCC 17978	20	50
A550	20	50
A552	20	50
A553	20	50
A554	20	50
A555	20	50
A556	20	100
A558	20	50
A560	30	50
A561	2.5	20
A563	30	100
A564	30	100
A568	30	50
A569	30	100
A570	20	50
A571	20	50
A572	20	50
A573	20	50
A574	20	50
A578	20	50
A580	20	50
A583	20	50

MIC_{100}, minimum inhibitory concentration able to inhibit 100% of growth of the isolates; MHB, MBC, minimum bactericidal concentration; Mueller–Hinton broth.

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since there were no remaining viable cells. Similarly, stationary-phase cells of *A. baumannii* ATCC 17978 and the five clinical isolates were killed by 200–600 μg/mL MMC. In addition, the minimum concentration of MMC needed to eradicate *A. baumannii* biofilm cells was 250 μg/mL for *A. baumannii* ATCC BAA-747 and 250–400 μg/mL for the clinical isolates (Table 2).

3.3. *In vivo* activity of mitomycin C in a G. mellonella model of A. baumannii infection

The in vivo effect of MMC was tested using larvae of *G. mellonella* infected with a lethal dose of *A. baumannii*. The results indicated that MMC administered at 13–16 mg/kg of weight was able to

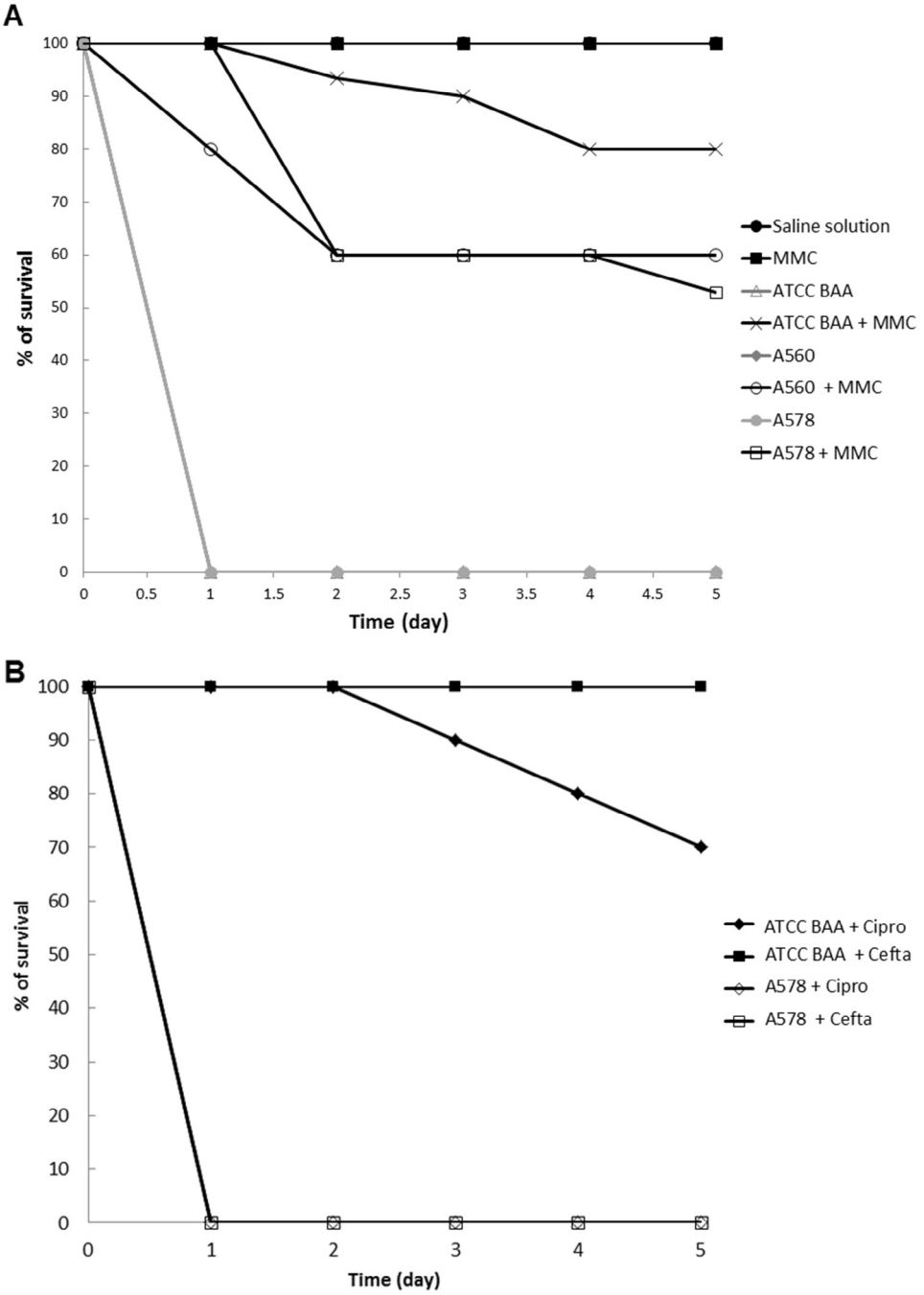

Fig. 1. (A) Mitomycin C (MMC) increases *Galleria mellonella* larvae survival against a lethal infection of *Acinetobacter baumannii*. Lethal doses of *A. baumannii* were 4×10^5 CFU for ATTC BAA-747 strain (Δ) and the clinical isolate A560 (♦) and 8×10^5 CFU for the clinical isolate A578 (●). MMC (8 μg per larva, equivalent to 13–16 mg/kg of weight) was administered by diluting it in sterile saline solution. The bacteria were injected first and the drug was injected 2–5 min following inoculation. ×, MMC-treated ATTC strain BAA-747; ○, MMC-treated strain A560; and □, MMC-treated strain A578. Controls included the drug (■) and saline solution (●) alone. Each point is the mean of three independent experiments using five worms each time. Survival plots were generated by the Kaplan–Meier method and were analysed by log-rank test using IBM SPSS Statistics for Windows v.20.0 (IBM Corp., Armonk, NY). Statistically significant differences ($P < 0.05$) were observed between infected larvae and infected larvae plus treatment with MMC. (B) The antibiotics ceftazidime and ciprofloxacin increase the survival of worms infected with the *A. baumannii* ATCC BAA-747 strain but not those infected with the multidrug-resistant (MDR) A578 strain. Results show the mean of two groups of five worms inoculated with independent cultures. Antibiotics were administrated at 1 mg dissolved in ethanol or dimethyl sulfoxide (DMSO). No effect on worm survival was seen with administration of the diluents or the antibiotics alone (data not shown). Survival plots were generated by the Kaplan–Meier method and were analysed by log-rank test using IBM SPSS Statistics for Windows v.20.0. Statistically significant differences ($P < 0.05$) were observed only for *A. baumannii* ATCC-BAA 747 between infected larvae and infected larvae plus antibiotics.

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Table 2
In vitro antibacterial properties of mitomycin C against two *Acinetobacter baumannii* laboratory strains and five clinical strains.

Strain	MIC_{50} in LB medium (μg/mL)	MIC_{100} in LB medium (μg/mL)	MIC_{100} in MHB (μg/mL)	MBC in MHB (μg/mL)	Minimum concentration to kill 100% of stationary-phase cells (μg/mL)	Minimum concentration to eliminate biofilm (μg/mL)
ATCC BAA-747	7	25	2.5	20	200	250
ATCC 17978	<5	10	20	50	200	250
A553	<5	10	20	20	600	400
A554	8	50	20	20	600	400
A558	5	25	20	50	600	400
A560	6	25	30	50	200	400
A578	7	50	20	50	600	400

$MIC_{50/100}$, minimum inhibitory concentrations for 50% and 100% of growth, respectively; LB, Luria–Bertani; MHB, Mueller–Hinton broth; MBC, minimum bactericidal concentration.

increase the survival of *G. mellonella* infected with an otherwise lethal dose of *A. baumannii* ATTC BAA-747, the MDR strain A560 (sensitive only to amikacin and colistin) and the MDR strain A578 (sensitive only to colistin, imipenem and meropenem) from 0 to 53–80% (Fig. 1A). In contrast, the antibiotics ciprofloxacin and ceftazidime were only able to increase the survival of larvae infected with *A. baumannii* ATCC BAA-747 but failed to rescue any larvae infected with the MDR strain A578 (Fig. 1B).

Furthermore, CFU determination in the haemolymph of the worms showed that without treatment, viable bacteria increased 50-fold at 24 h. In contrast, MMC treatment decreased the CFU to almost undetectable levels (Supplementary Fig. S2). Hence, these data provide further evidence for repurposing MMC for the treatment of *A. baumannii* infections. The potential of MMC is remarkable since the antibiotics ceftazidime and ciprofloxacin were only able to increase survival when worms were infected by *A. baumannii* ATCC BAA-747 but not with the MDR strain A578.

4. Discussion

Since to date virtually no effective antibiotics against several *A. baumannii* strains are available, except for colistin that is highly nephrotoxic [17], the search for new suitable antimicrobials is mandatory. In this regard, other compounds such as gallium nitrate have been identified as promising against this bacterial species, including MDR strains, with an MIC_{100} of 0.5–20 μg/mL [13], which is comparable with MMC. However, gallium nitrate administrated at sub-MIC_{50} concentrations promotes the production of virulence factors in *P. aeruginosa* since it generates iron deficiency that increases virulence [18]; whether the same phenomenon could be observed for *A. baumannii* is still unknown. Other compounds including novel antimicrobial peptides such as dendrimer G3KL [19] and vitroprocines [20] have also demonstrated promising results but were tested only in vitro and, more importantly, these molecules are still experimental. Remarkably, the effective concentrations of MMC are comparable with those of novel compounds such as the antibacterial peptide dendrimer G3KL ($MIC_{50/90}$ of ca. 8 μg/mL) [19], vitroprocines (MIC_{100} of 8–64 μg/mL), but in contrast to these novel compounds MMC has been successfully used for the treatment of several kinds of cancers for many years. Hence, dosages, administration protocols and the pharmacology for this drug are known. Although new experiments including testing MMC in models that more closely resemble human physiology are needed, the current results demonstrate that MMC has remarkable antibacterial potential against *A. baumannii*. Hence, repurposing it for treating *A. baumannii* infections may be a suitable alternative in the clinic.

5. Conclusion

Remarkably, MMC was able to kill stationary-phase cells, to eradicate biofilms and persister cells, and to protect *G. mellonella* larvae against an otherwise lethal *A. baumannii* infection. Hence, we propose MMC as a suitable candidate for repurposing as an antimicrobial agent.

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Competing interests: None declared.

Ethical approval: Not required.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2016.08.022.

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