Effects of tumour necrosis factor-\(\alpha\) in
the human forearm: blood flow
and endothelin-1 release

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ABSTRACT

Increased circulating concentrations of tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)) are seen in several pathological conditions associated with vascular disease. TNF-\(\alpha\) induces the synthesis of endothelin-1 (ET-1), a potent vasoconstrictor, by the endothelium. However, there is profound vasodilatation in sepsis, where circulating levels of both ET-1 and TNF-\(\alpha\) are elevated. The details of the interaction between ET-1 and TNF-\(\alpha\) and the predominant resulting haemodynamic effect in healthy humans are unclear. The aim of the present study was to determine the effects of intra-arterial TNF-\(\alpha\) on ET-1 spillover, vascular tone and endothelial function in the healthy human forearm. Brachial arterial and deep venous blood samples, forearm plasma flow measurements and blood flow responses to acetylcholine and sodium nitroprusside were obtained in six healthy subjects before and during a 6 h infusion of TNF-\(\alpha\) into the brachial artery. Forearm blood flow was significantly greater than baseline during exposure to TNF-\(\alpha\) [median (lower quartile, upper quartile): baseline, 2.6 (2.1, 2.8) ml \(\cdot\) min\(^{-1}\) \(\cdot\) 100 ml\(^{-1}\); TNF-\(\alpha\), 4.6 (4.5, 5.1) ml \(\cdot\) min\(^{-1}\) \(\cdot\) 100 ml\(^{-1}\); \(P < 0.05\)]. The rate of release of ET-1 was significantly greater than baseline after 30 and 260 min of TNF-\(\alpha\) infusion [median (lower quartile, upper quartile): baseline, 0.8 (0.6, 1.1) pg \(\cdot\) min\(^{-1}\) \(\cdot\) 100 ml\(^{-1}\); 30 min, 2.4 (1.9, 3.2) pg \(\cdot\) min\(^{-1}\) \(\cdot\) 100 ml\(^{-1}\); 260 min, 4.1 (3.1, 4.2) pg \(\cdot\) min\(^{-1}\) \(\cdot\) 100 ml\(^{-1}\); \(P < 0.05\)]. The vasodilatory response to acetylcholine was diminished during TNF-\(\alpha\) infusion, whereas the response to sodium nitroprusside remained unchanged. We thus demonstrate for the first time that local TNF-\(\alpha\) increases ET-1 spillover from the human forearm and impairs endothelium-dependent vasodilatation. In spite of this action, TNF-\(\alpha\) has a vasodilatory effect, resulting in an increase in forearm blood flow.

INTRODUCTION

Tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)) has many effects on vascular function. It contributes to the hypotension seen in septic shock [1], and increases the risk of occlusive vascular events via its action on endothelial function [2]. Furthermore, elevated circulating concentrations of TNF-\(\alpha\) are common in states of increased vascular risk, such as obesity, diabetes and smoking [3–5]. In experimental studies, TNF-\(\alpha\) has been shown to induce the...
endothelial production of both endothelin-1 (ET-1) [6–9] and nitric oxide (NO) [2]. There is evidence that the TNF-α-stimulated release of ET-1 results in vasoconstriction in certain vascular beds [10,11] and that, in humans, there are regional differences in vascular sensitivity to ET-1 [12]. The interaction between local TNF-α and ET-1 and the resulting regional haemodynamic action in healthy humans have not been fully investigated.

The aim of the present study was to determine the effects of local TNF-α on various indices of endothelial function and metabolism in the healthy human forearm. We wished to test the hypothesis that exposure to intra-arterial TNF-α stimulates ET-1 release, resulting in vasoconstriction and a reduction in blood flow. Exposure to TNF-α in animals is associated with impaired endothelial capacity to respond to agonist-mediated vasodilators [13]. Therefore, we also measured agonist-mediated responses to endothelium-dependent and -independent vasodilators [14,15] at intervals during the course of the study to determine whether TNF-α has the same effects on the human arterial endothelium. TNF-α has well documented effects on the production of other cytokines, as well as on glucose and lipid metabolism, and we wished to explore whether other known effects of TNF-α would be induced locally. We therefore measured the effects of local TNF-α on interleukin-6 (IL-6) release [16], triacylglycerol clearance [17] and glucose uptake [18].

METHODS

The protocol was approved by the Ethics Committee of the Whittington Hospital. Six healthy subjects were recruited (mean age ± S.E.M. 56 ± 4 years; male/female, 5:1). None had any current or previous significant health problems, and none were taking any medication. All subjects gave written informed consent.

Subjects arrived on the day of the study following an overnight fast. All cannulation was done with the use of local anaesthetic. To facilitate simultaneous arterial blood sampling and intra-arterial infusion of TNF-α, the Seldinger technique [19] was used to cannulate the brachial artery, retrogradely, with a double-lumen catheter (Hydrocath™; Ohmeda, Swindon, U.K.). Samples were taken via the central lumen of the catheter while the TNF-α was infused though the peripheral lumen, which delivered the infusate ‘downstream’ from the sampling site.

A catheter was inserted in a retrograde fashion into a deep forearm vein of the same arm. After a 30 min period of stabilization, baseline blood samples were taken simultaneously from the artery and deep vein. Forearm blood flow was measured using a Hokanson venous occlusion plethysmograph (PMS Instruments) [20]. In order to exclude the hand circulation, a wrist cuff was inflated to 200 mmHg 1 min before the start of blood sampling and/or blood flow measurements, and was kept inflated throughout the measurement period.

The arterial and venous blood samples were taken and kept on ice until centrifugation. Thereafter, plasma samples were promptly frozen at or below −20 °C. Baseline arterial and venous samples were taken in duplicate. Baseline blood flow was measured twice, and remained stable for a 45 min period before the start of the TNF-α infusion. Blood flow was also measured in the contralateral arm as a control.

An infusion of human recombinant TNF-α (Bachem, St. Helens, U.K.), 10 μg reconstituted in 60 ml of 0.9% (w/v) NaCl, was then started into the brachial artery through the peripheral (downstream) lumen of the catheter, and was continued at a constant rate for 360 min. Based on the results of previous work [21] and our own pilot study, in which increasing doses of TNF-α were administered intra-arterially in a healthy volunteer, we aimed to achieve a local concentration of TNF-α of 200 pg·ml⁻¹. The actual TNF-α infusion rate for each individual subject was calculated on the basis of their forearm volume and baseline blood flow. Heart rate, blood pressure and core temperature were measured before and repeatedly during the TNF-α infusion.

Forearm blood flow measurements and deep venous and arterial blood samples were taken at baseline and during the course of the TNF-α infusion at 30, 120 and 260 min. At baseline and at 120 min, blood flow dose–response curves to acetylcholine (ACh) and sodium nitroprusside (SNP) were constructed as previously described [14,15]. The doses used were 0.15, 0.45, 1.5, 4.5 and 15.0 μg·min⁻¹·100 ml⁻¹ for ACh, and 1.0, 2.0 and 4.0 μg·min⁻¹·100 ml⁻¹ for SNP. At 30, 330 and 440 min (i.e. 80 min after the cessation of the TNF-α infusion), forearm blood flow responses to single doses of ACh (1.5 μg·min⁻¹·100 ml⁻¹) and SNP (2.0 μg·min⁻¹·100 ml⁻¹) were determined using the same protocol.

ET-1 concentrations were determined using a high-sensitivity ELISA method (R&D Systems, Minneapolis, MN, U.S.A.). Concentrations of IL-6 and TNF-α were determined using high-sensitivity two-site ELISAs from R&D Systems (Oxford, U.K.). Glucose and triacylglycerol concentrations were measured enzymically on a centrifugal analyser [22].

ET-1 and IL-6 release rates were calculated as:

\[
\text{Release} = (V - A) \times \text{blood flow} \times (1 - \text{haematocrit})
\]

where V is the venous concentration and A is the arterial concentration. Triacylglycerol and glucose uptake were calculated as described previously [22].

Values are presented as median (interquartile range), as some values were not normally distributed. Changes with time were analysed by ANOVA, after logarithmic transformation where necessary. If the ANOVA showed
significant changes, post hoc Wilcoxon signed rank tests were undertaken to compare baseline and infusion conditions.

RESULTS

TNF-α concentrations
These are shown in Table 1. There was a small rise in the arterial TNF-α concentration (P < 0.02; ANOVA), with a greater increase in the venous concentration (P < 0.001). There were no significant changes in blood pressure, core temperature or heart rate (Table 2).

Blood flow
There was a significant difference between the control and study arms (P < 0.001; ANOVA), and a significant change in blood flow when comparing baseline and infusion conditions (P < 0.05). In the control arm there was no significant change in blood flow between baseline and infusion conditions (P = 0.91; ANOVA), while in the study arm the blood flow increased during the infusion (P < 0.05) (Figure 1).

Responses to ACh and SNP
These are shown in Figures 2 and 3. Prior to TNF-α infusion, there was a clear vasodilatory response to increasing doses of ACh (P < 0.01; ANOVA). In contrast, there was a loss of this response to increasing doses of ACh after 120 min of TNF-α infusion (P = 0.17).

Table 1 Arterial and venous concentrations of TNF-α and ET-1, and arterial concentrations of glucose, triacylglycerol and IL-6
Values are given before (baseline) and after 30, 120 and 260 min of TNF-α infusion. Data are expressed as median (lower quartile, upper quartile). Significant differences from baseline: *P < 0.05 (Wilcoxon signed rank test).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>30 min</th>
<th>120 min</th>
<th>260 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial TNF-α (pg·ml⁻¹)</td>
<td>3.1 (2.8, 3.4)</td>
<td>8.1 (5.0, 17.1)</td>
<td>8.5* (5.0, 10.8)</td>
<td>16.0* (9.5, 18.4)</td>
</tr>
<tr>
<td>Venous TNF-α (pg·ml⁻¹)</td>
<td>2.5 (2.4, 2.8)</td>
<td>45.4* (28.7, 53.7)</td>
<td>31.1* (27.9, 52.9)</td>
<td>31.0* (25.1, 36.5)</td>
</tr>
<tr>
<td>Arterial ET-1 (pg·ml⁻¹)</td>
<td>1.1 (0.9, 1.5)</td>
<td>1.5 (1.2, 1.7)</td>
<td>1.4 (1.2, 1.5)</td>
<td>1.4 (1.2, 1.5)</td>
</tr>
<tr>
<td>Venous ET-1 (pg·ml⁻¹)</td>
<td>2.0 (1.9, 2.2)</td>
<td>2.6* (2.3, 3.0)</td>
<td>2.0 (1.8, 2.5)</td>
<td>3.0* (2.7, 3.4)</td>
</tr>
<tr>
<td>Arterial glucose (mmol·l⁻¹)</td>
<td>5.3 (5.1, 5.7)</td>
<td>5.2 (4.8, 5.4)</td>
<td>4.8 (4.7, 5.3)</td>
<td>4.7 (4.5, 4.9)</td>
</tr>
<tr>
<td>Arterial triacylglycerol (mmol·l⁻¹)</td>
<td>1.4 (0.9, 2.1)</td>
<td>0.7 (0.7, 1.3)</td>
<td>1.3 (0.7, 1.9)</td>
<td>0.9 (0.7, 1.6)</td>
</tr>
<tr>
<td>Arterial IL-6 (pg·ml⁻¹)</td>
<td>4.3 (4.4, 8.5)</td>
<td>4.5 (1.3, 7.0)</td>
<td>4.5 (4.0, 5.8)</td>
<td>12.0 (8.0, 15.0)</td>
</tr>
</tbody>
</table>

Table 2 Systolic and diastolic blood pressure, temperature and pulse
Values are given before (baseline), during (30, 115 and 255 min) and after (440 min) a 6 h TNF-α infusion. Data are expressed as median (upper quartile, lower quartile). Significant differences from baseline: *P < 0.05 (Wilcoxon signed rank test).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>30 min</th>
<th>115 min</th>
<th>255 min</th>
<th>440 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>124 (115, 132)</td>
<td>130 (106, 140)</td>
<td>126 (104, 138)</td>
<td>138 (108, 154)</td>
<td>130 (109, 149)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82 (72, 92)</td>
<td>84 (66, 86)</td>
<td>77 (62, 92)</td>
<td>75 (56, 82)</td>
<td>85 (67, 91)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>36.5 (36.1, 36.9)</td>
<td>36.5 (36.2, 36.9)</td>
<td>36.1 (36.7, 37.0)</td>
<td>37.0 (36.7, 37.2)</td>
<td>36.9 (36.9, 37.1)</td>
</tr>
<tr>
<td>Pulse (beats·min⁻¹)</td>
<td>63 (58, 71)</td>
<td>65 (56, 73)</td>
<td>67 (58, 73)</td>
<td>67 (62, 99)</td>
<td>70 (60, 76)</td>
</tr>
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</table>
Figure 2 Forearm blood flow dose–response curves to ACh and SNP at baseline (0 min) and at 120 min

Error bars show the interquartile range. Significance of differences: *P < 0.05 (Wilcoxon signed rank test) for response to ACh compared with baseline (BL), or for response to SNP compared with washout (WO). Doses A1–A5 are 0.15, 0.45, 1.5, 4.5 and 15.0 μg · min⁻¹ · 100 ml⁻¹ ACh respectively; doses S1–S3 are 1.0, 2.0 and 4.0 μg · min⁻¹ · 100 ml⁻¹ SNP respectively.

(Figure 2). There was a clear vasodilatory response to increasing doses of SNP, both before and after 120 min of the TNF-α infusion (P < 0.01 for both).

The blood flow responses to SNP were preserved during the course of the study, while the response to ACh at dose A3 (1.5 μg · min⁻¹ · 100 ml⁻¹) was significantly diminished both during and after cessation of the TNF-α infusion (Figure 3).

ET-1 release

The rate of release of ET-1 increased significantly following the start of the TNF-α infusion (P < 0.001; ANOVA) (Figure 1, lower panel). The increase in the ET-1 release rate was not due entirely to the increase in blood flow during TNF-α infusion. Venous concentrations of ET-1 increased during the course of the study (P < 0.02), while the arterial ET-1 concentration did not change significantly throughout the duration of the study (Table 1).

IL-6, triacylglycerol and glucose

Table 3 shows the IL-6 release rate and the rates of uptake of glucose and triacylglycerol both before and during the TNF-α infusion. Local infusion of TNF-α was associated with an increase in IL-6 release from the forearm (P < 0.05). There were no significant changes in forearm glucose or triacylglycerol uptake during TNF-α infusion.

DISCUSSION

The present study is the first to examine the sequelae of local TNF-α infusion into a human artery. The amount of TNF-α infused was sufficiently low to avoid any changes in pulse, blood pressure or temperature. We found that TNF-α infusion into the forearms of healthy humans was followed by (a) increased blood flow, (b) a reduction in responsiveness to ACh, while responses to SNP were unaffected, (c) increased release of ET-1, and (d) stimulation of IL-6 release.

The dose and duration of infusion of TNF-α that we used were chosen on the basis of results from previous in vitro and in vivo work [7,8], and were designed to avoid systemic symptoms. Our experiments were designed so that a short duration of exposure to a modest dose of TNF-α could be used, to minimize possible spillover into the systemic circulation with resultant symptoms in our volunteers. We intended to produce a concentration of approx. 200 pg · ml⁻¹ in the blood perfusing the forearm, and observed a concentration of approx. 40 pg · ml⁻¹ in venous blood draining the forearm. It is difficult to determine the actual arterial concentration achieved. Although the venous concentration was 20% of our predicted arterial concentration, this may have been due to local receptor binding and clearance of TNF-α before it reached the venous circulation. Although there was a small increase in the arterial TNF-α
TNF-\(\alpha\), forearm blood flow and ET-1

Forearm blood flow increased within 30 min of exposure to TNF-\(\alpha\), while flow in the non-infused arm did not change. This increase in flow is in contrast with studies of the rat coronary vasculature, in which TNF-\(\alpha\) (3000–4000 pg \(\cdot\) ml\(^{-1}\)) caused ET-1-mediated vasoconstriction [10,11]. The time course of the vasodilatory responses seen in the present study agrees with the findings of other workers: in one study, exposure to TNF-\(\alpha\) resulted in a response within 20 min [24], and in another study the response became maximal at 4 h [25].

TNF-\(\alpha\) induces vascular relaxation \textit{in vitro} and in animals [24], while patients with malignant disease given TNF-\(\alpha\) systemically at high doses become hypotensive [21]. TNF-\(\alpha\) causes arterial vasodilatation in experimental animals as a result of induction of inducible NO synthase and NO release [25]. Similarly, cytokines stimulate inducible NO synthase and NO release in human arteries \textit{ex vivo} [26], while local administration of TNF-\(\alpha\) in human veins results in NO-mediated venodilation either directly or in synergy with other cytokines [27,28]. The forearm vascular bed is not a common site for atherosclerotic disease, and its responses to infused vasoactive substances can be very heterogeneous. Consequently it is difficult to know the extent to which the forearm model is relevant to disease states or to other healthy arterial beds. However, we have demonstrated for the first time in healthy humans \textit{in vivo} an arterial vasodilator response to local exposure to TNF-\(\alpha\) which provides some insight into the role that cytokines may play in the regulation of blood flow.

Exposure to TNF-\(\alpha\) in animals is associated with impaired endothelial capacity to respond to agonist-mediated vasodilators [13]. We observed a loss of responsiveness to ACh during the TNF-\(\alpha\) infusion. As can be seen from Figure 2, at time 0 min (i.e. before TNF-\(\alpha\)), doses of ACh of 1.5, 4.5 and 15 pg \(\cdot\) min\(^{-1}\) \(\cdot\) 100 ml\(^{-1}\) induced vasodilatation. During TNF-\(\alpha\) infusion (at time 120 min), ACh at 1.5 or 4.5 pg \(\cdot\) min\(^{-1}\) \(\cdot\) 100 ml\(^{-1}\) pro-

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<td>IL-6 release</td>
<td>0.42 (0.45, 0.5)</td>
<td>24.8* (15.1, 44.6)</td>
<td>68.5* (49.1, 79.3)</td>
<td>353.1* (255.5, 436.9)</td>
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<td>Triacylglycerol uptake</td>
<td>78.7 (51.6, 122.1)</td>
<td>98.4 (48.6, 141.1)</td>
<td>72.2 (46.4, 113.0)</td>
<td>103.4 (41.0, 160.4)</td>
</tr>
<tr>
<td>Glucose uptake</td>
<td>0.50 (0.38, 0.62)</td>
<td>0.67 (0.57, 0.68)</td>
<td>0.46 (0.43, 0.79)</td>
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concentration, the total amount of TNF-\(\alpha\) administered (less than 2 \(\mu\)g per subject) was much lower than that known to have significant haemodynamic or metabolic effects [21,23], and no systemic effects were seen in our subjects.
duced no vasodilatation, and even a dose of 15 μg·min⁻¹·100 ml⁻¹ did not consistently result in vasodilatation in all subjects, so that none of the ACh doses produced statistically significant dilatation. Although it is possible that the loss of responsiveness to ACh was due to the increase in basal blood flow, with a consequent fall in its local concentration, previous work has shown a positive correlation between basal blood flow and response to ACh [14].

Venous endothelial function in humans is adversely affected by exposure to concentrations of TNF-α over the range 300–1000 pg·ml⁻¹ [28,29]. In the present study, we have observed an impairment of endothelial function after exposure to lower concentrations of TNF-α than used previously in human studies. We are not aware of other studies of the effects in vivo of TNF-α on the arterial responsiveness to ACh and SNP. The pattern of the defect induced in our study by TNF-α is usually taken to mean that the endothelial release of NO has been impaired, while the smooth muscle response to the NO donor SNP is intact [14]. Although suggestive, our present study cannot confirm that this is the mechanism of action of TNF-α. Further studies involving the co-infusion of TNF-α with a NO synthase inhibitor, such as N⁶-monomethyl-L-arginine, would be valuable in clarifying the mechanism of the endothelial defect observed.

In rats, intravenous infusion of TNF-α (at doses higher than have been administered to humans) increases circulating levels of ET-1 within minutes [6,11,30]. ET-1 has been identified in constitutive secretory vesicles [31]. However, ET-1 is mainly synthesized de novo when required. Indeed, studies of cultured endothelial cells show a significant increase in ET-1 mRNA and released peptide within 15 min of stimulation by TNF-α [32]. Although our study was not designed to establish the mechanisms of TNF-α-induced ET-1 release, the changes observed at 30 min are compatible with the release of newly synthesized ET-1 from the endothelium.

Previous work in vitro has shown maximal production of ET-1 by endothelial cells after 2–8 h of exposure to TNF-α, and our results showing increased ET-1 spillover after 260 min support the finding that TNF-α stimulates ET-1 synthesis at the level of gene expression [7,8].

During TNF-α infusion, IL-6 secretion increased significantly, as expected [16]. However, glucose and triacylglycerol uptake did not change significantly. This may have been because the local concentration of TNF-α achieved was not high enough to influence glucose or lipid metabolism.

We used the non-infused arm as a control for the measurement of blood flow. The ideal control would be to infuse saline into the brachial artery; however, we wished to avoid the hazardous procedure of inserting a second double-lumen intra-arterial catheter for this purpose. A study of the diurnal variation in forearm blood flow and endothelium-dependent vasodilatation in healthy subjects suggested that there is a fall in baseline blood flow and responsiveness to ACh at around 20:00 hours [33]. The changes in blood flow and ACh responsiveness seen in our study occurred much earlier in the day, suggesting that our findings were not due to normal diurnal variations.

In summary, we have demonstrated for the first time in humans, in vivo, that TNF-α stimulates the release of ET-1 and that, in spite of the potent vasoconstricting activity of ET-1, TNF-α has a predominantly vasodilatory action in the healthy human forearm. We also show endothelial dysfunction, suggesting that TNF-α-induced vasodilatation occurs independently of endothelial NO release. Further studies in humans are needed to elucidate the mechanisms involved.

ACKNOWLEDGMENTS

This work was supported by grants from The European Association for the Study of Diabetes, The Wellcome Trust and Diabetes U.K.

REFERENCES


Received 23 May 2002; accepted 12 July 2002