Dimercaptosuccinic acid in combination with carbapenems against isogenic strains of *Escherichia coli* producing or not producing a metallo-β-lactamase *in vitro* and in murine peritonitis

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Background: Carbapenemase-producing Enterobacterales represent a major therapeutic challenge. MBLs, requiring zinc at their catalytic site, could be inhibited by meso-dimercaptosuccinic acid (DMSA), a heavy metal chelator already widely used for treating lead intoxication.

Objectives: To evaluate the activity of carbapenems alone or combined with DMSA against MBL-producing *Escherichia coli* in a severe murine peritonitis model.

Methods: Isogenic strains of wild-type *E. coli* CFT073 producing the MBLs NDM-1, VIM-2 and IMP-1, and the control serine carbapenemases OXA-48 and KPC-3 were constructed. MIC determinations and time–kill assays were performed for imipenem, meropenem and ertapenem alone or in combination with DMSA. Infected mice were treated intraperitoneally for 24 h with imipenem, DMSA or their combination. Bacterial counts in peritoneal fluid and spleen were assessed at 24 h.

Results: DMSA in combination with each carbapenem caused a significant decrease in the MICs for all MBL-producing strains, in a concentration-dependent manner, but did not provide benefit against non-MBL strains. In mice infected with the NDM-1-producing strain, the combination of imipenem and DMSA significantly reduced bacterial counts in peritoneal fluid ($P = 0.0006$) and spleen ($P < 0.0001$), as compared with imipenem alone, with no benefit against the KPC-3-producing and CFT073 strains. DMSA concentrations in plasma of mice were comparable to those obtained in humans with a standard oral dose.

Conclusions: DMSA restores the activity of carbapenems against MBL-producing strains, and its combination with carbapenems appears to be a promising strategy for the treatment of NDM-producing *E. coli* infections.

Introduction

Antimicrobial resistance is a significant public health concern, leading to millions of deaths and high costs across the globe.[1,2] Notably, the spread of ESBL-producing Enterobacterales, resistant to third-generation cephalosporins and causing both community-acquired and healthcare-associated infections, has led to an increase in consumption of carbapenems.[3,4] More recently, carbapenem-resistant Enterobacterales have emerged worldwide, especially carbapenemase-producing Enterobacterales (CPE), which represent a major therapeutic challenge.[5] CPE often carry plasmids encoding resistance to carbapenems as well as to quinolones, aminoglycosides or co-trimoxazole,[6] leaving few therapeutic options and leading to high mortality rates for CPE infections.[7,8] These MDR-carrying plasmids are now found in *Escherichia coli*, which is both a major commensal and the most frequent pathogen responsible for urinary tract and intestinal infections.[9] Thus, finding alternative therapeutic options against CPE is an urgent medical need.

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Among carbapenemases, New Delhi metallo-β-lactamase 1 (NDM-1) is of particular concern because of its rapid spread, particularly in *E. coli*, and the limited therapeutic options.[10] Indeed, NDM-1 is an MBL that hydrolyses all β-lactams except aztreonam,[11] including the newly developed combinations of a cephalosporin and β-lactamase inhibitor. Those novel combinations such as ceftazidime/avibactam are effective only against some types of CPE, i.e. serine carbapenemase producers (OXA-48/KPC).[8,12,13] Even though several *in vitro* studies have described different strategies to inhibit the activity of NDM-1, no specific clinical inhibitor is available to date.

In order to hydrolyse β-lactams, MBLs require zinc ions at their active site. Meso-dimercaptosuccinic acid (DMSA; generic name: succimer) is an orally administered heavy metal chelator approved for years in many countries for the treatment of lead and mercury intoxication.[14,15] It is safe in children and adults,[15,16] with a large therapeutic window, as it is less toxic than other chelators that have been used therapeutically.[17] We hypothesized that DMSA might have a similar chelating activity on the zinc–carbapenemase binding, allowing, when combined with a carbapenem, restoration of the activity of the carbapenem against MBL producers.

This work aimed to evaluate the activity of carbapenems alone or in combination with DMSA against MBL-producing *E. coli* both *in vitro* and in a severe murine peritonitis model.

Materials and methods

In vitro studies

Bacterial strains, plasmids and growth conditions

Five clinical isolates were used as the source of carbapenemases genes for the construction of isogenic strains: *Klebsiella pneumoniae* NDM-1 (R2726), *Pseudomonas aeruginosa* VIM-2 (R51), *P. aeruginosa* IMP-1 (R409), *K. pneumoniae* OXA-48 (11978) and *K. pneumoniae* KPC-3 (GF). Chemically competent *E. coli* TOP10 was used for cloning experiments, and uropathogenic *E. coli* CFT073 (O6: K2: H1), previously used in murine peritonitis models,[18,19] served as the recipient strain.[18,19] Plasmid pACYC184 (New England Biolabs, Ipswich, MA, USA), which carries chloramphenicol (*cat*) and tetracycline (*tet*) resistance genes, was used for cloning experiments. In order to ensure plasmid maintenance, all subcultures were performed in the presence of ampicillin 100 mg/L and chloramphenicol 20 mg/L. Regions corresponding to structural genes and promoters of bla_{NDM-1}, bla_{VIM-2}, bla_{IMP-1}, bla_{OXA-48} and bla_{KPC-3} were amplified by PCR and cloned into pACYC184 (primers used are described in Table S1, available as Supplementary data at *JAC* Online). *E. coli* CFT073 was transformed with recombinant plasmids by electroporation, resulting in CFT073-pACYC184-NDM-1, CFT073-pACYC184-VIM-2, CFT073-pACYC184-IMP-1, CFT073-pACYC184-OXA-48, CFT073-pACYC184-KPC-3 and CFT073-pACYC184 (non-recombinant and cloning plasmid). They will be referred to as CFT073-NDM-1, CFT073-VIM-2, CFT073-IMP-1, CFT073-OXA-48, CFT073-KPC-3 and CFT073, respectively.

Minimum inhibitory concentrations

MICs of imipenem (Imipenem Mylan®, Saint Priest, France), meropenem (Meropenem Kabi®, Sèvres, France) and ertapenem (Invanz®, Merck, Haarlem, the Netherlands) alone or in the presence of DMSA powder (Sigma–Aldrich, Saint-Quentin-Fallavier, France) at different concentrations (0.3, 1.5, 3, 6, 9 and 12 mM), were determined by the broth microdilution method, according to EUCAST (http://www.eucast.org/), in triplicate.

Bactericidal combination study

The presence of an *in vitro* synergy between imipenem and DMSA was tested by performing time–kill assays against CFT073, CFT073-NDM-1, CFT073-OXA-48 and CFT073-KPC-3 with imipenem at a concentration equal to the MIC against the studied strain and DMSA at 6 mM, alone or in combination, as previously described.[20] Standard Mueller–Hinton (MH) broth test tubes were used as controls. A bactericidal effect was defined as a decrease of ≥ 3 $\log_{10}$ in cfu counts after 24 h compared with the initial inoculum. A synergistic effect was defined as a ≥ 2 $\log_{10}$ decrease in cfu counts after 24 h between the combination and its most active constituent.[20] All *in vitro* experiments were repeated at least three times, and the median values are reported.

Bacterial fitness

Bacterial fitness was determined by measuring growth rates at 37°C in MH broth as described.[21] For each strain and condition, maximal growth rate and time to achieve maximal growth rate were measured in three independent experiments and the median values were reported for each strain.

Murine peritoneal infection model

Ethics

Animal experiments and laboratory protocols were approved by the Departmental Direction of Veterinary Services (agreement No. 75-861). The peritonitis protocol was approved by the French Ministry of Research and by the ethics committee for animal experiments (No. APAFIS 4949-2016021215347422).

Peritonitis model

We used the lethal murine intra-abdominal infection model previously developed by our group.[18,19] Five-week-old Swiss female mice weighing 25–30 g were inoculated by the IP route with 250 μL of a bacterial suspension in porcine mucin 10% (Sigma–Aldrich), corresponding to a final inoculum of approximately 2.5×10^7 cfu/mL. The strains tested were CFT073, CFT073-NDM-1 and CFT073-KPC-3. Two hours after inoculation, at least five mice per strain were sacrificed to determine the pre-therapeutic bacterial loads, and referred to as start-of-treatment controls, since all untreated mice died within 24 h, preventing the use of end-of-treatment controls. For the treated mice, IP injections of the tested compounds were started 2 h after inoculation and continued for 24 h (identical preliminary results were obtained using the SC route of administration of antibiotics). Mice were sacrificed by IP injection of 200 μL of pentobarbital 40% (Euthasol Vet®, Dechra Veterinary Products, France) 4 h after the last antibiotic injection. Immediately after death, a peritoneal wash was performed by IP injection of 2 mL of sterile saline solution, followed by a gentle massage of the abdomen and opening of the peritoneum to collect 1 mL of peritoneal fluid. The spleen was extracted and homogenized. Samples were plated on LB agar supplemented with chloramphenicol 20 mg/L and ampicillin 100 mg/L to ensure plasmid maintenance. Colony counts were then determined after 24–48 h of culture. Results were expressed as $\log_{10}$ cfu/g of spleen and $\log_{10}$ cfu/mL of peritoneal fluid. The limit of detection was 1 $\log_{10}$ cfu/g or mL.

Therapeutic regimens

An imipenem dosing regimen of 100 mg/kg q4h was selected in order to reach the same peak plasma levels and the same percentage of time during which free drug plasma levels exceeded the MIC ($fT_{>MIC}$) as obtained in humans against a susceptible strain with standard IV regimens.[22,23] The DMSA dosing regimen was determined according to a previous study that found the LD_{50} of DMSA to be approximately 2500 mg/kg IP.[24] We first determined the two highest dosages of DMSA that could be used in

uninfected mice: 400 mg/kg q4h IP (2400 mg/kg per 24 h) close to the previously reported LD_{50} and 200 mg/kg q4h IP (1200 mg/kg per 24 h). As no death or any abnormal symptom occurred for either dosing regimen after 24 h of treatment, the 200 mg/kg q4h regimen was selected for safety reasons as the highest DMSA dosing regimen for therapeutic experiments. First, three different therapeutic groups were evaluated in mice infected with each of the three study strains: imipenem 100 mg/kg q4h alone, DMSA 200 mg/kg q4h alone and the combination of imipenem 100 mg/kg q4h + DMSA 200 mg/kg q4h. For imipenem-based regimens, at least 10 mice per group were used (5 for DMSA alone). Then, the lowest effective dosing regimen of DMSA in combination with imipenem against CFT073-NDM-1 was determined: 100 mg/kg, 50 mg/kg and 10 mg/kg q4h (10 mice minimum per group).

Assessment criteria

Assessment criteria for each strain and each treatment regimen were: (i) the bacterial load in peritoneal fluid and spleen; (ii) the mortality rate due to infection; (iii) the percentage of sterilization of peritoneal fluid and spleen samples; and (iv) the selection of resistant mutants.

Drug concentrations

Imipenem/cilastatin

After a single IP injection of imipenem/cilastatin 100 mg/kg, four mice per time measurement (15 min, 30 min, 1 h, 3 h, 4 h and 6 h) were anaesthetized and blood samples were obtained by intracardiac puncture before sacrifice. In addition, peritoneal washes were obtained from infected and treated mice ($n = 4$) 4 h after the last imipenem/cilastatin injection. Imipenem concentrations were measured in plasma by HPLC coupled with spectrophotometric detection at 237 nm.[25]

DMSA

Blood samples of healthy mice were collected by intracardiac puncture 30 and 60 min after a single IP dose of DMSA 400 mg/kg, 200 mg/kg, 100 mg/kg, 50 mg/kg and 10 mg/kg, and stored at −80°C.

An analytical technique of dosage of DMSA was developed. For each mouse, 80 µL of plasma was sampled into a 1.5 mL microtube and mixed with 20 µL of 10% trichloroacetic acid (TCA) to precipitate the proteins and extract the component. Samples were vortexed for 5 min, then placed in an ultrasound bath for 1 min and centrifuged to sediment the proteins (15 000 **g**, 5 min at 16°C). Supernatants were analysed using MS coupled with a triple quadripole Shimadzu LC-MS 8030 (LC-MS/MS).

The separation was performed on a kinetex polar column 2.6 µm C18 100 A 50×2.1 mm (Phenomenex) at 40°C with an injection volume of 10 µL. The mobile phase consisted of a mix of A (0.5% formic acid in water) and B (acetonitrile). Initial conditions were 99% A and 1% B. After 0.5 min, the gradient decreased until 1.2 min to 5% A and 95% B and remained as such for 0.2 min. It then rapidly came back to the initial composition between 1.42 min and 2.8 min. The mobile phase flow was 0.5 mL/min.

DMSA is an unstable molecule in plasma. All the compound was degraded after a few minutes as it oxidizes completely into a dimer of two molecules linked by a di-sulfur bridge. We therefore quantified the degradation compound rather than DMSA. The multiple reaction monitoring (MRM) analysis on precursor and fragmented ions of DMSA was chosen for quantification. The analysis was performed by negative ionization, and the precursor ion of the oxidized compound corresponded to 360.90 m/z and the chosen fragmented ions to 102.90 m/z and 146.90 m/z. For DMSA, they were, respectively, 181.1, 103.0 and 147.0 m/z. The limit of detection was 0.05 µM and the mean variation for each sample determination was 9%.

Zinc

Zinc concentrations were measured in MH broth and mouse sera, by inductively coupled plasma MS (ICP-MS) on an X-Series II® from Thermo Scientific.[26]

Statistical analyses

Continuous variables were expressed as the median and ranges (minimum to maximum) and compared using non-parametric test (Kruskall–Wallis). Proportions were compared using the Fisher exact test. Statistical analyses were performed with R® software (version 3.6.0). A P value ≤0.05 was considered significant.

Results

In vitro experiments

MICs of carbapenems alone or in combination with DMSA

The MICs of imipenem, meropenem and ertapenem alone or in combination with increasing concentrations of DMSA against study strains (susceptible strain *E. coli* CFT073 and isogenic derivatives producing NDM-1, VIM-2, IMP-1, OXA-48 or KPC-3) are shown in Table S2. DMSA supplementation generated a significant reduction in carbapenem MICs in a concentration-dependent manner and a full recovery of susceptibility to carbapenems for MBL-producing strains at a maximum concentration of 6 mM (~1.1 g/L). The concentration of DMSA that allowed each carbapenem to reach its susceptibility breakpoint varied depending on the carbapenemase and the carbapenem, from 6 mM for NDM-1-, 1.5–3 mM for VIM-2- and 0.3–1.5 mM for IMP-1-producing strains. No significant effect was observed against non-MBL-producing strains or the WT strain. The median concentration of zinc in MH broth was 1.1 mg/L (0.02 mM) (range 1.09–1.18 mg/L).

Time–kill assays

The results of time–kill curves for the three strains studied *in vivo* are represented in Figure 1. A beneficial bactericidal effect between imipenem at the MIC and DMSA 6 mM was observed against CFT073-NDM-1, with a > 3 $\log_{10}$ cfu/mL reduction in bacterial load at 24 h, but not against the susceptible or non-MBL-producing resistant strains.

Bacterial fitness

No difference of the maximal growth rate or time to achieve maximal growth rate was observed for each strain in MH broth containing DMSA or not at concentrations up to 6 mM (data not shown).

Murine peritoneal infection model

Activity of imipenem and DMSA alone or in combination

DMSA alone at the highest dose tested (200 mg/kg q4h) had no antibacterial effect in mice against any of the study strains (Figure 2). Since the mortality in DMSA-alone treatment groups was 100%, no peritoneal fluid samples were available. In contrast, imipenem alone at a dose of 100 mg/kg q4h showed a significant antibacterial activity in peritoneal fluid and spleen as compared with controls against the susceptible strain and against the NDM-1 strain, but no significant effect in spleen against the KPC-3 strain
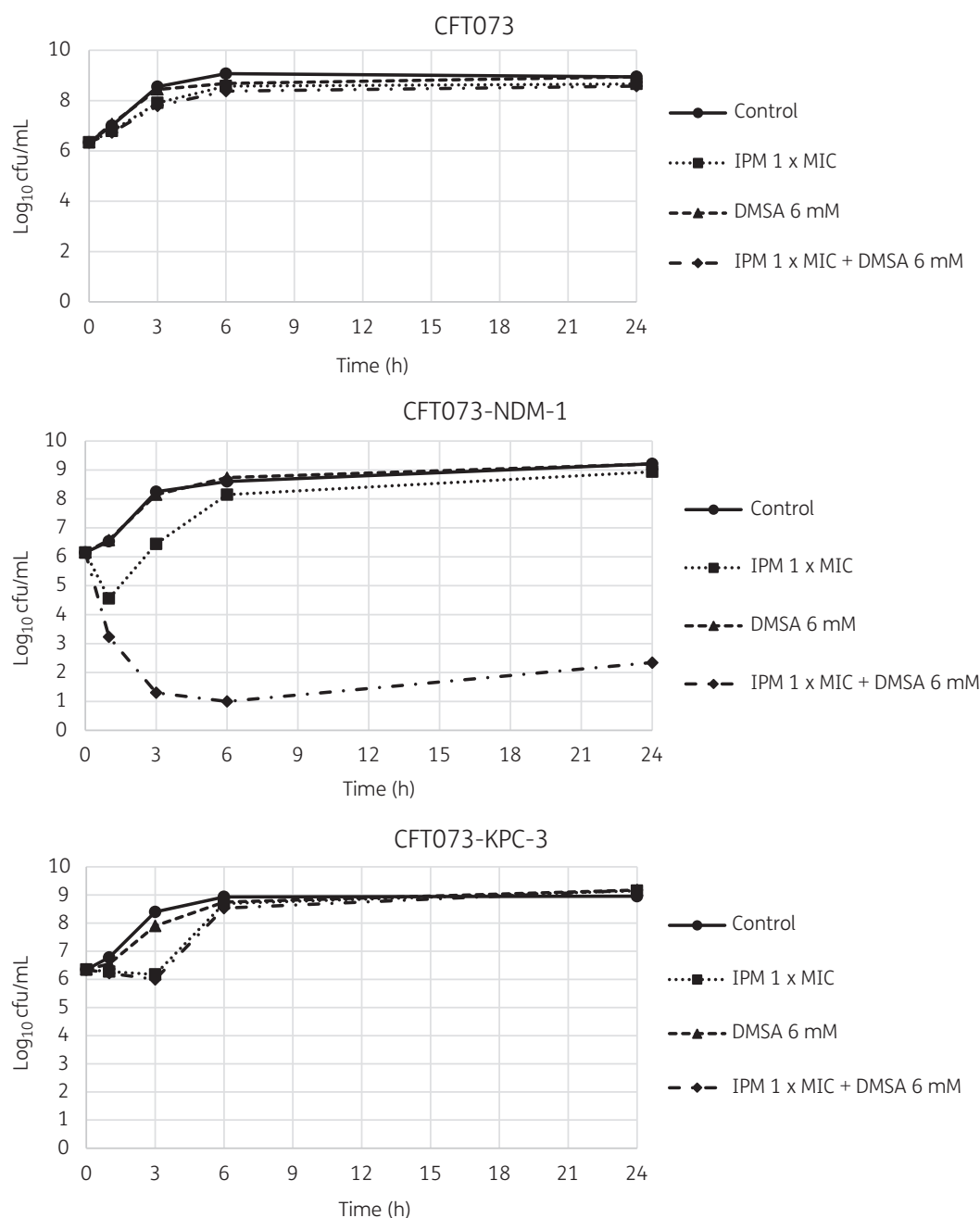
Figure 1. Time–kill curves of imipenem (IPM) and DMSA alone or in combination against WT CFT073 (top), CFT073- NDM-1 (middle) and CFT073-KPC-3 (bottom). Imipenem MIC was respectively, 0.125, 64 and 8 mg/L for CFT073, CFT073-NDM-1 and CFT073-KPC-3.

(Figure 2). In CFT073-NDM-1-infected mice, the combination of imipenem and DMSA produced a significant reduction of bacterial load in peritoneal fluid and spleen at 24 h as compared with imipenem alone (-1.35 $\log_{10}$ cfu/mL in peritoneal fluid, $P = 0.0006$ and -1.04 $\log_{10}$ cfu/g in spleen, $P < 0.0001$). No significant benefit of the combination was achieved in mice infected with the non-MBL-producing strains CFT073 and CFT073-KPC-3 as compared with imipenem alone. The dose–response study performed in CFT073-NDM-1-infected mice with decreasing DMSA dosing regimens of 100, 50 and 10 mg/kg q4h showed that a significant reduction in bacterial counts was still achieved with the 50 mg/kg q4h dosing regimen in the spleen in contrast to peritoneal fluid where the 200 mg/kg q4h regimen was the only tested DMSA dose found to be effective (Figure 3).

Sterilization rates

No spleen sterilization was obtained for any strain and therapeutic regimen. In peritoneal fluid, the imipenem sterilization rate was 0% (0/12) against NDM-1 and 12% (2/17) in combination with
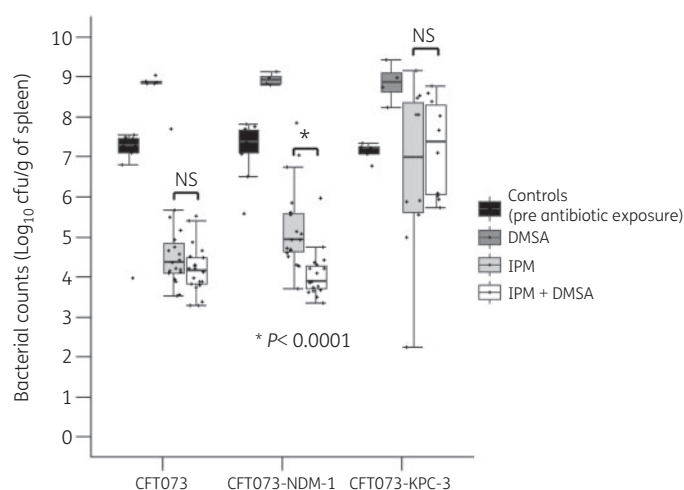
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Figure 2. Bacterial counts in the spleen from mice infected with *E. coli* CFT073, CFT073-KPC-3 and CFT073-NDM-1 strains before antibiotic exposure (Controls, pre-antibiotic exposure) and after 24 h of treatment with DMSA alone (DMSA), imipenem alone (IPM) or in combination (IPM + DMSA). Each point corresponds to a mouse. Boxplots represent the median (horizontal black line), IQR and minimum/maximum values. Mice were infected with *E. coli* CFT073, CFT073-NDM-1 or CFT073-KPC-3 strains just before antibiotic exposure (Controls, $n = 11$, 10, and 5, respectively) and after treatment with DMSA alone (DMSA, $n = 5$, 4, and 4, respectively), imipenem alone (IPM, $n = 19$, 19, and 10 respectively) or in combination (IPM + DMSA, $n = 20$, 19, and 10, respectively) for 24 h. NS, no significant difference.

DMSA ($P = 0.49$), similar to the 13% (2/15) obtained with imipenem alone against the susceptible strain.

Mortality rates

The mortality rates in groups treated by DMSA alone were 100% for all strains within 24 h. No significant reduction of mortality was obtained with the combination of imipenem and DMSA as compared with imipenem alone against any of the three tested strains. In particular, the mortality rate for CFT073-NDM-1 was 37% (7/19) for imipenem alone versus 11% (2/19) for the combination ($P = 0.13$).

Pharmacokinetics in mice

Imipenem/cilastatin pharmacokinetics

Peak levels of imipenem/cilastatin in plasma were achieved 15 min after IP injection. The median concentration was 86 mg/L (range 10–125), corresponding to a free concentration of 57 mg/L. The $fT_{>MIC}$ values in mice were 66%, 0% and 27% for strains CFT073, CFT073-NDM-1 and CFT073-KPC-3, respectively. Imipenem concentrations were below the detection limit (0.5 mg/L) 4 h after the end of the treatment in infected mice.

DMSA pharmacokinetics

DMSA peak levels in plasma were achieved 30 min after a single IP dose of 100, 200 or 400 mg/kg ($n = 3$ for each dose) in uninfected mice and it was not detected after 2 h. Results are shown in
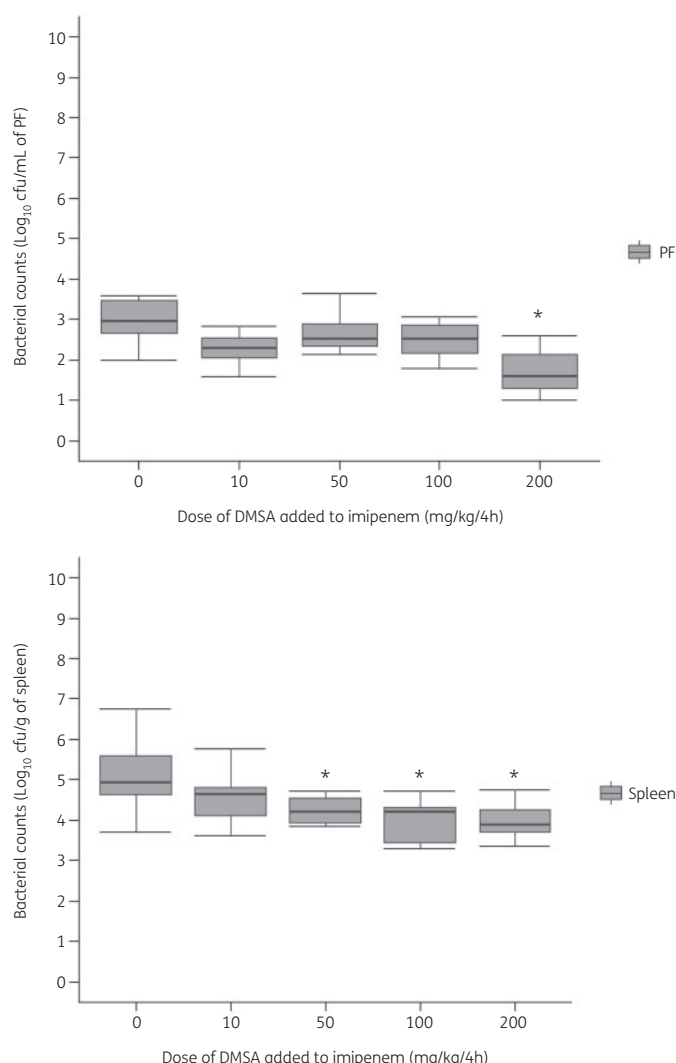

Figure 3. Bacterial counts after a 24 h treatment with imipenem alone or in combination with increasing doses of DMSA in the peritoneal fluid (PF; top) and in the spleen (bottom) from mice with peritonitis due to the *E. coli* CFT073-NDM-1 strain. Boxplots represent the median (black line), IQR and minimum/maximum values. The number of mice was 19, 10, 10, 10, 19 and 17, 10, 9, 8, 12 for DMSA 0, 10, 50, 100 and 200 mg/kg/4 h, respectively, in spleen and PF, respectively. *$P < 0.05$ compared with DMSA 0 mg/kg/4 h (imipenem alone).

Table S3 and compared with human values after a single oral dose of 10 mg/kg.

Zinc concentrations

The median concentration of zinc in the sera from healthy mice was 2.02 mg/L (range 1.59–3.36) and was 2.72 mg/L (range 1.94–3.87) in infected mice treated during 24 h with imipenem and DMSA ($P = 0.41$).

Discussion

In this study, DMSA allowed a recovery of the *in vitro* activity of car-bapenems against MBL-producing *E. coli* strains, and achieved a

significant benefit in combination with imipenem on the reduction of bacterial loads in peritoneal fluid and spleen in a lethal murine peritonitis model against an NDM-1-producing *E. coli* strain. This effect was not found with isogenic non-MBL-producing strains (OXA-48 or KPC producers), suggesting a specific effect on MBL.

An unexpected finding was that the beneficial antibacterial effect of adding DMSA to imipenem was achieved *in vivo* with plasma concentrations in the micromolar range whereas *in vitro* concentrations necessary to achieve this effect were in the millimolar range (1000-fold higher) (Tables S2 and S3). At least two hypotheses might explain this result. First, the rapid oxidation of DMSA observed in plasma could lead to the production of a more active dimer, thus enhancing its chelator activity *in vivo*. Indeed, the dimer is still a chelator as the carboxylic acid groups are not modified and the di-sulfur is still available. All the functions that bind to metal ions are still present in the new molecule. Additional studies are thus required to test the specific activity of the dimer, relative to DMSA. Second, the unexpected activity of imipenem *in vivo* against the NDM-1 strain could be associated with a decreased requirement of DMSA concentrations to achieve a beneficial activity of the combination in mice. This unexpected activity of imipenem *in vivo* corroborates results obtained by others in the thigh murine infection model without clear explanation.[27,28] Such an effect could not be explained by a fitness defect of the NDM-1-producing strain, nor by the IP route, as imipenem pharmacokinetics in plasma were similar to those with the SC route.[25] The pharmacokinetic/pharmacodynamic profile of imipenem in mice could not be an explanation since the $fT_{>MIC}$ was 0% for CFT073-NDM-1 herein, suggesting that mechanisms other than antimicrobial activity of supra-MIC concentrations of carbapenems could be involved.[29] Differences in zinc concentrations and consequently of carbapenemase activity were excluded, since similar concentrations of zinc were measured in plasma and in MH medium.

In vitro, the combination of DMSA with imipenem, meropenem or ertapenem produced a significant decrease of their MIC values against MBL-producing strains in a concentration-dependent manner. Similar results were obtained with clinical isolates of NDM-1-*E. coli* (unpublished data, P. Nordmann). This effect was specific to MBL, as no effect was observed against the non-MBL-carbapenemase-producing strains. Moreover, it was not related to bacterial fitness modifications. MICs of carbapenems against each type of carbapenemase in the constructed isogenic strains were consistent with available data on clinical strains.[27,28,30,31] The IMP-1-producing strain seemed more susceptible to the effect of DMSA than others, suggesting a higher sensitivity of the IMP-1 enzyme to the amount of zinc in its environment. Of note, zinc concentrations in MH medium used for the determination of MICs were approximately 1 mg/L, close to human concentrations in plasma.[32]

These *in vitro* results were mirrored by the *in vivo* results obtained in a severe peritonitis model using the constructed CFT073-NDM-1 strain. The combination of imipenem with DMSA produced a significant reduction in bacterial counts in peritoneal fluid and spleen as compared with imipenem alone, but had no effect against the non-MBL CFT073-KPC-3 strain or the susceptible control. The benefit observed with DMSA is likely to be related to its activity as a chelator of zinc, a metallic element that is essential for the hydrolytic activity of MBLs.[33–35] Indeed, unlike serine-β-lactamases (such as KPC and OXA-48-type enzymes), MBLs need the coordinated action of one or two zinc ions and a water molecule to open the β-lactam ring.[34,36] Several studies have described a potential interaction between DMSA and zinc.[37,38] Further *in vitro* studies may be performed to study the intimate interactions between NDM-1, DMSA and zinc. We may study the hydrolysis of NDM-1 in the presence of increasing concentrations of DMSA followed by increasing concentration of zinc to see if the inhibition of hydrolysis of NDM-1 is reversible or irreversible.

Due to the current lack of data on the use of DMSA in such a murine model, the choice of the final DMSA dosing regimen of 1200 mg/kg/day was based on a 100% survival rate with no symptoms of toxicity. Therefore, the benefit of the addition of DMSA to imipenem was obtained for doses of DMSA much lower than the lethal doses reported in the literature.[24] However, it must be acknowledged that the evaluation of the toxicity of DMSA was limited to 24 h, corresponding to the duration of therapy in our study. A more prolonged exposure with repeated doses, closer to a standard antibiotic treatment duration, would be necessary before application in humans.

Pharmacokinetic data in humans concerning DMSA are scarce, but available studies indicate mean peak plasma concentrations of about 20–50 μM after a standard 10 mg/kg oral dose (Table S3).[16,39,40] These concentrations are comparable to those obtained in mice with 200 mg/kg, the dose that demonstrated a beneficial effect in combination with imipenem *in vivo*. Thus, an *in vivo* benefit was obtained with plasma concentrations comparable to those achieved in humans with the standard oral dose of DMSA of 10 mg/kg.

In conclusion, we provide here the proof of concept of the efficacy of DMSA in combination with carbapenems against MBL-producing *E. coli* in a lethal murine peritonitis model. Its development is following a repurposing strategy, which is intended to speed up the development of antibiotics[41] that may fulfil an unmet clinical need.

Transparency declarations

None to declare.

Supplementary data

Tables S1 to S3 are available as Supplementary data at *JAC* Online.

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