Acute myocardial infarction induces hypothalamic cytokine synthesis

Joseph Francis,1 Yi Chu,1 Alan Kim Johnson,2 Robert M. Weiss,1,3 and Robert B. Felder1,3
1Departments of Internal Medicine and 2Psychology, University of Iowa, Iowa City 52242;
and 3Veterans Affairs Medical Center, Iowa City, Iowa 52242

Submitted 10 November 2003; accepted in final form 26 January 2004

Francis, Joseph, Yi Chu, Alan Kim Johnson, Robert M. Weiss, and Robert B. Felder. Acute myocardial infarction induces hypothalamic cytokine synthesis. Am J Physiol Heart Circ Physiol 286: H2264–H2271, 2004; 10.1152/ajpheart.01072.2003.—The inflammatory milieu of acute myocardial infarction (MI) is theoretically conducive to enhanced cytokine synthesis within the brain. We tested the hypothesis that synthesis of tumor necrosis factor-α (TNF-α), an indicator of proinflammatory cytokine activity, increases in brain after MI. MI was induced in rats by ligating the left anterior descending coronary artery and confirmed by echocardiography. Plasma and tissue levels of TNF-α were measured using ELISA; TNF-α mRNA was measured with real-time PCR. Heart, brain, and plasma samples were obtained 0.5, 1, 4, or 24 h or 4 wk after MI. TNF-α synthesis increased in the brain, heart, and plasma within minutes to hours after MI and was sustained over the interval tested. Among the brain tissues examined, TNF-α increased selectively in hypothalamus. Chronic treatment with pentoxifylline prevented the increases in TNF-α in brain, heart, and plasma measured 4 wk after MI. MI-induced cytokine synthesis in the hypothalamus and its prevention by pentoxifylline have important implications in the context of the development of heart failure after MI.

Tumor necrosis factor-α; sympathetic nerve activity; hypothalamic-pituitary-adrenal axis; heart failure

ACUTE MYOCARDIAL INFARCTION (MI) induces synthesis of the proinflammatory class of cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 in the injured and uninjured myocardium (25, 28, 46, 50, 65). Plasma levels of TNF-α, IL-1β, and IL-6 are increased. These myocardial and blood-borne cytokines can directly affect cardiac structure and function (5, 16, 31, 63) and so may contribute to the progression to heart failure. High levels of circulating cytokines after MI have additional effects on the brain that may promote the development of heart failure. TNF-α, IL-1β, and IL-6 share a common property of activating the hypothalamic-pituitary-adrenal axis (HPA) (6, 12, 40, 60), and recent studies from our laboratory suggest that blood-borne TNF-α stimulates behaviors favoring volume accumulation (unpublished observations) and increases sympathetic nerve activity (67).

The proinflammatory cytokines are normally synthesized at low levels by neurons and glial cells in the brain (41, 48). By mechanisms not fully understood, inflammation or injury of peripheral tissues induces an increase in brain cytokine synthesis (48, 58, 59). The present study was undertaken to determine whether MI induces an increase in proinflammatory cytokine synthesis within the brain, particularly in the hypothalamic region. The study was performed in a rat model in which the progression to heart failure after MI has been well characterized (24). Because the individual components of this class of cytokines are not expressed in isolation, and TNF-α appears early in the proinflammatory cytokine “cascade” (6), we measured TNF-α as an indicator of the general state of proinflammatory cytokine synthesis. Pentoxifylline (PTX), which generally inhibits proinflammatory cytokine synthesis but is most effective for TNF-α (8, 10, 38, 55, 61), was used to manipulate TNF-α levels after MI. The expected increase in TNF-α production in the heart (25, 28, 46, 50, 65) served as a positive tissue control for measurements of TNF-α production in the brain.

METHODS

Animals

Adult male Sprague-Dawley rats (3–4 mo old) weighing 350–375 g were obtained from Harlan Sprague Dawley (Indianapolis, IN). They were housed in temperature- (23 ± 2°C) and light-controlled (lights on between 7 AM and 7 PM) animal quarters and were provided with rat chow ad libitum. These studies were performed in accordance with the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings” (1). The experimental procedures were approved by the University of Iowa Institutional Animal Care and Use Committee.

Surgical Procedures

Recovery surgery was performed with the use of an aseptic technique. After each procedure, the animals recovered from anesthesia under observation in the laboratory before returning to the cages. They received buprenorphine (0.1 mg/kg sc) immediately after surgery and then 12 h later for management of postoperative pain.

Induction of MI. MI was induced by ligating the left anterior descending coronary artery under ketamine + xylazine anesthesia (90 and 10 mg/kg ip), as previously described (24). Sham-operated rats underwent the same surgery but did not undergo coronary ligation. Postsurgically, animals were given benzathine penicillin (30,000 units im).

Implantation of osmotic minipumps for drug infusion. Immediately after echocardiography (see Echocardiographic Assessment of Left Ventricular Function), osmotic minipumps (Alzet; Cupertino, CA) were implanted in the abdominal cavity as previously described (23). Twenty-four hours before the infusion, the minipumps were filled with PTX (Sigma; St. Louis, MO) to deliver a dose of 400 μg/h (equivalent to ~30 mg·kg⁻¹·day⁻¹) or its vehicle (sterile water; Veh) and then placed in 0.9% saline solution at room temperature to ensure a constant infusion rate at the time of implantation.

Echocardiographic Assessment of Left Ventricular Function

Twenty-four hours after coronary artery ligation, echocardiography was performed under ketamine (25 mg/kg ip) sedation, as previously described (24) by using a clinical imager (Sequoia model 256, Acuson; Mountain View, CA) fitted with an 8-MHz sector-array probe.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
which generates two-dimensional images at a rate of ~100/s. Short-and long-axis images of the left ventricle (LV) were analyzed. The ischemic zone (IZ) was estimated by planimetry of the region of the LV endocardial silhouette, which demonstrated akinesia or dyskinesia, expressed as a percentage of the whole (%IZ). %IZ, LV ejection fraction (LVEF), and LV end-diastolic volume (LVEDV) were reported. After two-dimensional imaging was completed, pulse-wave Doppler interrogation of mitral inflow was performed to determine heart rate. Only animals with large infarctions (39–67% IZ) were used in the study.

Blood and Tissue Sampling

The rats were decapitated while under pentobarbital anesthesia (50 mg/kg) for collection of trunk blood. The heart and brain tissue was then harvested.

Blood collection. Trunk blood was collected in chilled EDTA tubes. Plasma samples were separated and stored at −70°C until assayed for TNF-α.

Hypothalamic dissection. The method for hypothalamic dissection has been described previously (21). Briefly, the hypothalamus was removed using the posterior part of the optic chiasm as the anterior limit, the anterior part of the mammillary bodies as the posterior limit, and the lateral hypothalamic sulci as the lateral limits.

Heart dissection. The heart was cut into three cross sections. The right ventricular and LV tissues were separated, and the LV tissue was separated into infarct and peri-infarct tissue. The peri-infarct tissues were taken at least 1 mm away from the infarct tissues.

Measurements of TNF-α

Plasma and tissue TNF-α levels in rats were measured using an ultrasensitive rat TNF-α ELISA kit (Biosource International; Camarillo, CA) according to manufacturer instructions. Plasma TNF-α levels. The details of methodology have been described previously (20, 22). The minimum detectable concentration of TNF-α was <0.1 pg/ml.

Tissue levels of TNF-α. Tissue TNF-α levels were measured in the myocardium and hypothalamic tissue as described by Irwin et al. (28) with modifications (20). The tissues were homogenized in denaturizing buffer containing PBS, protease inhibitors (20 mmol/l leupeptin and 3.1 mmol/l aprotinin), 1% Triton X-100, 0.1% bacitracin, and 1% Trition X-100. The samples were centrifuged at 15,000 rpm for 15 min. The supernatant was collected into fresh tubes and stored at −70°C until assayed for TNF-α. The protein content of the samples was measured with the use of a protein assay kit (Bio-Rad Laboratories) with bovine serum albumin used as standard.

Tissue mRNA for TNF-α. mRNA for TNF-α was measured using real-time PCR using protocols described previously (7, 28, 62) with modification. The hypothalamus and heart tissues were dissected and homogenized in TriReagent to extract total RNA. RNA (0.1 pg/ml) for collection of trunk blood. The heart and brain tissue was removed using the posterior part of the optic chiasm as the anterior limit, the anterior part of the mammillary bodies as the posterior limit, and the lateral hypothalamic sulci as the lateral limits.

Heart dissection. The heart was cut into three cross sections. The right ventricular and LV tissues were separated, and the LV tissue was separated into infarct and peri-infarct tissue. The peri-infarct tissues were taken at least 1 mm away from the infarct tissues.

Measurements of TNF-α

Plasma and tissue TNF-α levels in rats were measured using an ultrasensitive rat TNF-α ELISA kit (Biosource International; Camarillo, CA) according to manufacturer instructions. Plasma TNF-α levels. The details of methodology have been described previously (20, 22). The minimum detectable concentration of TNF-α was <0.1 pg/ml.

Tissue levels of TNF-α. Tissue TNF-α levels were measured in the myocardium and hypothalamic tissue as described by Irwin et al. (28) with modifications (20). The tissues were homogenized in denaturizing buffer containing PBS, protease inhibitors (20 mmol/l leupeptin and 3.1 mmol/l aprotinin), 1% Triton X-100, 0.1% bacitracin, and 1% Trition X-100. The samples were centrifuged at 15,000 rpm for 15 min. The supernatant was collected into fresh tubes and stored at −70°C until assayed for TNF-α. The protein content of the samples was measured with the use of a protein assay kit (Bio-Rad Laboratories) with bovine serum albumin used as standard.

Tissue mRNA for TNF-α. mRNA for TNF-α was measured using real-time PCR using protocols described previously (7, 28, 62) with modification. The hypothalamus and heart tissues were dissected and homogenized in TriReagent to extract total RNA. RNA (0.1 pg/ml) was reverse transcribed using random hexamers as primers and Moloney murine leukemia virus RT in 20-μl volume at 37°C for 90 min. PCR primers and TaqMan probes for TNF-α were designed using the software program from Perkin-Elmer. The primers and probe for TNF-α are the following: sense primer (495–515 bp) 5′-CCAGGAAAGAATGACCTCT-3′; antisense primer (561–581 bp) 5′-TCATACAGGGCCTCAGGCTC-3′; and probe (sense) (527–552 bp) 5′-AGACCCCTGGCCCTAAGGACACCC-3′.

With the use of this primer sequence, a RT-PCR reaction was carried out with hypothalamic and heart tissue and an 87-bp product was detected and sequenced to confirm the product as TNF-α.

As an internal control for RNA input and RT efficiency, 18S rRNA was also quantitated for each sample with the use of a TaqMan kit (PE Biosystems). Thus the end results of real-time RT-PCR are expressed as copies of mRNA per nanogram of total RNA and the ratio of mRNA of interest and 18S rRNA.

Protocols

Study I: time course of TNF-α response to MI. In this study, rats underwent coronary artery ligation (CL) or sham CL (Sham) and were euthanized at 30 min, 1, 2, 4, or 24 h, or 4 wk. For most time points, ischemic injury was confirmed by echocardiography. The 30-min time point was too early to permit an echocardiogram. In these animals, blanching of the anterior LV wall at the time of CL and macroscopic postmortem examination of the injured ventricle were taken as evidence of acute MI. The animals that were euthanized at the 4-wk time point underwent echocardiography 24 h after CL. At the time of death, trunk blood was collected for measurement of TNF-α using ELISA. The hypothalamus and heart tissue were harvested for the measurement of TNF-α, mRNA, and protein.

Study II: effect of PTX on TNF-α response to MI. In this study, rats underwent CL or sham CL, with ischemic injury confirmed by echocardiography within 24 h. MI and sham animals were treated with PTX or Veh intraperitoneally via osmotic minipump for a period of 4 wk. At the end of the 4-wk treatment interval, the animals were euthanized and trunk blood was collected for the measurement of circulating TNF-α levels. The hypothalamus and heart were harvested and prepared for mRNA measurement using real-time RT-PCR or for measurement of TNF-α protein using ELISA.

Statistical Analysis

Each value is expressed as a means ± SE. Changes in circulating and tissue levels of TNF-α levels between the groups were analyzed by two-way repeated-measures ANOVA, followed by post hoc Fisher’s least-significant difference test. Differences between groups in circulating and tissue levels of TNF-α were analyzed using one-way ANOVA followed by Fisher’s least-significant difference test.

RESULTS

Survival

Eighteen of 85 rats (22%) undergoing coronary artery ligation died within 24 h of complications of the surgery and were not included in the study. All 32 rats undergoing sham coronary ligation survived the procedure. Among the rats assigned to study I, two coronary ligation rats and one sham-operated rat died before the designated time point. All rats assigned to study II survived to the completion of the protocol. Thus, over the time course of this study, PTX treatment had no apparent influence on survival.

Study I: Time Course of TNF-α Response to MI

The echocardiographic data for study I are displayed in Table 1. Compared with Sham animals, the MI rats studied at each point had lower LVEF, higher LVEDV, and higher LVEDV-to-LV mass ratio. The %IZ was similar at all time points, ~60% of the LV.

MI resulted in a progressive increase in plasma TNF-α (pg/ml), compared with the baseline level (2.8 ± 0.37) in the sham-operated rats. As shown in Fig. 1, plasma TNF-α had already increased significantly (6.32 ± 1.01) 30 min after MI. The greatest increase (87.8 ± 8.2) was observed at the 4-wk time point.

Thirty minutes after MI, expression of mRNA for TNF-α (copies/ng of mRNA) had already increased in the brain and heart (Fig. 2). In the Sham rats, mRNA for TNF-α was barely detectable in the hypothalamus (2.7 ± 1.0) and in the heart (LV: 63 ± 20 and right ventricle: 36 ± 6.5). Relative to these baseline values, the most dramatic early increase in mRNA for
MYOCARDIAL INFARCTION AND HYPOTHALAMIC CYTOKINES

Tumor necrosis factor-α (TNF-α) occurred at 30 min (85 ± 12), with further and even more striking increases at 24 h and at 4 wk (265 ± 28). Notably, there was no change in mRNA for TNF-α in brain cortex. In the heart, the early increases were significant but less impressive, with the LV infarct zone (109 ± 11) and peri-infarct zone (112 ± 13), and right ventricle (86 ± 12) expressing approximately twofold increases at 30 min. The highest levels of mRNA expression in myocardium were measured at 24 h in the LV infarct zone (665 ± 187), the peri-infarct area (1,246 ± 212), and the right ventricle (747 ± 230). At 4 wk, however, the mRNA for TNF-α in the cardiac tissues was still substantially higher than the baseline (Sham) value.

Study II: Effect of PTX on TNF-α Response to MI

The MI and Sham animals assigned to treatment with PTX versus Veh were well matched with regard to echocardiographically defined LV function. Compared with Sham rats (Fig. 3), MI rats had reduced LVEF and increased LVEDV. The %IZ was 54.6 ± 2.4 for MI rats assigned to Veh treatment, and 56.5 ± 3.2 for MI rats assigned to PTX treatment. Heart rate was not different among treatment groups: Sham + Veh, 407.9 ± 10.4; MI + Veh, 414.7 ± 14.9; MI + PTX, 416.2 ± 9.3; and Sham + PTX, 402.7 ± 11.1.

PTX treatment had a profound effect on plasma TNF-α (pg/ml) levels, measured 4 wk after MI (Fig. 4). In the vehicle-treated MI rats (MI + Veh, n = 15), the TNF-α levels had risen significantly (83.3 ± 11.6), closely resembling the 4-wk time-point measurement of circulating TNF-α (87.8 ± 8.2) in study I. In marked contrast, the PTX-treated MI rats (MI + PTX; n = 14) had plasma TNF-α levels (3.1 ± 0.6) that were not different from those of the two sham-operated groups (Sham + PTX: 3.3 ± 0.9, n = 14; Sham + Veh: 3.1 ± 0.5, n = 13). Thus at 4 wk after MI, PTX treatment had normalized plasma TNF-α.

PTX treatment had similar effects on the mRNA for TNF-α (copies/ng mRNA) in the hypothalamus and in the myocardium (Fig. 5). Four weeks after MI, hypothalamic expression of TNF-α mRNA (282 ± 45) had increased in the vehicle-treated rats to a level comparable to that observed at 4 wk in study I (265 ± 28). In the PTX-treated MI rats (MI + PTX), expression of mRNA in hypothalamus (6.2 ± 2.8) was comparable to that of the Sham + Veh group. Similarly, at 4 wk after MI, the expression of mRNA for TNF-α was increased in the LV (infarct zone: 645 ± 157; peri-infarct zone: 672 ± 140) and in the right ventricle (395 ± 42) in vehicle-treated rats (MI + Veh), but was normalized in PTX-treated rats. PTX treatment in the sham-operated rats had no effect on hypothalamic or myocardial mRNA for TNF-α.

Four weeks of treatment with PTX also normalized tissue TNF-α levels (pg/mg of protein) in the hypothalamus and myocardium in MI rats (Fig. 6). MI rats treated with vehicle (MI + Veh) had a severalfold and comparable increase in TNF-α levels in the hypothalamus (9.8 ± 0.38), LV (infarct: 9.44 ± 2.2; peri-infarct: 10.9 ± 1.4), and right ventricle (5.9 ± 0.34), compared with sham-operated controls (Sham + Veh: hypothalamus, 2.65 ± 0.19; LV, 2.86 ± 0.21; RV, 1.87 ± 0.26). TNF-α levels in the cortex did not increase after MI. In
the PTX-treated MI rats (MI + PTX), the tissue TNF-α levels (hypothalamus, 2.9 ± 0.15; LV infarct, 3.4 ± 0.4; LV perinfa
cart, 3.18 ± 0.38; RV, 2.13 ± 0.29) were not different from
those of the sham-operated controls (Sham + Veh). PTX treat-
ment in the sham-operated rats (Sham + PTX) had no effect on
tissue levels of TNF-α, compared with vehicle treatment
(Sham + Veh).

DISCUSSION

The principal new findings of this study are the following: 1) the hypothalamic synthesis of TNF-α increases within minutes of MI and persists for at least 4 wk, the duration of our study
protocols; 2) TNF-α appears at about the same time in plasma as in brain and heart, suggesting that increased synthesis of proinflammatory cytokines in these tissues is a prodrome to the generalized inflammation that characterizes heart failure after MI; 3) treatment with PTX prevents the MI-induced increases in TNF-α in the brain, heart, and plasma. Extrapolating from the known behavior of cytokines (6, 60), it seems reasonable to
suggest that the hypothalamic expression of other members of
the proinflammatory cytokine family, including IL-1β and IL-6, is similarly altered by acute MI and by PTX treatment after acute MI.

Most novel yet perhaps least surprising, in the context of existing literature, is the observation that MI induces proinflammatory cytokine synthesis in the hypothalamus. Among
brain tissues, the hypothalamus is particularly sensitive to the
influences of inflammatory stress and peripheral cytokine pro-
duction. For example, low doses of intraperitoneal lipopolysac-
caride induce the expression of mRNA for proinflammatory
cytokines (particularly IL-1β and IL-6) in the hypothalamus
(49, 64) with minimal effect in the cortex (49). The present
study, using TNF-α as a marker, demonstrates a similar dis-
tribution of cytokine expression in response to MI. TNF-α,
IL-1β, and IL-6 also activate hypothalamic neurons, particu-
larly those in the paraventricular nucleus (PVN) of the hypo-
thalamus that regulate the HPA via the blood-borne route (6,
12, 41). Although too large to readily cross the blood-brain
barrier, these circulating proinflammatory cytokines are thought to stimulate endothelial cells of the blood-brain barrier
to produce prostaglandin E2 (PGE2), which then diffuses into
brain tissue to activate the HPA (13, 41, 53). Although other
mechanisms have been proposed, a notable aspect of this
particular hypothesis is the possibility that PGE2 crossing into
the brain may induce local synthesis of proinflammatory cyto-
kines (41, 44).

The signal leading to augmented hypothalamic cytokine
synthesis after MI was not identified in the present study. The
early appearance of TNF-α in the hypothalamus suggests a
mechanism, perhaps stimulated by chemical and me-
chanical changes in the injured and inflamed myocardium. The
cardiac branch of the vagus nerve conveys mechanosensitive
and chemo-sensitive information to cardiovascular regions of
the brain (11), including the PVN (36), and so might well signal
the brain to activate mechanisms to counteract cardiac
injury. In a similar scenario, the abdominal vagus nerve has been implicated in the induction of hypothalamic IL-1β mRNA in response to intraperitoneal endotoxin (33) or IL-1β (26). Another likely source of ascending information regarding the state of the heart is the cardiac sympathetic innervation, which senses mechanical and chemical changes in the myocardium (3, 34, 47) and conveys cardiac pain sensation to the central nervous system (17). It seems reasonable to suggest that the cytokine-laden (29, 46, 51, 65) post-MI heart might provide a stimulus to one or both of these visceral sensory afferent systems that would signal the hypothalamus to increase cytokine production.

Of course, one cannot exclude the possibility that circulating cytokines, acting via one or more of the mechanisms suggested above, might account for the increased hypothalamic synthesis of TNF-α observed in this study. Because tissue and plasma samples were obtained simultaneously, any causal relationship that might exist between increases in plasma TNF-α and the increases in brain and heart TNF-α would be masked. The appearance of TNF-α in plasma implies excessive tissue production, and the injured heart seems an obvious source of blood-borne cytokines that might stimulate the brain. On the other hand, the brain is a well-recognized source of circulating TNF-α (52), and prior work from our laboratory (22) has demonstrated that central blockade of mineralocorticoid receptors prevents the rise in plasma TNF-α induced by this same coronary ligation protocol. Moreover, the present data demonstrate an impressively high ratio of TNF-α mRNA to TNF-α protein in the hypothalamus, compared with heart, suggesting that overproduction in brain tissue may have contributed significantly to the measured plasma levels. Of course, other tissue sources not tested in this study may also have contributed to the early increase and the later escalating levels of plasma TNF-α. It is a well-known property of the proinflammatory cytokines to amplify their own secretion (6). For example, the plasma collected from the coronary sinus of healthy donors (39). Thus perpetuation of the plasma cytokine response likely depends on the induction of synthesis in diverse tissues.

An increase in TNF-α and other proinflammatory cytokines (including IL-1β and IL-6) in the infarcted and noninfarcted heart tissue has been reported previously (25, 28, 46, 50, 65), although the mechanism for increased TNF-α production in uninjured cardiac tissues is still not well understood. Increased wall stresses may be a contributing factor (30), resulting from increased cavity size and dysfunctional contraction of the LV, with increased afterload on the right ventricle secondary to high LV end-diastolic pressures. In the present study, LVEDV to LV mass ratio, an indicator of wall stress, was already increased in the animals studied at the 1-h time point (Table 1). The MIs were large in these animals (Table 1), and others have reported right ventricular hypertrophy as a sign of the severity of LV failure (45, 54). Countering the chronic wall stress hypothesis is a recent study (4) demonstrating that sustained aortic occlusion in mice induced only a transient increase in myocardial TNF-α, IL-1β, and IL-6 lasting <72 h. In the present study, cytokine synthesis in the heart seemed to peak at 24 h (the last “early” time point checked) but was sustained at a lower level at 4 wk. Other potential explanations for this finding of persistent increased cytokine synthesis in the uninjured myocardium might be chronic exposure to increased catecholaminergic stimulation, which facilitates TNF-α production by the heart (43, 50), and the influence of persistent high circulating cytokines.

The augmented synthesis of proinflammatory cytokines in the hypothalamus early after MI has important pathophysiological implications. Whereas TNF-α, IL-1β, and IL-6 vary somewhat in their physiological effects, they share the property of inducing of local PGE2 synthesis (41). In the hypothalamic region, PGE2 has several important effects. With regard to the HPA, it induces the expression of mRNA for corticotropin-releasing hormone (CRH) as well as the expression of the CRH type 1 receptor in PVN (32), and it promotes exocytosis of CRH granules at the median eminence (40). With regard to the sympathetic nervous system, intracerebroventricular injection of PGE2 increases PVN neuronal activity (67), induces a sympathetically mediated pressor and tachycardic response (27), and increases circulating norepinephrine (42). Microinjection of PGE2 directly into the PVN increases the activity of neurons in the rostral ventrolateral medulla and increases renal sympathetic nerve activity, arterial pressure, and heart rate (67). Thus whereas the direct effects of augmented proinflammatory cytokine synthesis in the hypothalamus are not clear at this point, the indirect effects via induction of secondary mediators are potentially detrimental in the setting of acute MI.

From a clinical perspective, our data suggest that the increased production of proinflammatory cytokines in the heart, brain, and blood might be prevented if treatment with PTX were initiated early after acute MI. PTX has the property of inhibiting synthesis of proinflammatory cytokines in general, although existing data suggest it may be most effective in inhibiting synthesis of TNF-α (8, 10, 38, 55, 61). Because TNF-α appears early in the cytokine cascade (6), and induces the synthesis of IL-1β and IL-6, TNF-α inhibition may be a reasonable clinical goal. However, no clinical study has addressed the potential therapeutic benefit of PTX treatment early

Table 1. Echocardiographic measures for study I group

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Heart Rate, beats/min</th>
<th>LVEDV, ml</th>
<th>LVEDV/LV Mass, ml/g</th>
<th>Cardiac Output, ml/min</th>
<th>Ischemic Zone, %</th>
<th>LVEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>6</td>
<td>398.9 ± 19.4</td>
<td>0.30 ± 0.04</td>
<td>0.37 ± 0.07</td>
<td>105.7 ± 2.5</td>
<td>0</td>
<td>0.87 ± 0.04</td>
</tr>
<tr>
<td>MI + 1 h</td>
<td>7</td>
<td>383.8 ± 14.4</td>
<td>1.11 ± 0.08*</td>
<td>1.06 ± 0.01*</td>
<td>116.5 ± 12.0</td>
<td>58.5 ± 2.0*</td>
<td>0.39 ± 0.05*</td>
</tr>
<tr>
<td>MI + 4 h</td>
<td>8</td>
<td>389.1 ± 24.4</td>
<td>1.08 ± 0.07*</td>
<td>1.03 ± 0.02*</td>
<td>112.3 ± 8.5</td>
<td>59.0 ± 3.5*</td>
<td>0.37 ± 0.04*</td>
</tr>
<tr>
<td>MI + 24 h</td>
<td>8</td>
<td>401.7 ± 19.1</td>
<td>1.14 ± 0.05*</td>
<td>1.08 ± 0.05*</td>
<td>103.1 ± 9.7</td>
<td>57.2 ± 1.6*</td>
<td>0.37 ± 0.03*</td>
</tr>
<tr>
<td>MI + 4 wk</td>
<td>8</td>
<td>396.6 ± 16.2</td>
<td>1.13 ± 0.06*</td>
<td>1.05 ± 0.03*</td>
<td>108.7 ± 10.3</td>
<td>58.7 ± 1.7*</td>
<td>0.38 ± 0.02*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats per group. Sham, sham operation; MI, myocardial infarction; LV, left ventricular; LVEDV, LV end-diastolic volume; LVEF, LV ejection fraction. *P < 0.05 vs. Sham.

AJP-Heart Circ Physiol • VOL 286 • JUNE 2004 • www.AJPheart.org
after MI. Beyond its traditional use to treat peripheral vascular disease (18), PTX has been employed to counter the cytokine effects in patients with inflammatory liver disease (2), and in small clinical studies in patients with heart failure (56, 57), in which a high level of circulating TNF-α is a recognized marker of poor prognosis (9), PTX seems to have had a beneficial effect. In contrast, two large industry-directed clinical studies (RENAISSANCE and RECOVER) of etanercept, a large synthetic receptor complex that binds circulating TNF-α, were terminated early due to lack of efficacy in heart failure (35). Etanercept is too large to cross the blood-brain barrier, and thus unable to influence brain production of TNF-α (or other proinflammatory cytokines), except perhaps indirectly by reducing stimulation of endothelial PGE2 synthesis by circulating TNF-α.

Whereas the present study clearly demonstrates the effectiveness of PTX as an inhibitor of tissue production of TNF-α, it is important to note that it does not address the physiological significance of these reductions in TNF-α (or other proinflammatory cytokines). At this point, it is not clear whether or at what time point blocking or reducing the production of brain and/or heart cytokine production might prove beneficial in preventing the progression to heart failure after MI. In considering this issue, it is important to remember that the inflammatory response to myocardial injury is a critical component of the healing process (15). Nevertheless, persistent excessive activation of compensatory neurohumoral systems is one of the hallmarks of congestive heart failure, and treating these excesses has proven to be the most effective therapy to date (19).

In the present study, although brain and plasma TNF-α rose early in response to an acute event, they continued to rise throughout the 4-wk protocol, ultimately becoming dissociated from the inciting event. The highest levels of mRNA for TNF-α in heart were observed at 24 h, likely reflecting the proximity in time to the acute tissue injury and associated stress, but the highest levels of plasma TNF-α and of hypothalamic mRNA for TNF-α were observed at the 4-wk time point. These observations suggest a continued stimulus to cytokine production that extends well beyond the immediate myocardial injury. Thus persistent activation of the immune system, with its adverse long-term influences on both neurohumoral regulation and peripheral factors affecting myocardial function (16, 37) may be a valid target for therapeutic intervention. It is unknown whether early intervention with PTX (or a similar inhibitory agent) might help prevent the progression to heart failure while permitting the healing influence of the inflammatory process.

In summary, the present study has demonstrated that a large MI elicits the early appearance of proinflammatory cytokines not just at the site of cardiac injury but also remotely in a brain region that is critical for the cardiovascular adaptation to physiological stress. In this region of the brain, where the proinflammatory cytokines subserve many of the same functions as the active products of the renin-angiotensin-aldosterone system (14, 66), their presence may signal the continued activation of central neural mechanisms that drive sympathetic nerve activity and volume accumulation despite existing therapies targeting the renin-angiotensin-aldosterone system. In this study in rats with MI, PTX prevented the increases in TNF-α, and, by extrapolation, the increases in related proinflammatory cytokines in the brain, heart, and plasma. However, the significance and the optimal timing of such an intervention in the progression toward heart failure after acute MI in humans remains to be determined.

ACKNOWLEDGMENTS

The authors thank K. Zimmerman for diligent and expert assistance in the performance and interpretation of the echocardiograms.

Present address for J. Francis: Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute (NHBLI) Program Project Grant RO1 HL-014388 (to F. M. Abboud and R. B. Felder), NHBLI Grants RO1 HL-63915 and RO1 HL-73786 (to R. B. Felder), NHBLI Cardiovascular Interdisciplinary Research Fellowship HL-07121, and American Heart Association Scientist Development Grant 0330191N to J. Francis.

REFERENCES


