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ORIGINAL INVESTIGATIONS

Presence of Tumor Necrosis Factor in Humans Undergoing Cardiopulmonary Resuscitation With Return of Spontaneous Circulation

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Tumor necrosis factor-alpha (TNF) was measured in 21 patients who presented to the emergency department in normothermic, nontraumatic cardiopulmonary arrest and who had eventual return of spontaneous circulation (ROSC). Tumor necrosis factor was measured by an enzyme-linked immunosorbent assay in serum collected at various time points after the initiation of cardiopulmonary resuscitation (CPR). Tumor necrosis factor was not detected within 4 hours post-ROSC in any patient, but was detected in all nine patients in whom survival post-ROSC was 6 hours or

more. Six of nine patients demonstrated TNF presence at 6 hours. No patient demonstrating TNF at 6 hours survived longer than 27 hours post-ROSC. In patients surviving at least 12 hours, production of 400 pg/mL TNF was associated with a 100% mortality. Tumor necrosis factor is released in cardiopulmonary arrest patients who survive at least 6 hours post-ROSC and may play a role in the global ischemia-reperfusion phenomena of CPR.

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TUMOR NECROSIS FACTOR (TNF) is a 17,000-d, biologically active polypeptide hormone that is a primary mediator in the pathogenesis of cellular injury and inflammation, with pleiotropic effects on a variety of cells and organs.^{1,2} The major effect of TNF depends on its concentration; at low levels, it appears to participate in normal physiologic homeostasis and immunoregulation. However, when released in high concentrations, TNF is a potent cytokine mediator that has been strongly linked to septic shock, the adult respiratory distress syndrome (ARDS), and multiorgan system failure (MOSF).³⁻¹¹

Various inflammatory mediators have been implicated in models of ischemia-reperfusion injury, including lipids, prostaglandins, leukotrienes, platelet-activating factor, reactive oxygen metabolites, complement, adherence proteins, and proteases.¹²⁻¹⁷ Recent evidence suggests that in an animal model of liver isch-

emia-reperfusion, TNF is present in suprahepatic blood and is associated with significant hepatic and pulmonary abnormalities during the reperfusion phase.^{18,19} This data suggests that hepatic ischemia-reperfusion results in local TNF production, which is associated with distal organ injury.

Prolonged resuscitation in cardiopulmonary

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arrest (CPA) patients who have return of spontaneous circulation (ROSC) serves as an *in vivo* model of global anoxia/ischemia and subsequent reperfusion injury. Survivors of resuscitation may develop a clinical picture not unlike septic shock (postresuscitation syndrome) with similar hemodynamic and metabolic derangements, cellular and tissue injury, and, eventually, MOSF.²⁰ The exact etiology of the postresuscitation syndrome is unknown, but probably involves some or all of the putative factors involved in ischemia-reperfusion injury models.²¹⁻²⁴

We demonstrate here a potential correlation of circulating TNF in CPA patients who survived greater than 6 hours after resuscitative intervention.

PATIENTS AND METHODS

The study protocol was approved by the Human Rights Committee of the Henry Ford Health Systems. The subjects studied were adult patients who had an out-of-hospital CPA and who were brought into the emergency department (ED) of Henry Ford Hospital by either emergency medical services (EMS) or family members. Cardiopulmonary resuscitation (CPR) was begun at the CPA site by a family member or a bystander or when the EMS arrived and was continued until the patient was brought to the ED. History was obtained from family members or from other available records. Patients with a history of recent or current infection, renal failure, or malignancy were excluded from the study. Twenty-one patients were recruited that fulfilled the study guidelines.

On arrival to the ED, all patients were resuscitated by advanced cardiac life support guidelines. The majority of the patients (17 of 21; 81%) were resuscitated by standard advanced cardiac life support maneuvers; four patients received high-dose epinephrine (0.2 mg/kg). Five milliliters of blood were drawn from a central venous catheter (subclavian right atrial catheter) at initiation of CPR and subsequently from an arterial line at ROSC, and at 1, 2, 3, 4, 6, 12, and 24 hours post-ROSC. All blood was collected in serum separator tubes, allowed to clot at room temperature (37°C), then centrifuged at 2,000 rpm for 10 minutes. The serum was decanted and placed into freeze-resistant polypropylene tubes and immediately stored at -70°C. All samples were labeled with the patient's name, medical record number, and precise time of collection. In addition, biographic data were collected containing demographics, medical history, medications, disposition, sampling time, and mode of resuscitation. The death of the patient post-ROSC precluded the collection of blood at all designated time points. When there was deviation from the above protocol, the serum was discarded and the patient was excluded from the study.

All serum samples were assayed by the same investigator within 2 months of collection using a commercially available

enzyme immunoassay kit for the quantitative determination of TNF (Biokine TNF Test Kit; T-Cell Sciences, Cambridge, MA). Tumor necrosis factor was measured with a standard sandwich enzyme immunoassay that uses an enzyme-conjugated (horseradish peroxidase) murine monoclonal anti-TNF antibody to human TNF. A substrate solution using o-phenylenediamine resulting in the formation of a colored product in proportion to the amount of TNF present was then measured at 490 nm on an automated, dual wavelength, 96-well microtiter plate reader (Titertek Multiskan MCC/340, Lugano, Switzerland). A standard curve was prepared from five TNF standards (0, 44, 130, 425, and 925 pg/mL) and the unknown sample values were determined from the standard curve. All TNF standards and patient samples were prepared in duplicate, with the reported optical density being an average of the measured duplicate samples. All serum TNF concentrations were expressed in picograms per milliliter, with the detection limit of the assay being 10 pg TNF/mL. Interassay and intraassay coefficient of variation is less than 10%.

Data analysis was performed using either a chi-square test or a two-sample *t*-test. Differences were considered significant at $P < .05$. All values are expressed as the mean \pm standard error of the mean.

RESULTS

Twenty-one human subjects (11 men aged 65.2 ± 5.5 years and 10 women aged 65.4 ± 8.8 years) met entry criteria and were included in the study. Accurate quantitation of the time between out-of-hospital CPA and the arrival of the patient in the ED was hampered by incomplete history, suboptimal prehospital resuscitation, and poor documentation by family members or the EMS. Twelve of 21 patients (seven men and five women aged 67.7 ± 0.7 years; 57%) survived 4 hours or less post-ROSC (mean, 187.8 ± 25.30 minutes), while the remaining nine patients (four men and five women aged 62.1 ± 3.5 years; 43%) survived more than 4 hours post-ROSC. Seven of the nine patients in the group that survived more than 4 hours died in the hospital, a mortality rate of 78% in this subset of patients. There was no significant difference in either sex distribution or age in the two groups ($P > .05$) (Table 1). The mean time between initiation of CPR in the ED and ROSC in the group of patients who survived less than 4 hours was not significantly different from that for the group of patients who survived more than 4 hours (22.9 ± 1.0 minutes *v* 20.1 ± 2.6 minutes, respectively; $P > .05$).

In all 21 patients, TNF levels at time points ≤ 4 hours were undetectable (< 44 pg/mL). Tumor necrosis factor levels for the nine pa-

Table 1. Characteristics of Patients Surviving Less Than 4 Hours Versus Patients Surviving More Than 4 Hours

	<4-hr Survival Group (n = 12)	>4-hr Survival Group (n = 9)	P Value
Males	7	4	0.528*
Females	5	5	
Age (yr)	67.7 ± 0.7	62.1 ± 3.5	0.156*
CPR/ROSC Interval (min)	22.9 ± 1.0	20.1 ± 2.6	0.3334*

*Not significant.

tients surviving more than 4 hours are detailed in Table 2. Six of these nine patients demonstrated TNF presence at 6 hours. The remaining three patients, all of whom survived 20 days or longer post-ROSC, demonstrated TNF presence by 24 hours.

Six patients survived at least 12 hours post-ROSC. Of these, three survived 20 days or longer, whereas three died within 27 hours post-ROSC. All three patients who died within 27 hours post-ROSC demonstrated TNF levels greater than 400 pg/mL in the 24 hours post-ROSC. None of the patients surviving 20 days or longer demonstrated a TNF level greater than 253 pg/mL.

DISCUSSION

Tumor necrosis factor plays a major role in many immunologic and inflammatory processes, including overwhelming infection and sepsis,

ARDS, chronic inflammation, cachexia, the acquired immunodeficiency syndrome, and parasitic infections.^{7-9,25-29} The inflammatory effects of TNF on cells or tissues is likely mediated either by its direct effect or its ability to invoke injury via either activation of effector cells or through the release of a cascade of subsequent inflammatory mediators.³⁰

Tumor necrosis factor has been implicated as a proximal mediator in host responses to sepsis and septic shock, septic ARDS, and MOSF, as well as in certain animal models of ischemia-reperfusion injury.^{8-11,18,19} In sepsis and MOSF, detectable, circulating levels of TNF are responsible for multiple organ damage, higher lung injury scores, a greater incidence of ARDS, and an increased mortality rate.⁸⁻¹¹ Work by Damas et al suggests that TNF has not been associated with other shock states.⁸ In a rat model of ischemia-reperfusion injury, lung injury as assessed morphometrically and by changes in pulmonary capillary permeability were attributable to circulating TNF, since this cytokine could be detected in downstream blood and passive administration of TNF antisera prevented the observed changes.^{18,19}

Patients resuscitated from CPA often develop syndromes similar to septic shock with delayed MOSF (postresuscitation syndrome).^{20,21,23,24} Although the exact mechanisms are not known, reperfusion-induced release of

Table 2. Characteristics of Patients Surviving 6 Hours or More Post-Cardiopulmonary Resuscitation: Temporal Changes in Tumor Necrosis Factor

Patient No.	CPR/ROSC Interval (min)	Resuscitation Mode	TNF (pg/mL) Time						Clinical Data
			0 (ROSC)	2 hr	4 hr	6 hr	12 hr	24 hr	
1 (Q.J.)	18	ACLS and high-dose Epi	<44	<44	<44	<44	<44	253.0	ARDS, ATN; survived to hospital discharge
2 (M.M.)	33	ACLS	<44	<44	<44	25.6*	—	—	Respiratory failure, shock; died 6 hr post-ROSC
3 (J.M.)	20	ACLS and high-dose Epi	<44	<44	<44	<44	NC	26.7	MOSF, PVS; survived to hospital discharge
4 (L.S.)	19	ACLS	<44	<44	<44	<44	NC	40.1*	Sepsis, ARDS, MOSF, died 20 d post-ROSC
5 (W.F.)	29	ACLS	<44	<44	<44	43.3*	—	—	Respiratory failure, shock; died 6 hr post-ROSC
6 (D.C.)	22	ACLS	<44	<44	<44	39.0*	—	—	Respiratory failure, shock; died 6 hr post-ROSC
7 (R.P.)	19	ACLS and high-dose Epi	<44	<44	<44	43.7	170.8	426.9*	MOSF, died 27 hr post-ROSC
8 (M.C.)	15	ACLS and high-dose Epi	<44	<44	<44	15.5	468.5*	—	MOSF, died 12 hr post-ROSC
9 (O.H.)	6	ACLS	<44	<44	<44	245.6	339.4	438.2*(15h)	MOSF, died 15 hr post-ROSC

Abbreviations: ACLS, advanced cardiac life support; Epi, epinephrine; ARDS, adult respiratory distress syndrome; ATN, acute tubular necrosis; MOSF, multiorgan system failure; NC, not collected; PVS, persistent vegetative state.

*Patient died.

mediators, such as platelet-activating factor, prostaglandins, leukotrienes, reactive oxygen species, and cytokines, have been investigated.^{20,21,31} Cardiopulmonary arrest with ROSC following prolonged CPR provides a clinical model analogous to studies of whole-animal ischemia-reperfusion. As opposed to the sepsis syndrome, where it is difficult to temporally define the initiation and release of biologically active mediators such as TNF, sudden death episodes in previously healthy individuals offer a determinable start of injury that may be associated with cytokine release.

We have demonstrated that TNF is present in the serum of patients who suffered an out-of-hospital CPA. Tumor necrosis factor was not detected before 4 hours in any patient, yet was measurable at different times after ROSC in all patients (nine of nine patients; 100%) who survived at least 6 hours. This disparity could not be explained by another discriminator. The absence of TNF in patients surviving less than 4 hours (12 of 12 subjects; 100%) may only be a reflection of their short survival time and may be unrelated to other factors. A latency period of 90 to 180 minutes to peak plasma TNF levels has been reported in human subjects after endotoxin administration.²⁵ In a rat model of hepatic ischemia-reperfusion injury, a latency period of 30 to 180 minutes from reperfusion to peak TNF levels has been noted.¹⁸ This probably represents the time required by macrophages to synthesize and release TNF in detectable quantities into the plasma after stimulation. Therefore, it is possible that the stimulus for production of TNF had also occurred in patients surviving less than 4 hours, but their short survival time prevented sufficient time for production and/or release of detectable quantities of this cytokine. If TNF release post-CPA follows temporal kinetics similar to that following endotoxin administration, the time between ROSC and TNF detection in survivors would suggest a stimulus for TNF synthesis and release several hours after resuscitation occurs. This might suggest that the stimulus for TNF production in such patients may not be the primary ischemia-reperfusion event, but may be due to the secondary loss of intestinal mucosal integrity leading to activation of the reticuloendothelial system and TNF release.^{32,33}

Six of the nine patients surviving ≥ 6 hours demonstrated TNF at 6 hours. It is notable that all six patients died within 27 hours post-ROSC, whereas the remaining three patients (all surviving 20 days or longer) had no detectable TNF at 6 hours. The significance of this observation is unknown, but one may speculate that this would suggest a lesser initial stimulus for TNF production at the time of CPA in the three long-term survivors. In addition, the highest TNF level in the three patients surviving 20 days or longer was 253 pg/mL. All of the patients surviving at least 12 hours, but who died within 27 hours post-ROSC, demonstrated TNF levels greater than 400 pg/mL. This suggests that in patients surviving longer than 6 hours, TNF production greater than 400 pg/mL in the 24 hours post-ROSC carried negative prognostic importance.

Recent work in animal models of ischemia-reperfusion suggests that TNF is a key mediator in organ injury, but similar evidence is lacking in humans.^{18,19} Our data indicate that TNF is produced and released in CPA/ROSC patients, but is temporally not detected before 4 hours; other conclusions are only speculative. At present, the effects of the raised levels of TNF in this setting are unclear. Tumor necrosis factor-induced multiple organ damage in animals infused with TNF, and TNF-induced liver and lung damage in rat ischemia-reperfusion injury suggests a significant role for this cytokine in MOSF.^{11,18,19} The MOSF that occurs in patients who are successfully resuscitated (post-resuscitation syndrome) may be caused in part by TNF.^{20,31} Tumor necrosis factor in this setting may either directly activate proinflammatory cells or induce the synthesis of more distal inflammatory mediators.^{1-3,17,30} The relationships of temporal or absolute TNF levels to morbidity or mortality requires further investigation. Finally, if additional work demonstrates a significant correlation of TNF to morbidity or mortality associated with postresuscitation, then the future consideration for the use of monoclonal antibodies directed against TNF may be a novel approach to therapeutic intervention.

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