Interleukin-1 and tumour necrosis factor cause hypotension in the conscious rabbit

J. R. WEINBERG, D. J. M. WRIGHT AND A. GUZ
Departments of Medicine and Medical Microbiology, Charing Cross and Westminster Medical School, London

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SUMMARY

1. The cardiovascular effects of intravenous injections of interleukin-1 (IL-1) and tumour necrosis factor (TNF) have been investigated in the conscious rabbit. They have been compared with the effects of bacterial lipopolysaccharide (LPS) because both IL-1 and TNF are released from macrophages by LPS.

2. IL-1, TNF and Escherichia coli J5-LPS all caused hypotension when given intravenously in a dose with low mortality. The time course of the hypotension caused by IL-1 and LPS was similar, although the maximal fall in mean blood pressure occurred earlier after IL-1. TNF produced a more sustained fall in blood pressure. Hypotension was not accompanied by a compensatory tachycardia after any of the test substances. Hypotension was associated with a fever after TNF, hypothermia after LPS and no significant change in temperature after IL-1.

3. The packed cell volume did not change during hypotension in any of the study groups, implying that the hypotension was not due to fluid loss resulting from increased capillary permeability.

4. IL-1 and TNF are candidates for the role of effectors of LPS-induced hypotension.

Key words: hypotension, interleukin-1, septic shock, tumour necrosis factor.

Abbreviations: IL-1, interleukin-1; LPS, lipopolysaccharide; TNF, tumour necrosis factor.

INTRODUCTION

Bacterial infection may be associated with hypotension, multi-organ failure and fever, often termed 'septic shock' [1]. Bacterial lipopolysaccharides (LPS) have been identified as a cause and when given intravenously will induce a syndrome similar to that seen with natural infection [2]. Recent work suggests that LPS itself is not the cause of 'septic shock', but initiates the release of endogenous mediators that may be the effectors of the syndrome [3, 4]. Interleukin-1 (IL-1) and tumour necrosis factor (TNF), polypeptide lymphokines which are known to be pyrogens, are released by macrophages after stimulation with LPS [5, 6]. Human recombinant TNF infused into anaesthetized rats mimics the effects of LPS [7]. Animals become hypotensive, acidic and haemoconcentrated; death occurs as a result of respiratory arrest and the post-mortem findings are similar to those seen after the administration of fatal doses of LPS.

The hypotension in clinical 'septic shock' is associated with vasodilatation [8], and although the mortality is high it is not inevitable. Therefore we studied whether IL-1 and TNF are hypotensive in a dose with a low mortality and whether the time course of hypotension produced by these substances was similar to that produced by LPS. Furthermore, we wished to perform the study in an unanaesthetized animal to avoid any possible influence of anaesthesia on cardiovascular reflexes. We have used a previously described model of non-fatal hypotension after the intravenous injection of LPS in the restrained conscious rabbit [9].

EXPERIMENTAL

Methods

Male New Zealand White rabbits, 1.8–4 kg, trained to sit in stocks were studied. Studies were always performed at the same time of day in an air-conditioned room (21.5–23.5°C) after the animals had been starved overnight. Temperature was measured per rectum, by an electronic thermometer (YSLI) and recorded on a chart recorder (Gould); the thermocouple probe was introduced to a minimum of 7 cm. A 23G cannula (Wallace) was implanted into the central artery of the ear, which had been anaesthetized by infiltration of its root with 2% (v/v) lignocaine [10]. All lines were filled with heparinized
physiological saline (0.9% w/v, NaCl solution, 1000 units of heparin/l). Four animals were studied on any occasion; lines leading from the arterial cannulae were routed to a system of taps, such that the blood pressure of a selected animal could be sensed by a blood pressure transducer. Lines were flushed with heparinized saline and all animals received similar volumes of fluid (approx. 5 ml/h) during a study. Arterial blood packed cell volume was measured from blood taken from the cannula at a proximal tap; the first 2 ml of blood was discarded. Heart rate was measured from the blood pressure wave form. Mean blood pressure was computed as diastolic blood pressure plus one-third of (systolic – diastolic pressure). Blood pressure wave form, mean blood pressure and temperature were recorded.

The results for heart rate, blood pressure and temperature were grouped into consecutive 12 min ‘time bins’. The highest value measured during the 12 min was taken as representing that time bin. The arterial lines required intermittent flushing; however, in most 12 min periods (the time bins) a good pressure trace could be obtained. A clot partially obstructing the cannula could produce a falsely low mean blood pressure and therefore the highest recorded value rather than the average of all the values of blood pressure obtained in a time bin was taken to represent pressure during that time bin. The between-rabbit variability in resting mean blood pressure, heart rate and temperature meant that true falls in pressure were obscured when the raw data were used and therefore we chose to analyse the data as change from resting preinjection value. The values for the four time bins before the test substance injection (time bins 1, 2, 3 and 4, representing times 48–36 min before injection, 36–24 min before injection and so on until the test substance was injected) were averaged. All the values were subtracted from this average and recorded as change from resting, preinjection, mean blood pressure, heart rate and temperature, respectively.

Materials

LPS. Highly purified Escherichia coli J5 endotoxin (J5-LPS) was kindly provided by Dr N. Rapson (Wellcome Research Laboratories, Beckenham, U.K.). This was used in a dose of 20 g/kg. Stock solution was diluted with 0.9% (w/v) NaCl.

IL-1 Recombinant human interleukin-1 (10⁸ thymocyte mitogenesis units/mg, less than 1 ng of LPS/ml [11]) was kindly provided by Drs S. Gillis and C. Henney (Immunex Corporation, Seattle, WA, U.S.A.). A dose of 0.75 μg/kg was used. Stock solution was diluted with 0.9% (w/v) NaCl.

TNF. Human recombinant TNF was kindly provided by Dr M. Shepard (Genentech, San Francisco, CA, U.S.A.). A dose of 50 μg/kg was used in these studies. LPS content was less than or equal to 0.125 endotoxin units/ml (manufacturer’s data). Stock solution was diluted with 0.9% (w/v) NaCl.

Control. Physiological saline in the same volume of ml/kg as the test substance was used as the control injection.

Plan of study

Four animals were studied together, one of these four was a control. A total of 52 animals were studied (14 J5-LPS, 16 TNF, 6 IL-1, 16 controls). The control data from all the studies were pooled. Test or control substances were injected intravenously into a marginal vein of the ear opposite to the one with the arterial cannula at the end of time bin four; time bin five therefore represents 0–12 min after injection. In each study only animals which had had no previous exposure to the test substances were used. The dose used was determined empirically as a dose which caused significant hypotension with minimum mortality. The critical dose was determined in preliminary studies: animals were given increasing doses, beginning with the published fever-producing dose [12]. If the dose failed to produce obvious hypotension that dose was abandoned and doubled for the next study. This procedure was followed until the hypotensive dose was reached. The limited availability of the test substances meant that a formal dose–response study could not be undertaken. The study was not performed in a randomized fashion; however, a control animal was included in each run.

Statistical analysis

Missing data values were interpolated as the mean of the values for the preceding and following time bins. Table 1 shows that few values needed to be interpolated. If consecutive values were missing, the data set was discarded. Data from animals dying within 24 h of the study starting were discarded.

The results were analysed by means of an analysis of variance (BMDP, University of London) that permits comparison between groups of different sizes. Fisher’s least significant difference was calculated for each of the test substances and for each of the measured variables comparing treated animals with control animals. The control data used were from the pooled controls, such that each of the test substances was compared with the same controls. A P value of less than 0.05 in a two-tailed test was taken to imply that a significant difference existed.

RESULTS

General observation

After the injection of LPS and TNF the animals became unwell; they developed tachypnoea, a staring appearance and a flattened posture in the stocks. After the injection of IL-1 the animals became tachypnoeic but were less toxic. When animals died during the experiments (5/14 after J5-LPS, 5/16 after TNF, 0/6 after IL-1) it was usually after hypotension; however, sudden death without hypotension was seen in the LPS and TNF groups. One death was seen in a control rabbit which occurred when the air conditioning broke down during hot weather and the animal developed hyperthermia; all the results from this study were discarded.
Lymphokines are hypotensive agents

Table 1. Number of data interpolations
Abbreviations: BP, blood pressure; HR, heart rate.

<table>
<thead>
<tr>
<th>Data</th>
<th>Number of animals</th>
<th>Total no. of data points</th>
<th>No. of interpolated points</th>
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<td>1</td>
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<tr>
<td>BP</td>
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<td>160</td>
<td>1</td>
</tr>
<tr>
<td>Temperature</td>
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<td>120</td>
<td>10</td>
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<tr>
<td>J5-LPS</td>
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<td>160</td>
<td>1</td>
</tr>
<tr>
<td>BP</td>
<td>8</td>
<td>160</td>
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</table>

Fig. 1. Test substance (▲) against control (●). Results are means ± SD. Bold bars represent Fisher's least significant difference (LSD) between test and control after analysis of variance. *P*<0.05 (two-tailed test). Abbreviations: BP, blood pressure; HR, heart rate.
The group mean data from all the rabbits given a particular substance are presented graphically in Fig. 1. A bar represents Fisher's least significance difference between test and control at the level of \( P < 0.05 \).

**Blood pressure**

TNF, IL-1 and J5-LPS all caused significant falls in blood pressure. Blood pressure fell in the fifth time bin with all three substances; this fall was statistically significant by the sixth time bin. After IL-1 and J5-LPS the blood pressure returned to control values by the end of the study (bin 21). The nadir of the fall after IL-1 was during bin 7, whilst that after J5-LPS was between the nineth and thirteenth time bins. The fall in blood pressure after TNF was not as marked as that after J5-LPS or IL-1, but was sustained until the end of the study.

**Heart rate**

The heart rate rose after the injection of IL-1 but this was only significant in one time bin. There were no significant changes in heart rate after J5-LPS or TNF.

**Temperature**

After J5-LPS \((n = 8)\) the temperature of five rabbits fell and that of two rabbits rose by an amount greater than the least significant difference. The average temperature fell significantly by the ninth time bin and remained depressed until the end of the period of observation. After TNF \((n = 7)\) a rise in temperature was observed in five rabbits, and a fall in none. The average temperature had risen by a significant amount by time bin 6; it then remained elevated for the rest of the study. After IL-1 \((n = 6)\) the temperature of four rabbits rose while that of one fell. The average did not change significantly.

**Packed cell volume**

The packed cell volume did not show any systematic change during hypotension after any of the injections, although there was a tendency for it to fall. Measurements were made during time bin 1 (control) and time bin 6 (hypotensive). The results in three animals after J5-LPS were 42 to 46, 41 to 45, 40 to 38; the results for six animals after IL-1 were 39 to 37, 39 to 39, 40 to 39, 40 to 40, 43 to 43, 42 to 41; the results for three animals after TNF were 46 to 44, 45 to 44, 43 to 41.

**DISCUSSION**

This animal model has demonstrated the hypotensive effects of IL-1, TNF and LPS. It has the advantage that the animals are unanaesthetized and the action of the test substances can be seen without the confusing influence of exogenous pharmacological substances. It has the disadvantage that only a restricted amount of information can be obtained since the animals only have peripheral arterial and venous cannucae in situ. We decided to avoid chronic catheterization as it is impossible to be sure that chronically implanted lines have not become the site of a low grade infection [13]. This might alter the background levels of the lymphokines we are interested in, and therefore the response to them. As the animals were conscious and as we were interested in measuring blood pressure and heart rate responses, they were handled as little as possible. This meant that occasionally the rectal probe came out and data were lost. Using this model, non-fatal hypotension and hypothermia after the injection of *Escherichia coli* 055 LPS has been demonstrated [9]. A lag phase of about 45 min between the injection of LPS and the fall in blood pressure was shown, and this raised the possibility of the release of endogenous mediators which were the effectors of the hypotension.

In the present study the blood pressure fall after IL-1 had a time course similar to that after J5-LPS with an early fall followed by a return to resting pretreatment values, and a nadir reached earlier than after J5-LPS. We did not notice a clear lag phase after J5-LPS in this study. This difference from previous results may arise from the use of a different LPS. At these doses TNF caused a less profound but more sustained hypotension. Although the fall in blood pressure was greater in the IL-1-treated group than after TNF, the mortality was higher in the TNF group. These results extend to another species a previous report that TNF can produce hypotension and lethal shock in rats and mice [7, 14]. It has been suggested that TNF is the mediator of septic shock [15]. However, we have shown here that IL-1 is also a hypotensive agent, which is in agreement with a recent preliminary report [16] concerning IL-1. The time course of IL-1-induced hypotension resembles that of J5-LPS more closely than does the time course of TNF-induced hypotension. Exposure of vascular smooth muscle cells to IL-1 induces the expression of IL-1 message within 30 min [17]; the induction of IL-1 is likely to be equally rapid after exposure to LPS [18]. The delay seen in the hypotensive effect of J5-LPS as compared with IL-1 is consistent with the induction of local IL-1 production. These observations are consistent with both IL-1 and TNF being mediators of septic shock.

The temperature responses showed that J5-LPS produced a significant fall in temperature, while TNF produced a significant rise. The failure of IL-1 to show any significant change in temperature results from the problem of averaging as most animals did exhibit the expected fever. Variability in response of rabbits to endogenous pyrogen has been demonstrated previously [19].

The heart rate only rose significantly during one time bin after IL-1. We cannot be certain that the absence of a compensatory tachycardia is evidence of the failure of baroreceptor mechanisms as it was not possible to obtain electrocardiograms for analysis of rhythm in these unanaesthetized animals. Intradermal needle electrodes produced traces with an unacceptable amount of interference. Therefore the presence of an arrhythmia had to be deduced from the blood pressure wave form. Transient changes in rate were seen; bradycardias, atrial fibrillation and ventricular ectopics were recognized and episodes of heart block may also have occurred.
Packed cell volume did not change systematically during any of the studies. This suggests that the hypertension after J5-LPS, IL-1 and TNF is not due to the leakage of fluid from excessively permeable capillaries. In their study of fatal shock after TNF, Tracey et al. [7] found, in the rat, that the packed cell volume rose; this difference may be because a non-fatal dose of TNF was being used in the present study. Alternatively, a species difference may be responsible. Clinical observation suggests that early in the time course of septic shock the peripheral resistance is reduced and the cardiac output increased [20, 21]. To be considered as a mediator of septic shock, a candidate should reproduce this pathophysiological pattern.

LPS is known to induce macrophages to produce IL-1 and TNF [5, 6]. The situation is further complicated as TNF may induce the release of IL-1 [22] and IL-1 may induce TNF release [23, 24]. Therefore there may be synergistic interactions between these lymphokines. The present studies suggest that intravascular IL-1 and TNF could be responsible for hypotension with vasodilatation. Further studies are in progress to ascertain the mechanism of the hypotension using non-invasive techniques of measuring skin blood flow. It would be of interest to measure these lymphokines in serum during septicaemia measuring skin blood flow. It would be of interest to ascertain whether lymphokine-induced hypotension can be prevented by an appropriate antibody. Of even greater interest would be the demonstration that such antibodies prevent the hypotension subsequent to the injection of LPS itself. There is evidence that this may be so [27].

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REFERENCES