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Research Article

RESEARCH ARTICLE

Meso-tartrate inhibits intracellular replication of *Coxiella burnetii*, the causative agent of the zoonotic disease Q fever

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One sentence summary: The growth of the disease-causing bacterium *Coxiella burnetii* inside human cells can be significantly decreased by using a compound non-toxic to human cells.

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ABSTRACT

The zoonotic disease Q fever caused by the intracellular bacterium *Coxiella burnetii* remains a global health threat due to its high infectivity, environmental stability, the debilitating nature and the long duration of treatment. Designing new and potent drugs that target previously unexplored pathways is essential to shorten treatment time and minimise antibiotic resistance. Nicotinamide adenine dinucleotide (NAD) is an essential and ubiquitous cofactor in all living organisms. NadB, an L-aspartate oxidase catalysing the first step of the prokaryotic-specific NAD *de novo* biosynthetic pathway, is required for *C. burnetii* growth and replication inside host cells. In this study, *in vitro* enzyme assays utilising recombinant glutathione S-transferase tagged NadB (GST-NadB) demonstrated inhibition of the L-aspartate oxidase activity of NadB by meso-tartrate. Furthermore, meso-tartrate inhibits intracellular growth and replication of *C. burnetii* inside host cells in a dose-dependent manner, and has no effect on the viability of mammalian cells. Unexpectedly, meso-tartrate also inhibited growth of *C. burnetii* in axenic medium, and further reduces replication of the *nadB* mutant inside host cells, suggesting it is acting more widely than simple inhibition of NadB. Overall, these results suggest that the antibacterial activity of meso-tartrate warrants further study, including investigation of its additional target(s).

Keywords: Q fever; *Coxiella burnetii*; antimicrobial; meso-tartrate; NAD synthesis; enzyme inhibition

INTRODUCTION

Coxiella burnetii, an intracellular Gram-negative pathogenic bacterium, is the causative agent of Q fever in humans, a disease that presents in both acute and chronic forms and which is transmitted primarily by inhalation of contaminated aerosols. Disease in humans is usually acquired from animals, with ruminant livestock a major reservoir (Moffatt, Newton and Newton 2015). *Coxiella burnetii* is a pleomorphic organism with a biphasic life cycle comprised of the replicating large cell variant

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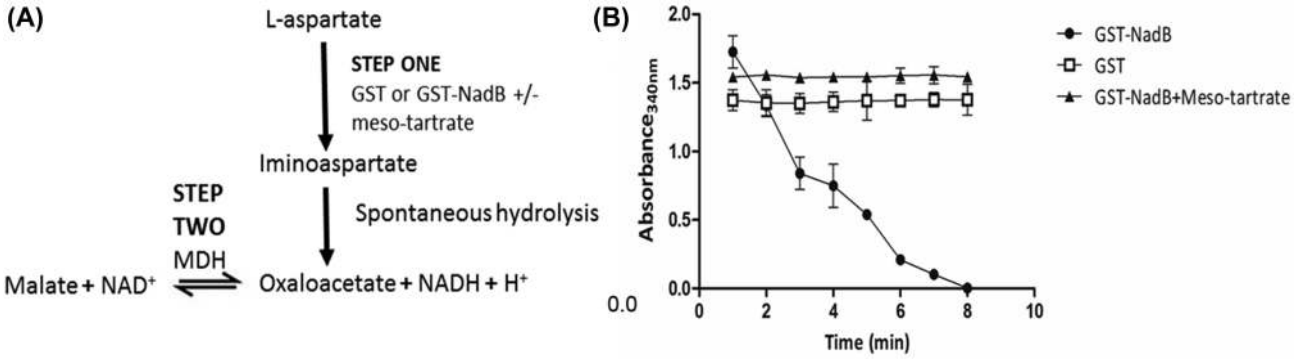

Figure 1. (A) Schematic of the two-step *in vitro* enzyme assay. In the first step of the *in vitro* assay, NadB converts L-aspartate to iminoaspartate, which then spontaneously hydrolyses to OAA. In the second step of the assay, commercial MDH was used to convert this OAA to malate, to measure if iminoaspartate was produced in step one. Conversion of OAA to malate is monitored by measuring the oxidation of the co-factor NADH to NAD^+ by measuring the change in absorbance at 340 nm. A fall in absorbance in the second step therefore indicates that NadB was active in the first step. **(B)** Meso-tartrate inhibits enzymatic activity of NadB. The change in absorbance at 340 nm when the conditions used in the first step were GST-NadB (closed circles), GST (open squares) and GST-NadB + 200 mM meso-tartrate (triangles), demonstrating that no OAA was present in the second step for GST-NadB + 200 mM meso-tartrate, and that meso-tartrate therefore inhibits NadB. The graph represents the mean ± SD of three independent experiments.

form and the environmentally stable but non-replicating small cell variant form (Shaw and Voth 2019). In a natural mammalian infection, *C. burnetii* has a high affinity for alveolar macrophages and once internalised replicates inside the *Coxiella*-containing vacuole (CCV), an acidic and highly oxidative vacuole containing active lysosomal proteases and lysosomal enzymes, including cathepsin D (van Schaik *et al.* 2013).

In order to survive and replicate inside the host cell, *C. burnetii* requires a functional Dot/Icm system (Beare *et al.* 2011; Carey *et al.* 2011), a type IV secretion system that translocates at least 130 effector proteins into the host cell to control various infection events (Moffatt, Newton and Newton 2015). These effector proteins are required for inhibition of apoptosis (Lührmann *et al.* 2010), stimulation of autophagy (Martinez *et al.* 2016; Latomanski and Newton 2018) and formation of the spacious replicative CCV (Larson *et al.* 2013; Weber *et al.* 2013; Newton *et al.* 2014; Latomanski *et al.* 2016; Crabill *et al.* 2018), and are the focus of many studies on *C. burnetii* molecular pathogenesis. However, intracellular proteins may also play crucial roles, and we recently demonstrated that *C. burnetii* NadB is an L-aspartate oxidase required for *de novo* nicotinamide adenine dinucleotide (NAD) synthesis, and that NadB is required for normal CCV biogenesis and *C. burnetii* intracellular replication (Bitew *et al.* 2018).

NAD is an essential molecule in all living organisms and is involved in numerous biological and core metabolic reactions (Gazzaniga *et al.* 2009; Osterman 2009). Maintenance of correct intracellular NAD+/NADH ratio is vital for survival (Mesquita *et al.* 2016). NadB is part of the prokaryotic-specific NAD biosynthetic pathway, and therefore an attractive potential therapeutic target. Meso-tartrate is a form of tartaric acid, which naturally occurs in numerous fruits, predominantly in grapes, but also in citrus, bananas and tamarinds (Duarte *et al.* 2012). It was previously shown to inhibit the activity of *Escherichia coli* L-aspartate oxidase (Nasu, Wicks and Gholson 1982). In this current study, *in vitro* enzyme assays demonstrated that meso-tartrate inhibits the L-aspartate oxidase activity of NadB in a dose-dependent manner. Furthermore, meso-tartrate inhibits *C. burnetii* replication inside both epithelial and macrophage-like mammalian cells, but has no effect on the viability of these mammalian cells. Unexpectedly, meso-tartrate also inhibited the growth of the *nadB* transposon mutant inside host cells in a dose-dependent manner, indicating that meso-tartrate is acting on at least one additional bacterial target.

RESULTS

Meso-tartrate inhibits the L-aspartate oxidase activity of NadB *in vitro*

Previously, recombinant GST-NadB was purified and demonstrated to have L-aspartate oxidase activity *in vitro* (Bitew *et al.* 2018) using a two-step assay (Fig. 1A). In the second step of this assay, oxaloacetate (OAA), produced by spontaneous hydrolysis of the iminoaspartate produced by NadB in the first step, is converted to malate by commercial malate dehydrogenase (MDH). MDH requires the cofactor NADH, and concurrent oxidation of NADH to NAD^+ can be measured by monitoring the change in absorbance at 340 nm. Absorbance decreases in the second step only when active NadB in the first step produces iminoaspartate (Bitew *et al.* 2018).

To investigate whether meso-tartrate inhibits enzymatic activity of NadB, the two-step assay was carried out with 200 mM of meso-tartrate added to the first step of the reaction. Similar to the GST control, no change in absorbance was observed in the second step, indicating that no iminoaspartate was produced in the first step and that meso-tartrate inhibits the L-aspartate oxidase activity of NadB (Fig. 1B).

Meso-tartrate inhibits intracellular replication of *C. burnetii* in both epithelial and macrophage-like cells

Enzymatically active NadB is required for intracellular replication of *C. burnetii* (Bitew *et al.* 2018). To investigate if meso-tartrate could inhibit NadB during infection of mammalian cells, and therefore reduce *C. burnetii* intracellular replication, HeLa cells and THP-1 cells were infected with *C. burnetii* and cultivated in medium containing 200 mM meso-tartrate. Replication of *C. burnetii* over 7 days was monitored using quantitative polymerase chain reaction (qPCR). *Coxiella burnetii* grown in HeLa cells cultivated in medium supplemented with meso-tartrate displayed significantly reduced bacterial replication compared to *C. burnetii* replication in control HeLa cells cultured in normal medium. Only a 50-fold increase in genome equivalents at 7 days post-infection occurred in the meso-tartrate positive conditions, whereas in the absence of meso-tartrate *C. burnetii* showed over 400-fold increase in genome equivalents at 7 days post-infection (Fig. 2A). The growth over 7 days in the presence

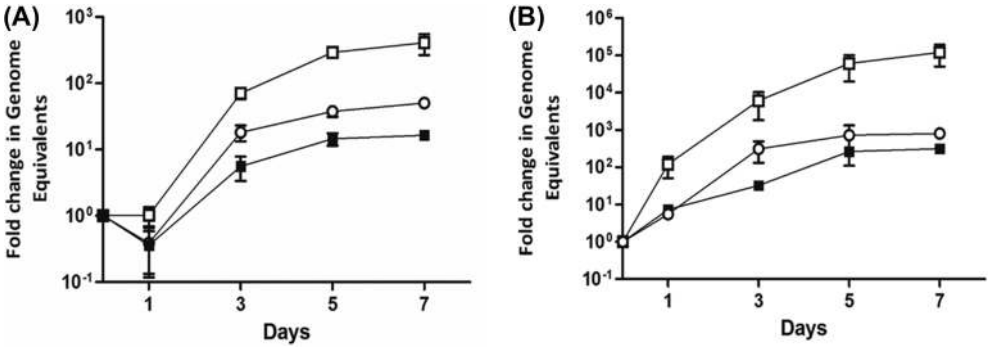

Figure 2. Meso-tartrate inhibits intracellular replication of *C. burnetii*. Intracellular growth of *C. burnetii* NMII (open squares), *C. burnetii* NM II + meso-tartrate (open circles) and *nadB*::Tn (closed squares) at days 1, 3, 5 and 7 post-infection in HeLa epithelial cells **(A)** and THP-1 macrophage-like cells **(B)**. The fold change in genome equivalents relative to day 0 of each strain was determined by qPCR using *ompA* specific primers. Both *nadB*::Tn and wild-type *C. burnetii* + meso-tartrate showed a statistically significant decrease in fold change in genome equivalents when individually compared to *C. burnetii* − meso-tartrate at days 3, 5 and 7 ($P < 0.05$, paired t-tests), demonstrating the inhibitory effect of 200 mM meso-tartrate in the host cell medium on *C. burnetii* growth and replication. This result represents three independent experiments that are displayed as mean +/- standard deviation of three independent infections.

of meso-tartrate was more similar to that of the *nadB* mutant. In a similar way, *C. burnetii* replicating within THP-1 cells cultured in medium supplemented with 200 mM meso-tartrate were significantly reduced in replication over 7 days, with only an 802-fold increase in genome equivalents at day 7 compared with a 1.2×10^5 fold increase in wild-type *C. burnetii* grown in THP-1 cells cultured in meso-tartrate negative medium (Fig. 2B). Replication of *C. burnetii* in THP-1 cells under meso-tartrate positive conditions was similar to that of the *nadB* mutant.

Qualitative analysis using fluorescence microscopy demonstrated that *C. burnetii* replicating in both mammalian cell lines in the presence of meso-tartrate displayed a smaller CCV containing fewer bacteria (Fig. 3A and B), similar to the *nadB* mutant, whereas *C. burnetii* grown in meso-tartrate negative conditions formed a much bigger vacuole containing many bacteria (Fig. 3A and B).

Meso-tartrate is not cytotoxic for HeLa or THP-1 cells

To investigate if the reduction in intracellular growth was due to a toxic effect of meso-tartrate on the mammalian cells themselves, a cytotoxicity assay was conducted for both HeLa and THP-1 cells. As the intracellular replication assays were carried out over 7 days, viability assays were performed at 24 h and 7 days after the addition of meso-tartrate to the mammalian cell culture medium. Meso-tartrate at concentrations of 50, 100, 150 and 200 mM did not significantly reduce the cellular viability of HeLa cells and THP-1 cells at 24 h and 7 days compared to untreated cells, as measured by the number of active metabolic cells present under each condition using a commercial cell viability kit (Fig. 4) that utilises soluble tetrazolium salt WST-8, which is reduced by cellular dehydrogenase present in viable cells into yellow coloured product (formazan). The formation of formazan dye is directly proportional to the number of living cells.

Meso-tartrate inhibits *C. burnetii* replication inside THP-1 cells in a dose-dependent manner

To confirm the inhibition of *C. burnetii* growth inside macrophage-like cells by meso-tartrate, a dose-response analysis was carried out in THP-1 cells. At day 7, all four concentrations of meso-tartrate tested (50, 100, 150 and 200 mM) significantly reduced *C. burnetii* compared to the untreated control. Furthermore, the growth inhibition induced by 200 mM meso-tartrate was significantly higher than that induced by 50 or 100 mM, demonstrating that the observed inhibition was dose dependent (Fig. 5A).

Meso-tartrate also inhibits replication of the *nadB* transposon mutant inside THP-1 cells, indicating that it acts on additional bacterial target(s)

Similar to its effect on *C. burnetii* NMII, meso-tartrate was, surprisingly, able to inhibit replication of the *nadB*::Tn mutant inside THP-1 cells in a dose-dependent manner (Fig. 5B). This suggests that meso-tartrate is acting on at least one other *C. burnetii* target to reduce intracellular replication of the bacterium. Similar inhibition was also observed for the complemented strain *nadB*::Tn pFLAG:*nadB* (Fig. 5C).

Meso-tartrate inhibits the growth of *C. burnetii in vitro*

Although enzymatically active NadB is required for optimal replication inside host cells, in the host-cell free medium ACCM-2 the *C. burnetii nadB* mutant replicates normally, demonstrating that active NadB is not required *in vitro* (Bitew *et al.* 2018). Further supporting the presence of additional targets for meso-tartrate, replication of *C. burnetii* in ACCM-2 was significantly inhibited by addition of 50, 100 and 150 mM of meso-tartrate, with a reduction in genome equivalents at day 7 when compared to the untreated *C. burnetii* control (Fig. 6B). In addition, replication of the *nadB*::Tn mutant was also inhibited in axenic medium, as was replication of the complemented strain *nadB*::Tn pFLAG:*nadB* (Fig. 5B and C), suggesting that meso-tartrate is able to inhibit other crucial processes.

DISCUSSION

Disrupting the NAD *de novo* biosynthesis pathway in *C. burnetii* results in significantly reduced replication inside host cells (Bitew *et al.* 2018), suggesting that targeting this prokaryotic specific pathway may result in novel therapeutics. Recent studies demonstrated that targeting NadD and NadE, enzymes downstream of NadB in the NAD *de novo* biosynthesis pathway, may lead to the production of highly effective drugs against the important human pathogen *Mycobacterium tuberculosis* (Rodionova *et al.* 2014; Osterman *et al.* 2019). In the current

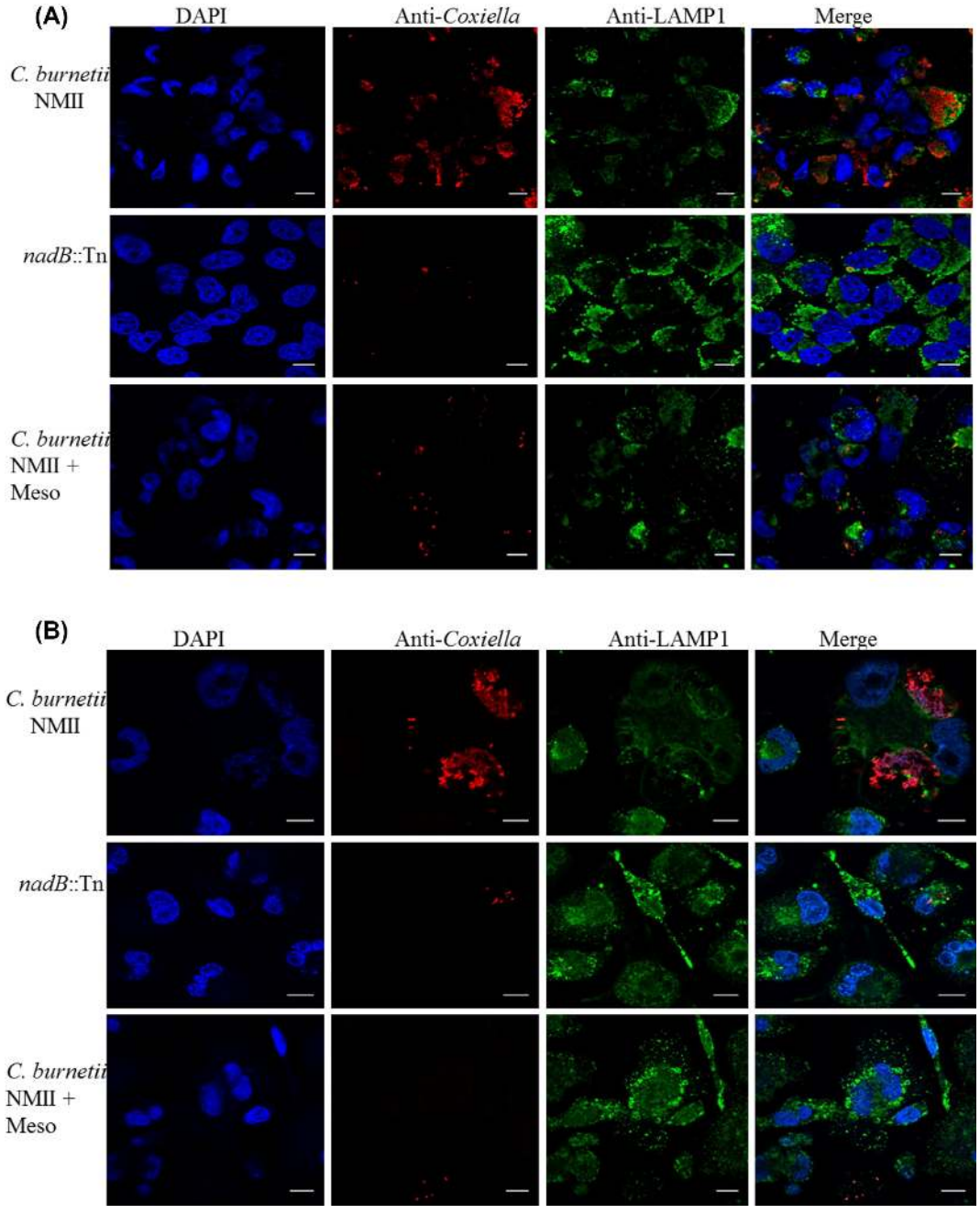

Figure 3. Representative micrographs demonstrating that meso-tartrate treatment of host cells results in smaller CCVs and fewer bacteria inside host cells. HeLa cells **(A)** and THP-1 cells **(B)** were infected with *C. burnetii* strains at multiplicity of infection (MOI) of 50 and 25, respectively. Three days post-infection the cells were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS). The cells were stained with rabbit anti-*Coxiella* antibody (red) and mouse anti-LAMP1 antibody (green) in blocking buffer and DNA stained with DAPI (blue) diluted in PBS. Both the *nadB* mutant and wild-type *C. burnetii* + meso-tartrate produced smaller vacuoles containing fewer bacteria, whereas wild-type *C. burnetii* − meso tartrate formed much bigger vacuoles containing a large number of bacteria. Image acquisition was conducted by Nikon A1R confocal microscope and the images are representative of three independent experiments. A 10 μm scale bar was used for these images. Meso = meso-tartrate.

study, meso-tartrate inhibited both NadB activity *in vitro* and intracellular replication of *C. burnetii* NMII, without causing cytotoxicity in host mammalian cells, suggesting it could have exciting potential as a therapeutic agent. However, and surprisingly, meso-tartrate also inhibited growth of *C. burnetii* NMII *in vitro*. NadB is not required for *C. burnetii* to replicate *in vitro,* presumably because NAD salvage pathways are able to compensate for the absence of *de novo* NAD synthesis in the rich growth medium used *in vitro* (Bitew *et al.* 2018). Combined with the observed inhibition of replication of the *nadB*::Tn mutant both inside host cells and in axenic medium, this strongly indicates that meso-tartrate is targeting other prokaryotic-specific pathways. A previous historical study observed that meso-tartrate inhibited the growth of certain *Salmonella* strains *in vitro* (Old, Alfredsson and Brown 1980), which was thought to be due to inhibition of the tricarboxylic acid (TCA) cycle. As the TCA cycle is also essential in mammalian cells, this may be why the therapeutic potential of meso-tartrate as an antibacterial agent has not been

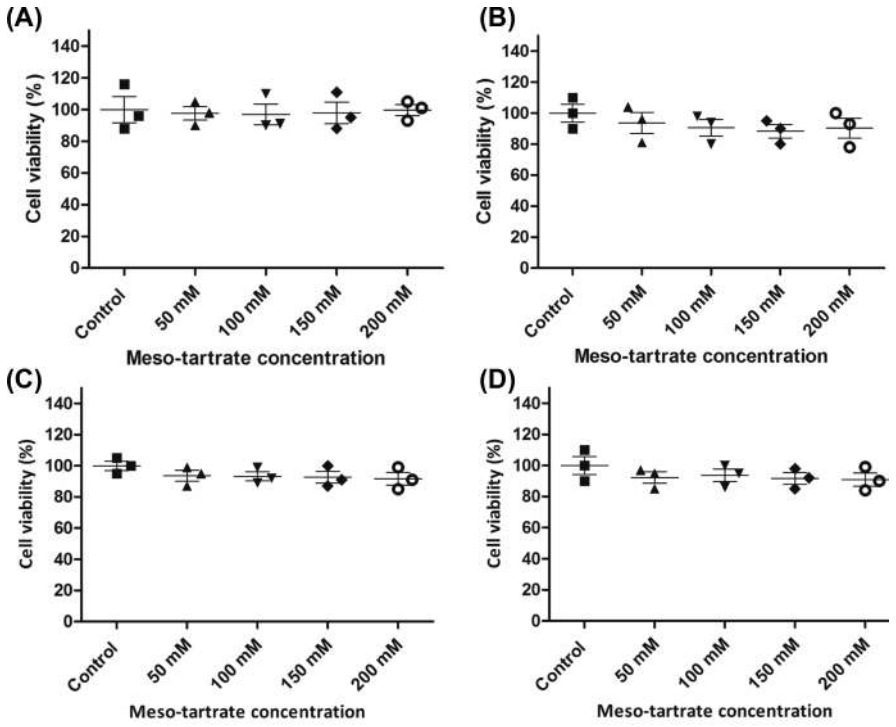

Figure 4. Cell cytotoxicity assay. Viability of HeLa cells at 24 h **(A)** or 7 days **(B)** and THP-1 cells at 24 h **(C)** or 7 days **(D)** post-treatment with meso-tartrate. Data are represented as the percentage of viable cells compared to the untreated control. Graphs represent the mean ± SD of three independent experiments, with the symbols indicating the individual mean of each biological replicate and the horizontal line the mean of all three replicates. No significant differences were observed (Student's *t*-test).

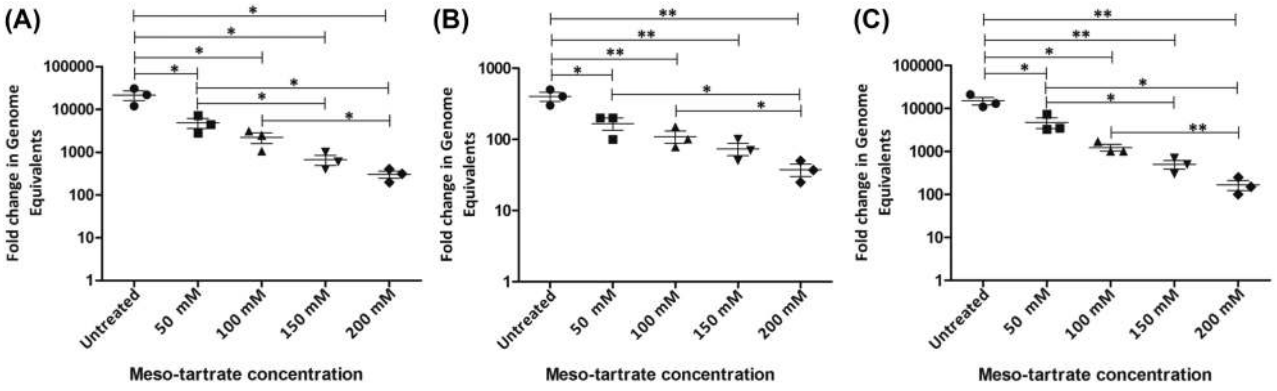

Figure 5. Meso-tartrate has a dose-dependent effect on intracellular replication of *C. burnetii*. THP-1 cells were infected with *C. burnetii* NMII **(A)**, the *nadB*:Tn mutant **(B)** and the complemented strain (*nadB*::Tn pFLAG:*nadB*) **(C)** at MOI of 25 and treated with 50, 100, 150 and 200 mM meso-tartrate. At day 7, the infected cells were harvested and genome equivalents were determined by qPCR and are represented as to inoculum. All meso-tartrate concentrations significantly reduced the fold change in genome equivalents compared to the control, and the highest concentration tested (200 mM) significantly inhibited replication more than either 50 or 100 mM, for all three strains tested. The graphs represent the mean ± SD of three independent experiments, with the symbols indicating the individual mean of each biological replicate and the horizontal line the mean of all three replicates. * $P < 0.05$, ** $P < 0.01$ (unpaired two-tailed *t*-test).

previously explored. However, the lack of toxicity to mammalian cells observed in the current study suggests that the additional targets inhibited by meso-tartrate are prokaryotic-specific.

Interestingly, NadB is not considered a therapeutic target in *M. tuberculosis* as in this bacterial species the enzyme is dispensable for virulence (Rodionova *et al.* 2014). However, as meso-tartrate appears to be acting more broadly it may be worth investigating its effect on mycobacteria, as well as investigating if it can inhibit the replication of a number of other intracellular pathogenic bacteria. The concentrations of meso-tartrate used in this study to achieve inhibition of replication are relatively high, suggesting that, despite its lack of toxicity on mammalian cells, in its current form meso-tartrate itself may not be a useful sole therapeutic agent. However, new compounds derived from meso-tartrate may be more potent and of use in either sole or combination therapies. Furthermore, future work identifying the other targets of meso-tartrate is likely to identify additional novel therapeutic targets.

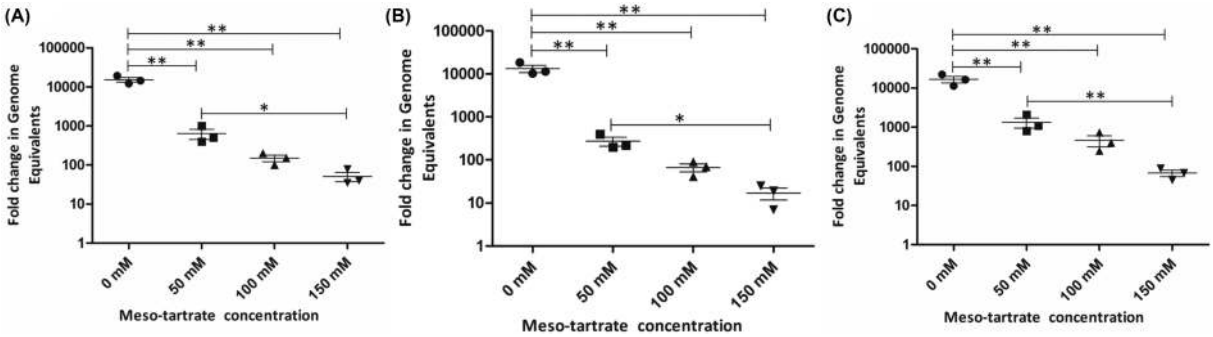

Figure 6. Meso-tartrate inhibits growth of *C. burnetii in vitro*. qPCR was used to quantify *C. burnetii* genome equivalents after 7 days of *in vitro* culture and the fold change in genome equivalents relative to the inoculum was determined. The growth of *C. burnetii* NMII **(A)**, the *nadB*:Tn mutant **(B)** and the complemented strain (*nadB*::Tn pFLAG:*nadB*) **(C)** were all significantly inhibited by supplementing medium with 50, 100 and 150 mM of meso-tartrate. The graphs represent the mean ± SD of three independent experiments, with the symbols indicating the individual mean of each biological replicate and the horizontal line the mean of all three replicates. * $P < 0.05$, ** $P < 0.01$ (unpaired two-tailed *t*-test).

MATERIALS AND METHODS

Cell strains and culture conditions

Plaque purified *C. burnetii* Nine Mile phase II (NMII) strain RSA 439 and the derivative strains *nadB*::Tn and *nadB*::Tn pFLAG:*nadB* (Bitew *et al.* 2018) were cultured in liquid Acidified Citrate Cysteine Medium (ACCM-2) (Omsland *et al.* 2009) at 37°C with 5% CO_2 and 2.5% O_2. Chloramphenicol at a final concentrations of 3 μg/ml was used for transposon selection when culturing the *C. burnetii nadB* mutant and kanamycin (350 μg/ml) used to select for pFLAG:*nadB* (Bitew *et al.* 2018). Expression and purification of recombinant GST-NadB and GST was carried out using *E. coli* JM109 strain. *Escherichia coli* was cultured in Luria–Bertani medium and ampicillin (100 μg/ml) added for plasmid selection. HeLa CCL2 cells were grown and maintained in Dulbecco's Modified Eagle's Medium (DMEM) with 5 or 10% fetal calf serum (FCS) at 37°C in 5% CO_2. THP-1 cells were grown in RPMI 1640 supplemented with 5 or 10% FCS at 37°C in 5% CO_2.

NadB inhibition assays

Recombinant GST-NadB was expressed in *E.coli* JM109 and purified as described previously (Bitew *et al.* 2018). The two-step enzyme assay to determine L-aspartate oxidase activity of NadB was carried out as described previously (Bitew *et al.* 2018). For the assays to determine the inhibitory effect of meso-tartrate, the first step of the assay consisted of 100 mM bicine, pH 8.0, 2μM FAD, 10 mM L-aspartate, 0.5 mg bovine serum albumin and 4.9 μg of NadB, 4.9 μg GST or 4.9 μg of NadB + 200 mM meso-tartrate (Sigma). The pH of the reaction mixture was confirmed to be 8.0 after the addition of meso-tartrate, to ensure any inhibitory effects observed were not due to a change in pH. After 20 min of incubation, the reaction was stopped by adding ice cold 1.7 M per chloric acid, centrifuged (1000 × *g*, 3 min, RT) to remove denatured protein and the supernatant transferred to a new tube and the reaction neutralised by adding 5 M potassium hydroxide. Secondly, 0.1 mM NADH, 2.5 mM EDTA and 100 mM bicine were added to a total volume of 1 ml. The second step of the reaction was initiated by adding 10 U of malate dehydrogenase (MDH; Sigma) and absorbance at 340 nm, 25 °C was measured at 1 min interval for 8 min and the average OD calculated at each time point following subtraction of the blank absorbance reading.

Axenic growth inhibition assay

Coxiella burnetii strains were inoculated into ACCM-2 supplemented with 0, 50, 100 or 150 mM of meso-tartrate (Sigma) at 1×10^4 genome equivalents/ml and incubated for 7 days at 37°C in 5% CO_2 and 2.5% O_2. Aliquots were taken from cultures and the *C. burnetii* genome equivalents were quantified by qPCR using *ompA* specific primers, as described previously (Jaton *et al.* 2013). Each experiment was conducted three times and significance determined by Student's *t*-test relative to untreated group.

Cell viability test

The cytotoxicity of meso-tartrate was determined by measuring mammalian cell viability using a Cell Counting Kit (CCK-8) assay (Sigma). HeLa CCL2 cells were seeded in 96-well plates at a density of 1×10^4 cells per well in DMEM + 10%FCS. The cells were pre-incubated for 24 h in a humidified incubator at 37°C, 5% CO_2. Three days prior to treatment, THP-1 cells were seeded at a density of 1×10^4 cells per well in RPMI + 10%FCS in 96-well plates and treated with 10 nM phorbol 12-myristate 13-acetate to induce differentiation into macrophage-like cells. Meso-tartrate (Sigma) was dissolved in DMEM (Dulbecco's Modified Eagle Medium) or RMPI (Roswell Park Memorial Institute medium) and a final concentration of 50, 100, 150 or 200 mM of this solution was added onto cells and incubated for 24 h or 7 days. To measure viability, 10 μl of CCK-8 reagent was added to each well and the absorbance at 450 nm was measured using microplate reader following 4 h incubation. Untreated cells were used as a control and the viability of treated cells was compared to these control cells. Three independent experiments were conducted.

Intracellular replication of *C. burnetii*

Intracellular replication assays were carried out as described previously (Newton *et al.* 2014; Fielden *et al.* 2017). Briefly, 5×10^4 HeLa CCL2 cells were seeded into 24-well plates in DMEM with 10% FCS (5 trays – day 0, 1, 3, 5 and 7; strains in duplicate) the day before infection. *Coxiella burnetii* strains were grown in ACCM-2 and pelleted and resuspended in 10 ml of DMEM + 5% FCS. *Coxiella burnetii* genome equivalents were quantified by qPCR amplifying *ompA* to determine the MOI of 50, as described previously (Jaton *et al.* 2013). *Coxiella burnetii* diluted in DMEM + 5% FCS

or DMEM + 5% FCS supplemented with 200 mM meso-tartrate was added to each well. After 4 h of infection, the media was removed from all trays and wells and washed with PBS to remove uninfected bacteria. For day 1, day 3, day 5 and day 7 plates, fresh DMEM + 5% FCS or DMEM + 5% FCS + 200 mM meso-tartrate was added whereas for the day 0 timepoint 500 μl of distilled water was added to the wells and incubated at RT for 10 min to lyse the cells. The cells were harvested by scraping using pipette tips and the harvested lysate was pelleted and resuspended in distilled water. The *C. burnetii* genome equivalents were quantified by qPCR using *ompA*-specific primers.

THP-1 cells were seeded at 5×10^5 cells/well 72 h before infection and treated with 10 nM phorbol 12-myristate 13-acetate to induce differentiation into macrophage-like cells. The cells were infected by *C. burnetii* strains at an MOI of 25 in RPMI + 5% FCS or RPMI + 5% FCS + 200 mM meso-tartrate, and intracellular growth assays were carried out in a similar manner as performed for HeLa cells.

Immunofluorescence microscopy and observation of CCV biogenesis

Both HeLa CCL2 and THP-1 cells were seeded in 24-well plates with 10 mm glass coverslips and infected with *C. burnetii* strains in a similar way as described for the quantitative assays. After 3 days post-infection, the media ware removed and the cells were fixed with 4% paraformaldehyde in PBS and blocked with PBS + 2% (w/v) BSA + 0.05% (v/v) saponin for 1 h. The cells were stained with rabbit anti-*Coxiella* and mouse anti-LAMP1 antibodies in blocking buffer at 1/10 000 and 1/500 dilutions, respectively. Antirabbit 568 (*Coxiella*) and antimouse 488 (LAMP1) diluted at 1/3000 in blocking buffer were used as a secondary antibodies. DAPI diluted at 1/10 000 in PBS was used to stain host DNA. Following staining, the coverslips were mounted on glass slides using Dako Fluorescent Mounting Medium. Nikon A1R confocal microscope was used to capture images and analysed by ImageJ.

Dose-dependent response analysis in THP-1 cells

To examine the effect of different concentrations of meso-tartrate on intracellular growth and CCV formation of *C. burnetii*, growth inhibition assays were carried out using differing concentrations of meso-tartrate. THP-1 cells were seeded in 24-well plates as described for the 7 days infection experiments and infected with *C. burnetii* strains at MOI of 25 and treated with 50, 100, 150 and 200 mM meso-tartrate in RPMI + 5%. Seven days post-infection the cells were harvested and the *C. burnetii* genome equivalents quantified by qPCR amplifying *ompA* and the fold change in genome equivalents determined relative to the inoculum. Three biological repeats were performed.

Conflict of interest. None declared.

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