Prevention of endotoxaemia by non-absorbable antibiotics in heat stress

P GATHIRAM,* M T WELLS, J G BROCK-UTNE, B C WESSELS, S L GAFFIN

From the Departments of Physiology,* University of Durban-Westville and University of Natal Medical School, Durban, South Africa

SUMMARY Four anaesthetised monkeys were given oral kanamycin (15 mg 1 kg 12 hourly) over five consecutive days before being heat stressed. Four other anaesthetised monkeys served as controls. The plasma lipopolysaccharide concentration in control primates increased initially from 0·044 (SEM 0·004) ng/ml to 0·062 (0·006) ng/ml as the rectal temperature increased from 37·5 to 39·5°C. A second increase in lipopolysaccharides started at 42°C and reached 0·308 (0·038) ng/ml (p < 0·01) at 44·5°C. Before heat stress the plasma lipopolysaccharide concentration in the primates who had been pretreated with kanamycin was 0·007 (0·006) ng/ml, and despite heating these animals to 44·5°C no increase in plasma lipopolysaccharide concentrations were seen in this group. The cardiovascular variable during heat stress were more unstable in the control group and began to deteriorate at a lower temperature than in the group receiving antibiotic. These data suggest that the increased plasma lipopolysaccharide concentration during heat stress originates mainly from the gut.

Recent evidence shows that endotoxins (lipopolysaccharides or LPS), the highly toxic components of the outer cell membrane of Gram negative bacteria, may have a role in heat stroke pathophysiology.1–6 The lumen of the mammalian gut always contains Gram negative bacteria, and hence LPS. Normally, the gut wall is impermeable or slightly permeable to LPS. Small amounts of LPS that may leak into the portal circulation are detoxified by the reticuloendothelial system.7 Damage to the gut wall by ischaemia, trauma, hyperthermia, vasoactive agents, ionising radiation, hypoxia, and viral gastroenteritis, however, enables LPS to leak rapidly into the portal and systemic circulations.3–8–13 Should the reticuloendothelial system of the liver and spleen become overwhelmed by the rapidly rising blood LPS concentrations or should the reticuloendothelial system function be inadequate to remove the LPS, then the plasma LPS concentration in the systemic circulation would rise and persist in the circulation, eventually causing vascular collapse, shock, and death.9–14

In a previous study we determined the time course of changes in circulating plasma LPS concentrations and cardiovascular variables in heat stressed monkeys.1 Furthermore, high concentrations of plasma LPS have been detected in a few cases of fatal heat stroke.2,5 In addition, reduction of gut flora with antibiotics has been found to increase an 18 hour survival in heat stressed dogs4 and to reduce the prevalence of endotoxaemia in heat stressed rabbits.9 We believe that during heat stress the reduced blood flow to the visceral regions15,16 coupled with the high core temperature leads to damage to the permeability properties of the gut mucosa and results in leakage of LPS into the portal and lymphatic circulation.

In this study an attempt has been made to show that the increase in LPS that occurs during heat stress is intestinal. The amount of endogenous Gram negative flora and hence LPS in the gut can be reduced by the oral administration of a non-absorbable antibiotic before heat stress.

Material and methods

Eight monkeys (Cercopithecus aethiops) of either sex, weighing between 2·7 and 6·8 kg were used. They were anaesthetised with ketamine (10 mg/kg, given intramuscularly). Thereafter, incremental intravenous bolus doses (5–10 mg/kg intravenously) of ketamine were used to maintain anaesthesia. The animals were divided into two groups. Four of them received kanamycin (15 mg/kg) (Kantrexil suspension, the B-M Group Ltd) every 12 hours over five consecutive days via a nasogastric tube prior to heat stress; four others served as controls.

After cleansing both the femoral areas with chlor-
hexidine gluconate catheters were introduced into both femoral arteries and a peripheral vein. One of the arterial catheters was connected to a B188 Stat- 
ham transducer for recording of blood pressure, while blood samples were collected at various times from the opposite catheter. Only equipment without pyro-
gen was used, and each heparinised plastic tube into 
which the blood was collected was stored on ice until centrifuged for LPS and anti-LPS IgG analyses. After 
each withdrawal of blood sample an equal volume of 
physiological saline was reinfused. Arterial pressure 
was recorded using a Honeywell CM 130 patient 
monitor system at five minute intervals during the 
experiment. Heart rate, systolic, diastolic, and mean 
arterial pressures were calculated electronically and 
presented as a printout with the blood pressure 
recording. The mean arterial pressure (MAP) was 
measured automatically from integration of the area 
under the arterial pressure curve.

Rectal temperatures were recorded continuously 
using a rectal probe inserted about 10 cm into the rec-
tum, and a telethermometer (Yellow Springs Instru-
mont 46-TUC) connected to a chart recorder.

After surgery a 30 minute stabilisation period was 
allowed and then baseline rectal temperature, arterial 
blood pressures, and room temperature were 
recorded, and 1 ml blood sample for LPS and anti- 
LPS analyses and 3 ml for serum enzyme analyses 
were collected in heparinised and non-heparinised tubes, 
respectively. Each animal was then positioned in 
a forced draft incubator, where the environmental 
temperature was maintained at 41·0 (0·3)°C and rela-
tive humidity close to 100%. During heat stress, 
blood samples for LPS and anti-LPS IgG analyses 
were taken at 15–30 minute intervals and for serum 
enzyme analyses when the rectal temperature reached 
40, 42, and 43°C. 

The blood samples for LPS and anti-LPS IgG assay 
were stored in ice until the end of each experiment. 
After centrifugation of the samples the plasma was 
removed under sterile conditions in a laminar flow 
hood. The samples were either analysed immediately 
or stored at −20°C for up to a week until analysed. 
Plasma LPS concentrations were determined using 
the chromogenic substrate modification of the limu-
lus amebocyte lysate (LAL) technique (MA Biopro-
ducts).17 18 We previously found a 95-1% recovery 
from spiked plasma samples using this method, cali-
brating the standard curve with LPS prepared from 
Escherichia coli 0111:B4.19 Relative plasma anti-LPS 
IgG concentrations were determined using an enzyme 
linked immunoabsorbent assay (ELISA).20 The con-
centration of anti-LPS IgG at a rectal temperature of 
37.5°C for each animal was taken as 100%. A detailed 
description of the procedure adopted has already 
been described.4 Boehringer kits were used for deter-
mining the activities of aspartate aminotransferase 
(AST), alanine aminotransferase (ALT), L-γ- 
glutamyl-transferase, alkaline phosphatase and the 
concentrations of bilirubin, albumin, and total pro-
tein in the serum.

Rectal swabs for bacteriological examination were 
taken from all animals after anaesthesia but before 
experimental heat stress. These sterile rectal swabs 
were initially moistened with sterile saline and care 
was taken to avoid perianal contamination. After 
storing in nutrient broth the swabs were individually 
plated out on deoxycholate citrate (DCA) Mac-
Conkey, and nutrient agars. These plates were then 
incubated aerobically for 18–24 hours at 37°C and 
farther subcultured on to DCA. Twenty four hours 
later each plate was examined for non-lactose fer-
menting colonies which were then isolated and 
identified by Gram stain, biochemical reactions, 
colony form and pigmentation.

Immediately after the termination of each exper-
iment the abdomen of each animal was opened. A 
6–7 cm section of the transverse colon was tied off at 
each end and removed. One gram of fecal contents 
was then emulsified in 100 ml of sterile peptone water 
under a laminar flow hood. Serial dilutions of 
10³–10⁸ were made up using an initial 1/100 dilution. 
Triplicate pour plates of 1 ml of each in nutrient agar 
were made for bacterial enumeration. Plates were 
incubated inverted at 37°C for 18–24 hours. Bacterial 
colonies were enumerated and results were reported 
as the number of colony forming units/g faeces.

All data were presented as a mean and (SEM) and 
compared using Student’s t test, the analysis of vari-
ance (ANOVA), and Duncan’s multiple range test21; 
a p value of <0·05 was taken as significant.

Results

The figure shows an initial plasma LPS concentration 
of 0·044 (0·004) ng/ml in the control group similar to 
the low values we have previously seen.21 As the rec-
tal temperature rose there was a small but 
insignificant increase in plasma LPS concentration 
near 39·5°C followed by a large rise at about 42°C 
and reaching 0·308 (0·038) ng/ml (p < 0·01) 
(ANOVA and Duncan’s multiple range test) at about 
44·5°C. Within a few minutes of reaching this rectal 
temperature each animal succumbed. In contrast, 
the group treated with kanamycin showed no significant 
change in plasma LPS concentrations during the 
entire heat stress period. Before this latter group was 
subjected to heat stress the plasma LPS concentration 
0·007 (SEM 0·008) ng/ml was significantly lower than 
that of the controls (p < 0·02) and after heat stress it 
never rose significantly above baseline, and a concen-
tration of 0·005 (0·002) ng/ml was measured just
before the monkeys died. The primates in the group treated with kanamycin succumbed at a significantly lower rectal temperature than those in the control group (44.1 ± 4.6°C) (p < 0.025).

As the core temperature rose the MAP in the control group (figure) increased from 85 (SEM 4.2) mm Hg at a rectal temperature of 37.5°C to 100 (5) mm Hg at 39–41°C. Above 41°C the MAP gradually declined until a rectal temperature of about 43°C when there was a rapid decline. This rapid fall in MAP coincided with a rapid rise in heart rate and shortly before the rapid rise in plasma LPS. In the group treated with kanamycin the MAP curve was similar to that of the controls but at a level about 10–20 mm Hg higher than the controls throughout the whole temperature range.

As the rectal temperature rose the heart rate (figure) in the control group increased rapidly from 113 (SEM 3) beats/minute at a rectal temperature of 37.5°C to 152 (SEM 16) beats/minute at 39°C. This increase was followed by a slight decline in heart rate to 128 (SEM 9) beats/minute at 40°C before rising steadily to 155 (SEM 9) beats/minute at 42°C. Thereafter, the heart rate increased rapidly to reach a peak of 303 (SEM 6) beats/minute (p < 0.001) at 44°C after which it declined rapidly until a temperature of about 44.5°C was recorded. In the group treated with kanamycin the heart rate increased steadily from

**Gathiram, Wells, Brock-Utne, Wessels, Gaffin**

126 (SEM 7) beats/minute at 37.5°C to 309 (SEM 7) beats/minute at 43.5°C, with a dip at 38°C. For any given rectal temperature between 39°C and 43.5°C, both the MAP and heart rate were higher than those recorded for the controls.

Except for a significant increase in serum AST concentration (p < 0.01) which increased from 47.55 (SEM 3.25) IU/l at 37°C to 75.9 (SEM 2.9) IU/l at 43°C in the control group, no significant changes in the serum enzymes ALT, L-γ-glutamyltransferase, and alkaline phosphatase were observed in either groups (table). Albumin, total protein, bilirubin concentrations and anti-LPS IgG titres were also not found to have changed significantly during heat stress (table).

Bacterial examination of rectal swabs from control animals yielded *Escherichia coli*, *Pseudomonas* sp, *Coliform bacilli*, *Proteus* sp, *Serratia* sp, *Staphylococcus* sp and *Lactobacilli* sp. In contrast, pretreatment with kanamycin resulted in an overgrowth of the Gram positive *Staphylococcus* sp and *Lactobacillus*, although *E coli* was found in some animals. Plate counts in the controls yielded a total of 12.56 (SEM 1.83) colony forming units × 10⁹/g faeces of Gram negative and Gram positive bacteria. In the group treated with kanamycin plate counts showed a significant reduction to 0.183 (SEM 0.04) colony forming units × 10⁹/g faeces (p < 0.001). This count would have been much lower had it not been for the overgrowth of Gram positive bacteria.

**Discussion**

Heat stroke is characterised by high body temperature, loss of consciousness, irritability, diarrhoea, vomiting and other symptoms. These symptoms are similar to those in endotoxic shock caused by high plasma concentrations of LPS. Hence LPS has been postulated to play a part in the pathophysiology of heat stroke. We have recently shown the time course of endotoxaemia in heat stressed monkeys.

In the control group of this study the plasma LPS concentration began to increase when the rectal temperature reached 42°C (figure). Initially this increase was gradual up to 43°C when a rapid rise occurred, reaching 0.308 (0.038) ng/ml (p < 0.001) just before death. The changes in plasma LPS concentration seen here were similar to those previously reported. On the other hand, in the experimental group pretreatment with the oral non-absorbable antibiotic completely prevented any significant increase in plasma LPS concentration. The active ingredients of Kantraxil suspension are kanamycin and kaolin. Although kanamycin is sensitive for most Gram negative bacteria and aerobic bacteria, together with kaolin, it suppresses the plasma LPS concentration.
Table Changes in concentration of serum enzymes, bilirubin, albumin, total protein and anti-LPS (IgG) in control and treated groups

<table>
<thead>
<tr>
<th>Concentration at rectal temperatures of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
</tr>
<tr>
<td>Control group:</td>
</tr>
<tr>
<td>AST (IU/l)</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/l)</td>
</tr>
<tr>
<td>L-γ-glutamyltransferase (IU/l)</td>
</tr>
<tr>
<td>Bilirubin (μmol/l)</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
</tr>
<tr>
<td>Anti-LPS (IgG)%</td>
</tr>
</tbody>
</table>

*Treatment group:|
|AST (IU/l) | 34.85 (7.85) | 32.0 (11.71) | 31.4 (6.17) | 31.83 (8.12) |
|ALT (IU/l) | 5.73 (2.38) | 8.68 (1.06) | 10.25 (3.86) | 4.85 (1.19) |
|Alkaline phosphatase (IU/l) | 431.2 (78.8) | 372.0 (64.7) | 413.1 (101.6) | 437.6 (105.9) |
|L-γ-glutamyltransferase (IU/l) | 26.53 (3.23) | 26.15 (3.65) | 27.78 (4.35) | 26.38 (3.6) |
|Bilirubin (μmol/l) | 4.18 (0.14) | 2.63 (0.86) | 3.5 (0.75) | 4.85 (0.83) |
|Albumin (g/l) | 27.8 (1.05) | 27.68 (2.38) | 27.85 (1.65) | 27.48 (1.83) |
|Total protein (g/l) | 54.78 (2.57) | 53.4 (3.19) | 56.48 (3.42) | 54.9 (3.74) |
|Anti-LPS (IgG)% | 100 | - | 87.8 (14.8) | 98.9 (21.3) |

*p < 0.05.

The LPS of anaerobic Gram negative bacteria which, incidentally, make up a large proportion of the gut flora, lack certain characteristics of classic endotoxins and as such are less toxic. In view of this no attempts were made either to suppress their activity in the gut or to determine their count in the faeces. Moreover, prophylaxis with kanamycin only has been advocated and used for sterilisation of the gut during surgery. Furthermore, pretreatment with oral kanamycin has been shown to reduce the incidence of persisting endotoxaemia, lung lesions, and mortality from 90% to 32% during temporary occlusion of the superior mesenteric artery, intravenous injection of endotoxin or bradykinin, and intraperitoneal injection of E coli in rabbits. Excessive and indiscriminate use of antimicrobes active against the anaerobic Gram negative gut flora may also reduce or eliminate the “interference phenomenon” and encourage invasion by pathogenic bacteria. Although prophylaxis with kanamycin used in this study significantly reduced the total bacterial count (p < 0.001), at the same time it promoted an overgrowth of the Gram positive bacteria. The reduction in count from 12.56 (183) colony forming units × 10^9/g faeces to 0.183 (0.04) colony forming units × 10^9/g faeces of total bacterial flora might, therefore, not be sufficient to have caused the reduction in the plasma LPS concentration observed in this study. The count shown would have been much lower had it not been for the overgrowth of the Gram positive bacteria.

In this study as the rectal temperature increased so did the MAP in both the groups. In the control group not only were the MAPs lower for any given rectal temperature but the MAP also began a downward trend at a lower rectal temperature than the group treated with kanamycin (40.5°C v 42°C) (p < 0.05). Gorman and Proppe also noticed a similar decline in MAP in the early period of heat stress in baboons. The higher MAP in the group treated with kanamycin may be partly explained by the anticholinergic properties of aminopentamide, one of the constituents of Kantrexil suspension; and this could also explain why the heart rates were higher in those primates pretreated with kanamycin, and also possibly why they succumbed at a lower rectal temperature.

Hyperthermia not only causes an increased blood flow to the skin but also a decreased visceral blood flow. These could result in an ischaemic gut wall and hence raised plasma LPS. High concentrations of plasma LPS have been reported in patients with fatal heat stroke and a reduction of gut flora with antibiotics and enemas have been found to increase the incidence of an 18 hour survival in dogs subjected to heat stress from 20% to 70-6%. Furthermore, rabbits pretreated with oral antibiotics have a reduced tendency toward developing endotoxaemia than untreated heat stressed rabbits. The standard treatment for heat stroke is rapid cooling, correction of fluid and electrolyte disturbances, and treatment of shock.

Our study suggests that the origin of the increased plasma LPS concentration seen during heat stress is mainly derived from the gut. Furthermore, gut derived LPS may be an important contribution to the
pathophysiology of heat stroke, and the use of anti-LPS antibodies may prove beneficial in heat stroke.

We thank Mr K Perumal and the late Mr G Moffett for their technical assistance, and Dr GTW Blake for his statistical expertise.

This research was supported by a grant from the Chamber of Mines, Johannesburg, South Africa.

References

Prevention of endotoxaemia by non-absorbable antibiotics in heat stress.

P Gathiram, M T Wells, J G Brock-Utne, B C Wessels and S L Gaffin

*J Clin Pathol* 1987 40: 1364-1368
doi: 10.1136/jcp.40.11.1364

Updated information and services can be found at: [http://jcp.bmj.com/content/40/11/1364](http://jcp.bmj.com/content/40/11/1364)

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to: [http://group.bmj.com/group/rights-licensing/permissions](http://group.bmj.com/group/rights-licensing/permissions)

To order reprints go to: [http://journals.bmj.com/cgi/reprintform](http://journals.bmj.com/cgi/reprintform)

To subscribe to BMJ go to: [http://group.bmj.com/subscribe/](http://group.bmj.com/subscribe/)