The efficacy of antibiotics in reducing morbidity and mortality from heatstroke – A systematic review

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TITLE PAGE

Title: The efficacy of antibiotics in reducing morbidity and mortality from heatstroke – a systematic review.

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Highlights

- Hyperthermia, e.g. from exercise or heat waves, is associated with significant morbidity and mortality
- Endotoxaemia presumed from the gastrointestinal tract is common in hyperthermia
- Antibiotics against intestinal bacteria may improve outcome after heatstroke in animals
- Conclusive evidence in humans is lacking

Declarations of interest

None

Author contributions

Both authors were involved in the conceptualisation, data collection and analysis, and the writing of the paper. Both authors have seen and approved the final version.

ABSTRACT

Severe hyperthermia, for example, classical heatstroke or exertional heatstroke from heatwaves or exercise respectively, or from drug ingestion or other non-infective pyrogens, is associated with a high mortality and morbidity, which may be chronic or permanent. Abolition of lipopolysaccharide, from gram-negative intestinal bacteria translocating into the systemic circulation via an intestinal wall rendered permeable from the hyperthermia, reduces the adverse effects, suggesting that antibiotics against the intestinal bacteria may have a similar effect. A systematic review searching Embase, MEDLINE and PubMed from the earliest date available until 2019 was conducted, according to PRISMA guidelines. Two papers were found which fit the criteria. In one, non-absorbable oral antibiotics were administered prior to the onset of heat stress, which reduced the cardiovascular dysfunction and rise in endotoxaemia, but animals succumbed at a lower temperature. In the second, non-absorbable oral antibiotics, in combination with a laxative and enema, given prior to the onset of heat stress, improved mortality; antibiotics administered after the heat stress did not, but the antibiotics used may have limited action against intestinal bacteria. Only two papers were found; both suggest an improvement in organ dysfunction or mortality after an episode of heat stress. No papers were found that investigate the sole use of antibiotics effective against intestinal bacteria given after the onset of heat stress, although biological plausibility suggest they warrant further research.

KEYWORDS

Antibiotic, hyperthermia, heat stress, endotoxaemia.

Abbreviations

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Footnotes

None

Review registration

Registered with PROSPERO database (reference 159923)

1. INTRODUCTION

A critically raised temperature may be due to a variety of infective and non-infective causes. The latter includes classical heatstroke (for example, in heatwaves), exertional heatstroke (for example, in athletes and the military, after strenuous physical activity) and drug-induced hyperthermia. The non-infective causes have a high mortality. Classical heatstroke (CHS) is associated with a mortality rate between 40% (Jimenez-Mejias et al., 1990) and 64% (Pease et al., 2009); exertional heatstroke (EHS), defined as a core temperature (T_{CORE}) above 40.5°C with neurological dysfunction, is the third commonest cause of death in athletes (Casa et al., 2017). In survivors of episodes of extreme hyperthermia, there is a risk of multi-organ failure, including renal (Carter et al.,2005; Dematte et al., 1998), liver (Alzeer et al., 1997) and neurological dysfunction, from which recovery may be delayed (Walter and Carraretto, 2016).

Increasing temperatures increase gastrointestinal paracellular permeability (Mosely et al., 1994), which can occur after as little as 60 min at temperatures above the critical thermal maximum (41.6-42.0°C)(Lambert et al., 2002), and at temperatures as low as 39°C (Dokladny et al., 2006). Strenuous exercise also increases intestinal permeability, but whether this is driven by the exercise or resulting hyperthermia is unclear (Pals et al., 1997). Translocation of intestinal bacteria into the systemic circulation may occur as intestinal wall permeability increases. Over 50% of patients with CHS show evidence of concomitant bacterial infections (Dematte et al., 1998). Furthermore, procalcitonin, which has a high sensitivity and specificity for detecting bacteraemia, was elevated in 58% of patients with CHS and was associated with mortality (Hausfater et al., 2008). Microbiological and clinical evidence of infection was not however significantly higher in these patients. and therefore it is unclear

whether this represents undiagnosed bacteraemia or procalcitonin elevated in the absence of infection.

Lipopolysaccharides (LPS) are large molecules forming part of the outer membrane of gram-negative bacteria, for example, in the gastrointestinal tract, and stimulate production of pro-inflammatory mediators if they enter the systemic circulation (Opal, 2007) with a reduction in systemic LPS significantly reducing cytokine production (Heled et al., 2013). Reductions in intestinal bacterial load by non-absorbable antibiotics reduces circulating LPS levels (Cuevas et al., 1972). LPS contains a hydrophobic domain, known as endotoxin, which appears to be responsible for some of the deleterious effects of hyperthermia, Heat stress is known to produce endotoxaemia in rodents (Hall et al., 2001), primates (Gathiram et al., 1988a) and humans (Bouchama et al., 1991). Administration of exogenous LPS can create diffuse endothelial injury, tissue hypoperfusion and refractory shock (Opal, 2007) and attenuation of systemic LPS by corticosteroids (Gathiram et al., 1988b) or by anti-LPS antibodies (Gathiram et al., 1987b) improves survival after heat stress.

The proposed pathway of endotoxaemia and subsequent pro-inflammatory response from gastrointestinal gram-negative bacteria entering the systemic circulation via a permeable gastrointestinal wall allows several potential sites for modulating the pathway and affecting the outcome from heat stress. It is proposed that antibiotics active against gastrointestinal bacteria may reduce the bacterial translocation and endotoxaemia, and therefore improve the outcome from non-infective hyperthermia.

2. METHODS

Evidence for the clinical effectiveness of antibiotics in the acute treatment of hyperthermia and heatstroke was assessed by conducting a systematic review of published research evidence. The review adhered to the PRISMA guidelines (Shamseer et al., 2015).

2.1. Identification of studies

Randomised controlled trials were identified by searching three electronic medical databases (MEDLINE, Embase and PubMed), from the earliest date until August 2019. In addition, the EU Clinical Trials Register and the Cochrane library were searched. Further attempts to identify studies were made by examining the reference lists of all retrieved articles and review articles identified by the original searches. Cancer terms were excluded from searches, to eliminate papers detailing hyperthermia as a treatment for malignancy.

The search terms used for the three searches are summarised as follows, and detailed in appendix A: The Embase database was searched from 1974 to August 2019, using the 'explosion' search terms of 'heat stress', 'heat injury', 'hyperthermia' and 'antibiotic agent'. The Medline database was searched from 1950 to August 2019, using the 'explosion' search terms of 'heat stress disorders' and 'anti-bacterial agents'. The PubMed database was searched from 1966 to August 2019, using the search terms 'antibiotic*', 'heat illness', 'heat stroke', and 'heat stress' in the titles or abstracts. No limits on any searches were set.

2.2. Inclusion and exclusion criteria

Two reviewers independently screened all titles and abstracts. Full-text papers of any titles and abstracts that were considered relevant by either reviewer were obtained where possible. The relevance of each study was assessed according to the inclusion criteria stated

in table 1. Studies that did not meet the criteria were excluded. Any discrepancies were resolved by consensus.

Study design	RCTs
Population(s)	Animal or human studies
Intervention(s)	Administration of antibiotic before or after exposure to hyperthermia or heat stress
Comparators	The intervention will be compared with the control group
Outcomes	Organ dysfunction or death

Table 1 – design and characteristics of published studies to be identified from the literature search. Studies meeting these criteria will be included in the initial (stage 1) screening.

2.3 Types of intervention

We sought published RCTs where antibiotics or antimicrobial agents were administered to animals or humans, compared against placebo. There were no restrictions on the class of antibiotic, or dose, timing or frequency of administration.

2.4 Outcome measures

We included trials where the antibiotics or antimicrobial agents were assessed against survival data, or evidence of organ dysfunction. We considered that these outcomes were the most clinically relevant. Survival data was taken as the primary outcome; organ dysfunction was taken as the secondary outcome.

2.5 Assessment of bias

The studies were assessed for bias by using the Cochrane-recommended ROB2 system (Sterne et al, 2019).

2.6 Measures of treatment effect

We included any measure of mortality, including survival time, temperature at which death occurred, and absolute survival numbers after cessation of heat insult or trial.

2.7 Exclusion

In vitro studies and studies not including a comparator (hyperthermic but did not receive antibiotics) group were excluded.

2.8 Subgroup analysis

Additional statistical analysis between the intervention and control groups was undertaken where appropriate if not reported in the study. The Student t-test was used for continuous outcome data; the chi-squared statistic for discrete outcome data.

3. RESULTS

Electronic searches identified 2749 citations. Hand searches revealed no further studies. Their titles and abstracts were assessed for their relevance to the review (stage 1 screening), resulting in 70 potential citations being retained. The full texts of these citations were obtained. After applying inclusion criteria to these full-text papers (stage 2 selection), 68 citations were excluded. Two citations were therefore included in the systematic review (appendix B, figure 1). No studies were found that investigated the secondary outcome that did not also investigate mortality.

Both studies were considered to have low risk of bias, according to the ROB2 bias assessment method (Sterne et al., 2019) (see table 4).

The studies were considered too heterogeneous in the methodologies and outcome measures for a meta-analysis to be performed. A narrative description is therefore presented.

Study	Date of Study	Country	Commercial / financial support
Gathiram	1987	South Africa	Chamber of Mines, Johannesburg, South Africa
Bynum	1979	US	None declared

Table 2 – study characteristics detailing the date, country and financial support of the papers included in the final analysis

Study	Intervention	Species	Number of control / intervention subjects	Measure of mortality outcome	Summary of findings
Gathiram (1987)	Kanamycin prior to heat stress	Monkey	4 / 4	Temperature at death	44.1°C (intervention)* 44.6°C (control)
Bynum (1979)	Group 1: heated control Group 2: unheated control Group 3: Neomycin, tetracycline, laxative and enema before, and penicillin after heat stress. Group 4: IV penicillin after heat stress	Dog	Group 1: 15 Group 2: 11 Group 3: 17 Group 4: 7 Total: 50	% survival	20.0% (control) 70.6%† (pre-insult antibiotics) 14.2% (post-insult antibiotics)(ns)

				Survival time	3.8 ± 1.2 h (control) 10.6 ± 2.7 h (pre-insult antibiotics)* 3.2 ± 0.9 h (post-insult antibiotics)(ns)

Table 3 – details of intervention, outcome to be measured and clinical effect of the administered antibiotic in the papers included in the final analysis

Key:

* statistically different to control ($p<0.025$)

ns = not significant ($p > 0.05$)

† statistically different to control ($p<0.005$)

Study	**R**	**D**	**Mi**	**Me**	**S**	**Overall**
Bynum (1979)	Low	Low	Low	Low	Low	Low

Gathiram (1987)	Low	Low	Low	Low	Low	Low

Table 4 – Details of risk of bias assessment of the papers included in the final analysis, according to the ROB2 method of assessment.

Key:

R: Risk of bias arising from the randomisation process

D: Risk of bias due to deviations from the intended interventions

Mi: Missing outcome data

Me: Risk of bias in measurement of the outcome

S: Risk of bias in selection of the reported result

3.1 Mortality

In the first study (Gathiram et al., 1987a), four anaesthetised monkeys were given 15 $mg.kg^{-1}$ oral kanamycin every 12 hours over five consecutive days before being heat stressed in a forced draft incubator at 41.0°C. Four other heat-stressed anaesthetised monkeys served as controls. No animals survived, but the antibiotic-treated animals died at a lower T_{CORE} (44.1°C) than did the controls (44.6°C) ($p < 0.025$). No definition of heat stress was documented in the study, but initial serum levels for enzyme activity and LPS were taken when the rectal temperature first reached 40°C.

In the second study (Bynum et al., 1979), fifty anaesthetised dogs in three intervention groups were heated by a water blanket until the rectal temperatures rose to 43.5°C. Animals were then cooled passively in room air at 28°C until death occurred or 18 h had elapsed, and were euthanized. The first and second groups did not receive manipulation of bowel contents; the first group of 15 animals was heated to induce heatstroke, while the second group were not heated. The third group (12 animals) was heated in a similar manner to the first, but in addition received 1000 mg neomycin and 250 mg tetracycline four times per day for four days prior to the heat stress, and a laxative and enema prior to the heat stress, and 1 million units of intravenous penicillin every four hours during the heating and cooling periods. The fourth group (7 animals) were heated in the same way as the first and third, but only received the intravenous penicillin during and after heating, and not the prior antibiotics and laxatives that the third group received. The third group, who had intestine stool and bacterial contents reduced showed an increase in 18-h survival from 20.0% to 70.6%. In the group where antibiotics were administered after heatstroke, survival was 14.2% and not significantly different to survival in the heated but untreated group. The

paper also reported average survival times of the animals who succumbed before the 18 h had elapsed in the pre-heat antibiotic group (10.6 ± 2.7 h, n = 5), post-heat antibiotic group (3.2 ± 0.9 h, n= 6) and the heated control group (3.8 ± 1.2 h, n = 12). There was a significant improvement in survival time between the group receiving pre-insult antibiotics, but not between the group receiving post-insult antibiotics and the control group. The authors used a core temperature of 43.5°C, citing previous work suggesting heatstroke occurs at this temperature.

3.2 Organ dysfunction

The study by Gathiram et al. (1987a) reported changes in cardiovascular and hepatic function between the two primate groups. Cardiovascular function, as measured by mean arterial blood pressure (MAP) and heart rate during heat stress were more unstable in the control group and began to deteriorate at a lower temperature than in the group receiving antibiotic. As the T_{CORE} rose, the MAP in the control group increased up to 41°C, then gradually declined until 43°C, after which there was a rapid decline. The MAP in the antibiotic group showed a similar pattern, but at a level about 10-20 mmHg higher than the controls throughout the whole temperature range. No statistical significance was reported. The rapid fall in MAP coincided with a rapid increase in heart rate and shortly before the rapid increase in plasma LPS. As T_{CORE} rose, the heart rate in the control group generally increased until 44°C after which it declined rapidly until a temperature of about 44 5°C. At any given T_{CORE} between 39°C and 43 5°C, the heart rate was higher than those recorded for the controls.

Aspartate aminotransferase (AST) levels were significantly raised at 43°C in the control group compared with 37°C, and compared with 43°C in the intervention group. With the

exception of AST, there was no significant change in other liver function biomarkers. No data on organ dysfunction were reported in the canine study (Bynum et al., 1979).

3.3 LPS

In the study by Gathiram et al. (1987a), the plasma LPS concentration increased seven-fold in the control group. No significant increase in LPS from baseline was seen in the intervention group. LPS levels when the rectal temperature reached 44-45°C were different between the intervention group (0.308 ± 0.038 $ng.ml^{-1}$) and the control group (0.005 ± 0.002 $ng.ml^{-1}$) .No data on cytokine or endotoxin level were reported in the canine study (Bynum et al., 25).

3.4 Bacterial load

Gathiram et al. (1987a) reported a significant reduction in the group pre-treated with antibiotics, compared with the control group. The total bacterial count fell from 12 56 ± 1 83 colony-forming units x 10^9/g faeces to 0.183 ± 0 04 x 10^9/g faeces between the two groups primarily due to a large reduction in Gram-negative bacteria. There was an increase in the Gram-positive bacterial count. No data on the bacterial load in the group receiving antibiotics after the heat stress were reported.

4. DISCUSSION

Given that only two animal studies met the search criteria of the effect of antibiotics on organ dysfunction or mortality, applications to humans during heat stroke are difficult. The first study suggested that antibiotic treatment against gut bacteria may reduce cardiovascular dysfunction and prevent deterioration in hepatocellular dysfunction, but not the temperature at which death occurred; the second suggests that antibiotics administered prior to, but not after the heat stress may be beneficial, thus limiting applicability when treating humans. The examined orally administered antibiotics, neomycin and kanamycin, are active against intestinal bacteria, but do not cross into systemic circulation in sufficient quantity to have systemic effect (Kunin et al., 1960), suggesting that any effect on the endotoxaemia would be from alteration in the intestinal microbiome.

In both studies, the animals received antibiotics prior to the heat insult. Part of the Bynum study was the only study where antibiotics were given after heat trauma. However, intravenous penicillin was administered, which has limited efficacy against the predominant Gram-negative bacteria in the intestinal tract, probably as a result of the penicillinases produced by the bacteria (Sutherland, 1964). We were unable to find any studies investigating the effect of antibiotics effective against intestinal bacteria where the antibiotics were given after the onset of heat stress. However, antibiotics have a proven efficacy when given after the onset of a septic insult, although with decreasing efficacy as time progresses (Kim et al., 2018); if the pathophysiology of heat stress involves bacterial translocation and endotoxaemia, then, in similarity with sepsis, post-insult antibiotics might also be effective after heat stress.

The first study also showed a significant reduction in systemic levels of lipopolysaccharide, similar to other studies (Hall et al., 2001; Gathiram et al., 1988a; Bouchama et al., 1991). The orally administered antibiotics in this study are very poorly absorbed, suggesting that the systemic LPS is likely to be derived from the gastrointestinal tract. LPS is associated with a poor outcome after heat stress (Opal, 2007; Gathiram et al., 1988b; Gathiram et al., 1987b). While there was improvement in cardiovascular function in the study by Gathiram et al. (1987a), the animals pre-treated with antibiotics died at a lower temperature. However, what is not known is the rate of rise of core temperature, which was not reported in the study but known to be important in heat stress. The first group of the study by Bynum et al. (1979) also received an enema in addition to the antibiotics, which may have reduced intestinal contents and affected the systemic absorption.

No human studies were found, and it is not clear whether the animal studies are relevant to humans. While primates show a similar heat shock response to humans (Bouchama et al., 2005a; Bouchama et al., 2005b), the possibility of interspecies differences remain. There are functional and morphological differences in the canine response to heat stress compared with humans (Bruchim et al., 2017), making comparison with human pathophysiology less reliable. This includes a hepatic sphincter which exaggerates the magnitude of the splanchnic response during shock (Zweifach, 1961). Finally, the effect of anaesthesia on the heat stress response is unclear. In particular, animals in the study by Gathiram et al. (1987a) were anaesthetised with ketamine, which appears to alter the intestinal microbiome (Serbanescu et al., 2019; Schoster et al., 2016). The effect on heat stress manifestation is

unclear. In addition, an anaesthetised animal would not demonstrate the cardinal feature of heatstroke of neurological obtundation.

5. CONCLUSION

In this systemic review on the efficacy of antibiotics, only two papers were found, which suggested an improvement in organ dysfunction or mortality after an episode of heat stress. No papers were found that investigate the sole use of antibiotics effective against intestinal bacteria given after the onset of heat stress, or in humans, although biological plausibility suggest they warrant further research. These papers suggest that gut bacteria and subsequent endotoxaemia have a role in pathophysiology of heat stress. Antibiotics need to be effective against intestinal bacteria.

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APPENDIX A – Search terms

# Database search	Database	Search term	Results
6	EMBASE	exp "HEAT STRESS"/	9843
7	EMBASE	exp "HEAT INJURY"/	7262
8	EMBASE	exp HYPERTHERMIA/	26550
9	EMBASE	(6 OR 7 OR 8)	41854
10	EMBASE	exp "ANTIBIOTIC AGENT"/	1325681
25	EMBASE	(9 AND 10)	2609
26	EMBASE	(cancer).ti,ab	2205759
27	EMBASE	25 NOT 26	2134
13	Medline	exp "HEAT STRESS DISORDERS"/	5306
14	Medline	exp "ANTI-BACTERIAL AGENTS"/	710545
15	Medline	(13 AND 14)	27
17	PubMed	(antibiotic*).ti,ab	360804
18	PubMed	(heat illness).ti,ab	556
20	PubMed	(heat stroke).ti,ab	3292
21	PubMed	(heat stress).ti,ab	54325
22	PubMed	(hyperthermia).ti,ab	237457
23	PubMed	(18 OR 20 OR 21 OR	290007

		22)	
30	PubMed	17 AND 20	13
31	PubMed	17 AND 18	1
32	PubMed	17 AND 21	574
33	PubMed	17 AND 22	17970
34	EMBASE, Medline	(antibiotic* AND heat stroke).ti,ab [Humans]	18

APPENDIX B

Figure 1 – PRISMA flowchart