CARDIOPULMONARY SUPPORT AND PHYSIOLOGY

INHIBITION OF THE TRANSCRIPTIONAL ACTIVATOR PROTEIN NUCLEAR FACTOR κ B PREVENTS HEMODYNAMIC INSTABILITY ASSOCIATED WITH THE WHOLE-BODY INFLAMMATORY RESPONSE SYNDROME

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Background: The transcription factor nuclear factor κ B mediates the expression of a number of inflammatory genes involved in the whole-body inflammatory response to injury. We and others have found that dithiocarbamates specifically inhibit nuclear factor κ B-mediated transcriptional activation in vitro. **Objective:** We hypothesized that inhibition of nuclear factor κ B with dithiocarbamate treatment in vivo would attenuate interleukin 1α -mediated hypotension in a rabbit model of systemic inflammation. **Methods:** New Zealand White rabbits were anesthetized and cannulated for continuous hemodynamic monitoring during 240 minutes. Rabbits were treated intravenously with either phosphate-buffered saline solution or 15 mg/kg of a dithiocarbamate, either pyrrolidine dithiocarbamate or proline dithiocarbamate, 60 minutes before the intravenous infusion of 5 μ g/kg interleukin 1α . Nuclear factor κ B activation was evaluated by electrophoretic gel mobility shift assay of whole-tissue homogenates. **Results:** Infusion of interleukin 1α resulted in significant decreases in mean arterial pressure and systemic vascular resistance, both of which were prevented by treatment with dithiocarbamate. Pyrrolidine dithiocarbamate induced a significant metabolic acidosis, whereas proline dithiocarbamate did not. Nuclear factor κ B-binding activity was increased within heart, lung, and liver tissue 4 hours after interleukin 1α infusion. Treatment with dithiocarbamate resulted in decreased nuclear factor κ B activation in lung and liver tissue with respect to that in control animals. **Conclusions:** These results demonstrate that nuclear factor κ B is systemically activated during whole-body inflammation and that inhibition of nuclear factor κ B in vivo attenuates interleukin 1α -induced hypotension. Nuclear factor κ B thus represents a potential therapeutic target in the treatment of hemodynamic instability associated with the whole-body inflammatory response. (J Thorac Cardiovasc Surg 1999;118:154-62)

The use of cardiopulmonary bypass may result in a severe systemic inflammatory syndrome characterized by a hyperdynamic circulatory state in the presence

of reduced systemic vascular resistance (SVR).¹ This systemic inflammatory response syndrome may contribute significantly to postoperative morbidity and

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mortality.¹⁻³ Systemic inflammatory response syndrome is thought to be initiated by contact activation of complement and monocytes within the bypass circuit, leading to the elaboration of various inflammatory mediators including interleukin 1 α (IL-1 α).⁴ Widespread circulation of IL-1 α and other proximal inflammatory cytokines results in the transformation of vascular endothelium to an activation phenotype that enhances vasomotor dysfunction, coagulation, and leukocyte adhesion, further amplifying the hemodynamic derangements associated with systemic inflammatory response syndrome.⁵

Quiescent monocytic and endothelial cells respond to inflammatory stimuli and cell injury by activating signal transduction pathways that transmit signals through the cytoplasm to the nucleus, resulting in the transcription, translation, and expression of several genes involved in systemic inflammatory states. Nuclear factor κ B (NF- κ B) is an inducible transcription factor that binds with high affinity to specific sites in the promoter region of several inflammatory target genes to stimulate gene expression in response to a diverse array of inflammatory stimuli in vitro.⁶ The precise role of NF- κ B activation in vivo, however, has not been clearly defined. In particular, it is unknown to what extent NF- κ B can be targeted transcriptionally to prevent dysregulation of systemic inflammation.

IL-1 α induces significant hypotension when administered systemically to laboratory animals.⁷ IL-1 α was therefore administered to rabbits to create a simple model of the hemodynamic insufficiency encountered in systemic inflammatory states. We hypothesized that IL-1 α would systemically activate NF- κ B in vivo and that pharmacologic inhibition of this activation would attenuate cardiovascular decompensation induced by the inflammatory cytokine. To test these hypotheses and assess any possible detrimental systemic effects of NF- κ B inhibition in vivo, the dithiocarbamate compounds pyrrolidine dithiocarbamate (PDTC) and proline dithiocarbamate (ProDTC) were used. Such compounds have been shown in vitro to be specific potent inhibitors of NF- κ B.⁸

Methods

Reagents. PDTC was obtained from Sigma (St Louis, Mo). ProDTC was provided by Norbert Frank, PhD (German Cancer Research Center, Heidelberg, Germany). Human recombinant IL-1 α was a gift of Frank Chizzonite, PhD (Hoffmann-LaRoche Inc, Nutley, NJ).

Rabbit model. New Zealand White rabbits weighing 3 to 4 kg were used in research protocols approved by the Animal Care Committee of the University of Washington, Seattle. All animals received humane care according to the "Guide for the Care and Use of Laboratory Animals" prepared by the

Table I. *Experimental groups and conditions*

Group	Experimental conditions	
	First treatment	Second treatment
1	PBS	Saline solution
2	PBS	IL-1
3	PDTC	IL-1
4	ProDTC	IL-1

Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication No. 86-23, revised 1985). The rabbits were anesthetized with an initial intramuscular injection of 35 mg/kg ketamine and 5 mg/kg xylazine. They were intubated with an endotracheal tube (inner diameter 3.0 mm) and maintained with inhaled 1% halothane anesthesia in 100% oxygen with a tidal volume of 15 mL/kg for the duration of the study with a small-animal ventilator. Minute ventilation was adjusted according to arterial blood gas measurements to maintain arterial pH between 7.45 and 7.50 while keeping pCO₂ between 25 and 35 mm Hg. Body temperature was maintained at 36°C to 38°C with a heating pad. In accordance with a standard cutdown technique, a 20-gauge flexible catheter was placed in the left common carotid artery to continuously measure heart rate and mean arterial pressure and to collect blood samples. A double-lumen thermodilution probe and an injectate catheter (model 94-011-3F; Baxter Healthcare Corporation Cardio-Vascular Group, Irvine, Calif) were placed through the left femoral artery and vein to measure core temperature and cardiac output by thermodilution. A maintenance drip of Ringer's lactate solution (4-5 mL · kg⁻¹ · h⁻¹) was administered and central venous pressure was measured with a 20-gauge flexible catheter inserted into the right external jugular vein. An additional 30 to 50 mL normal saline solution was administered through the flushing of lines to maintain patency throughout the experiments. The cardiac output determinations were performed in triplicate and the results were averaged. SVR was calculated as follows:

$$\text{SVR} = (\text{MAP} - \text{CVP}) \times 80 / \text{CO} \text{ (dyn} \cdot \text{s} \cdot \text{cm}^{-5}\text{)}$$

where MAP was mean arterial pressure, CVP was central venous pressure, and CO was cardiac output. After insertion of the catheters, rabbits' conditions were allowed to stabilize for 30 to 60 minutes before baseline values were obtained. Minute ventilation and tidal volume were not changed after this baseline was established.

Study groups. Twenty-four rabbits were randomly assigned to 4 groups (n = 6 per group; Table I) and monitored for 240 minutes. Animals were intravenously treated with 1.0 mL phosphate-buffered saline solution (PBS) and PDTC or ProDTC (15 mg/kg in 1 mL PBS) 60 minutes before intravenous infusion of IL-1 α (5 μ g/kg) or 1.0 mL saline solution alone. Group 1 received PBS followed by saline solution alone. Group 2 was given PBS before IL-1 α infusion. Group 3 received PDTC before the administration of IL-1 α . Group 4 was treated with ProDTC before IL-1 α infusion. Hemodynamic parameters and arterial blood gas values were record-

Fig 1. Hemodynamic parameters from rabbits in group 1 (PBS plus saline solution, *open squares*), group 2 (PBS plus IL-1 α , *filled squares*), group 3 (PDTC plus IL-1 α , *open circles*), group 4 (ProDTC plus IL-1 α , *filled circles*). Graphs depict absolute change from baseline value (–60 minutes) for mean arterial blood pressure (**A**) and heart rate (**B**). Data are presented as mean for 6 rabbits in each group. Statistical significances of differences in means (group effects) and time trends (time effects) were assessed for 4 groups as cross-sectional time series with generalized estimating equations. *P* values from these analyses are represented in tables accompanying graphs.

ed at –60 minutes (baseline) and at 0, 30, 60, 120, 180, and 240 minutes after administration of saline solution or IL-1 α . Blood samples removed from the carotid catheter were replaced with the same volume of saline solution. At the end of 4 hours all animals were killed with 120 mg/kg intravenously administered concentrated pentobarbital. At that time heart, lung, and liver tissues to be used for electrophoretic mobility shift assay were snap-frozen in liquid nitrogen and stored at –70°C.

Nuclear protein extractions. Aliquots of frozen tissue

were mixed with liquid nitrogen and ground to powder with a motor and pestle. A 4-mL volume of solution A (0.6% Nonidet P-40 [ICN Biomedical, Inc, Aurora, Ohio], 150 mmol/L sodium chloride, 10 mmol/L *N*-2-hydroxyethyl-piperazine-*N*-2-ethanesulfonic acid, pH 7.9, 1 mmol/L ethylenediaminetetraacetic acid, and 0.5 mmol/L phenylmethylsulfonyl fluoride) were added to the mortar. The contents of the mortar were placed in a Dounce tissue homogenizer (Kontes Glass Company, Vineland, NJ), and the cells were lysed with 5 strokes of the pestle. After transfer to a 15-mL

Fig 1. Cont'd. Graphs depict absolute change from baseline value (–60 minutes) for cardiac output (C) and SVR (D). For complete legend, see Fig 1.

tube debris was pelleted by centrifugation at 2000 rpm for 30 seconds. The supernatant containing intact nuclei was transferred to 50-mL Corex tubes (Corning Incorporated Science Products Division, Acton, Mass), incubated on ice for 5 minutes, and centrifuged for 10 minutes at 5000 rpm. Nuclear pellets were then resuspended in 300 mL of solution B (25% glycerol, 20 mmol/L *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid, pH 7.9, 420 mmol/L sodium chloride, 1.2 mmol/L magnesium chloride, 0.2 mmol/L EDTA, 0.5 mmol/L dithiothreitol, 0.5 mmol/L phenylmethylsulfonyl fluoride, 2 mmol/L benzamidine, 0.5 mg/mL pepstatin, 0.5 mg/mL leupeptin, and 0.5 mg/mL aprotinin) and incubated on ice for 30 minutes. The mixture was then transferred to microcentrifuge tubes and nuclei were pelleted by centrifugation at 14,000 rpm for 1 minute. Supernatants containing

nuclear proteins were saved, divided into aliquots, and stored at –70°C. Protein quantitation was performed with the Bradford assay.

Electrophoretic mobility shift assays. An oligonucleotide containing the consensus sequence motif for NF-κB binding, 5′-αGTTGAGGGGACTTTCCCAGGC-3′ (Promega Corporation, Madison, Wis) was end-labeled with γ-phosphorus 32-tagged adenosine triphosphate (Nymcomed Amersham plc, Little Chalfont, United Kingdom) by means of polynucleotide kinase. Samples of nuclear protein extracts (20 μg) were incubated for 20 minutes at 25°C with the phosphorus 32-end-labeled, double-stranded oligonucleotide probe, underwent electrophoresis in native 4% polyacrylamide gels, and were autoradiographed. Cold competition was performed by adding a 50-fold molar excess of specific unlabeled double-stranded

Fig 2. Acid-base status from rabbits in group 1 (PBS plus saline solution, *open squares*), group 2 (PBS plus IL-1 α , *filled squares*), group 3 (PDTC plus IL-1 α , *open circles*), group 4 (ProDTC plus IL-1 α , *filled circles*). Graph depicts absolute change from baseline value (–60 minutes) for calculated base deficit. Data are presented as mean for 6 rabbits in each group. Statistical significances of differences in means (group effects) and time trends (time effects) were assessed for 4 groups as cross-sectional time series with generalized estimating equations. *P* values from these analyses are represented in accompanying table.

probe to the reaction mixture for 20 minutes before the addition of the phosphorus 32–end-labeled oligonucleotide probe.

Statistical analysis. The hemodynamic and acid-base data were analyzed as cross-sectional time series with generalized estimating equations, a technique that is analogous to repeated-measures analysis of variance but has more flexibility, allowing unequal spacing, unbalanced groups, and gaps.⁹ For each of the 5 outcome parameters the absolute change from baseline (–60 minutes) was modeled as the dependent variable and the following elements were included as covariates: the absolute baseline value of the outcome variable, log transform of time, the group effect, and a set of group $\times$ time interaction terms to determine whether there was a statistically significant difference among treatment groups with respect to time trends in the outcome variable. The time trends for these 4 groups were compared overall and assessed for statistical significance. The following 2×2 comparisons were made among specific groups: group 1 versus group 2, group 2 versus group 3, group 2 versus group 4, and group 3 versus group 4. Because our a priori hypotheses limited us to this restricted set of 2×2 comparisons, multiple comparisons adjustments were not made.

Results

Attenuation of interleukin 1 α –induced hypotension in vivo by dithiocarbamates. The hemodynamic effects of IL-1 α administration and dithiocarbamate

advance treatment are demonstrated in Fig 1. Rabbits in group 1 (saline solution control group) remained in hemodynamically stable condition throughout the 5-hour study period. Infusion of IL-1 α (group 2) resulted in a statistically significant reduction in mean arterial pressure of approximately 10 mm Hg with respect to the saline solution control group. Advance treatment with ProDTC attenuated this cytokine-induced hypotension ($P = .08$; Fig 1, A), whereas PDTC appeared to have little effect on blood pressure. PDTC advance treatment resulted in a significant increase in heart rate and a reduction in cardiac output that was not seen with ProDTC advance treatment (Fig 1, B and C).

IL-1 α injection caused a significant reduction in SVR with respect to that in the saline solution control group (Fig 1, D). Dithiocarbamate advance treatment repressed this IL-1 α –mediated reduction in SVR. Advance treatment with PDTC appeared to have a slightly greater and more prolonged effect in this regard, but there was no statistically significant difference in SVR trends between the 2 dithiocarbamate-treated groups.

Advance treatment with ProDTC appeared to inhibit IL-1 α –mediated hypotension primarily through its effects on SVR. Advance treatment with PDTC had more complicated hemodynamic effects. The IL-

Fig 3. Autoradiographs from electrophoretic gel mobility shift assays of NF- κ B activation in heart (A), lung (B), and liver (C) tissue of rabbits after IL-1 α administration with or without PDTC. Intravenous treatment with 15 mg/kg PDTC had little effect on IL-1 α -induced NF- κ B activation in rabbit heart tissue but resulted in inhibition of IL-1 α -induced NF- κ B DNA-binding in rabbit lung and liver tissue 240 minutes after injection of 5 μ g/kg IL-1 α . Lane 1, PBS plus saline solution; Lane 2, PBS plus IL-1 α ; Lane 3, PDTC plus IL-1 α ; Lane 4, cold competition, PBS plus IL-1 α incubated with unlabeled probe. Position of inducible NF- κ B-DNA complex is marked.

Fig 4. Autoradiographs from electrophoretic gel mobility shift assays of NF- κ B activation in heart (A), lung (B), and liver (C) tissue of rabbits after IL-1 α administration with or without ProDTC. Intravenous treatment with 15 mg/kg ProDTC had little effect on IL-1 α -induced NF- κ B activation in rabbit heart tissue but resulted in inhibition of IL-1 α induced NF- κ B DNA-binding in rabbit lung and liver tissue 240 minutes after the injection of 5 μ g/kg IL-1 α . Lane 1, PBS plus saline solution; Lane 2, PBS plus IL-1 α ; Lane 3, cold competition, PBS plus IL-1 α incubated with unlabeled probe; Lane 4, ProDTC plus IL-1 α . Position of inducible NF- κ B-DNA complex is marked.

1 α -mediated reduction in SVR was partially blunted by PDTC advance treatment, but PDTC advance treatment did not affect IL-1 α -mediated hypotension, perhaps because of the apparent cardiodepressive action evidenced by its chronotropic and negative inotropic effects.

Induction of metabolic acidosis by pyrrolidine dithiocarbamate but not proline dithiocarbamate. There were no significant differences in base deficit, bicarbonate level, or arterial pH between the groups at baseline before the administration of any treatment. Infusion of IL-1 α precipitated a metabolic acidosis with a base deficit of approximately -10 mEq after 120 minutes (Fig 2). This change in the base deficit was sig-

nificantly greater than that seen in the saline solution-treated control animals. Rabbits that were treated in advance with PDTC demonstrated a significant augmentation of this IL-1 α -associated base deficit ($P = .02$), an effect that was not seen after advance treatment with ProDTC.

Prevention of nuclear factor κ B nuclear localization in vivo by dithiocarbamates. To characterize the ability of dithiocarbamates to inhibit intranuclear NF- κ B DNA-binding activity we used electrophoretic mobility shift assay on nuclear proteins isolated from tissue of rabbits in the IL-1 α study groups. In rabbit heart, lung, and liver, NF- κ B DNA-binding activity was markedly increased 240 minutes after IL-1 α infu-

sion (Figs 3 and 4). In unstimulated heart and lung tissue there was essentially no detectable NF- κ B activation. NF- κ B did appear to be constitutively expressed in hepatic tissue. Advance treatment with PDTC or ProDTC had little effect on NF- κ B binding activity in the heart (Figs 3, A, and 4, A). Advance treatment with dithiocarbamate completely abolished the response to IL-1 α in the lung (Figs 3 B, and 4, B). Hepatic tissue from rabbits treated with dithiocarbamate before IL-1 α injection showed a level of NF- κ B binding that was similar to constitutive levels and less than the NF- κ B activation observed in untreated rabbits that received IL-1 α (Figs 3 C, and 4, C). Addition of 50-fold molar excess unlabeled, consensus sequence NF- κ B oligonucleotide (cold competition) to the nuclear protein samples successfully competed for NF- κ B binding, eliminating the distinct band and indicating the specificity of the NF- κ B DNA-binding complex.

Discussion

The principal goals of therapy in the treatment of cytokine-induced shock should be prevention of host cell activation, inhibition of secondary mediators of cellular injury, and limitation of end-organ damage.¹⁰ Current therapeutic interventions for systemic inflammatory response syndrome have failed to clearly demonstrate a clinical benefit, perhaps because they focus on inhibition of single distal components of the overall inflammatory response. Inflammation, however, is initiated and amplified by many redundant and overlapping molecular pathways. We therefore focused on the common control points shared by these mechanisms as targets for novel therapies to treat systemic inflammatory response syndrome. Transcriptional regulation of a set of inflammatory genes activated by cytokines may be a focal point in this process.

The role of NF- κ B in the transcriptional activation of key inflammatory genes has been extensively studied in primary and transformed cell lines in vitro. We have shown for the first time that NF- κ B is activated in vivo in multiple organs in response to IL-1 α administration. In this animal model of systemic inflammation we demonstrated the ability of PDTC to inhibit the activation of this transcription factor within lung and liver tissue. Further, this study demonstrated the ability of the less toxic dithiocarbamate ProDTC to inhibit in vivo NF- κ B activity. The ability of ProDTC to inhibit NF- κ B has never before been examined.

ProDTC was able to attenuate IL-1 α -induced hypotension primarily by inhibiting the IL-1 α -mediated decrease in SVR. PDTC was less effective at modulating these cytokine-induced hemodynamic changes. The

observed attenuation of cytokine-induced hemodynamic decompensation was associated with a decreased nuclear appearance of NF- κ B complexes in rabbit tissue. Dithiocarbamates block NF- κ B and prevent the expression of vascular cell adhesion molecule 1 and intracellular cell adhesion molecule 1 in human endothelial and fibroblast cells in vitro.¹¹⁻¹³ Expression of chemotactic cytokines is also prevented by dithiocarbamate treatment.¹⁴ Disruption of NF- κ B nuclear binding activity in vivo may have interrupted leukocyte trafficking and reduced organ inflammation, subsequently preventing leukocyte-mediated damage to the microvasculature. The hemodynamic stability observed in ProDTC-treated animals may have resulted from reduced intravascular fluid extravasation and improved hemodynamics related to reduced neutrophil accumulation within the tissue microvasculature, presumably resulting in decreased loss of endothelial barrier function. Alternatively, dithiocarbamate treatment may have reduced production of nitric oxide by inducible nitric oxide synthase in response to IL-1 α . Multiple NF- κ B binding sites exist within the promoter region of the inducible nitric oxide synthase gene, and dithiocarbamates prevent induction of inducible nitric oxide synthase in cultured macrophages.¹⁵

One unexpected finding in this study was that although PDTC is effective at blocking NF- κ B in vitro, it induces a toxic metabolic acidosis in vivo. In contrast, no acidosis was noted with ProDTC treatment alone. Animals treated with saline solution or ProDTC did not show a significant acidosis until after they received IL-1 α , suggesting that the cytokine was primarily responsible for the moderate acidosis observed. These changes cannot be attributed to differences in ventilation because the ventilatory settings were not altered after similar and stable baseline blood gas measurements had been attained in each group. ProDTC is known to behave similarly to other dithiocarbamates, with much less toxicity and greater stability in vivo, increasing the availability of the parent compound.¹⁶ As a consequence compounds such as ProDTC might exhibit superior activity against NF- κ B activation in vivo with fewer toxic side effects.

We have shown for the first time that NF- κ B activation is associated with severe IL-1 α -induced hypotension. PDTC was previously shown to be a potent inhibitor of NF- κ B in vitro. We have now shown that ProDTC can also act as a powerful inhibitor of NF- κ B, and it appears to be both more active and less toxic in vivo than PDTC. This study indicates that dithiocarbamates can be used as pharmacologic inhibitors of NF- κ B to study the contributions of this transcription factor to systemic inflam-

mation and other disease states, including neointimal formation, arteriosclerosis, transplant rejection, and ischemia-reperfusion injury. The inhibition of transcription factor activity represents an important approach to understanding the pathogenesis of dysregulation of inflammatory disease processes in vivo.

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Discussion

Dr Donald Glower (*Durham, NC*). The study has several new aspects that I think are intriguing and of potential future importance. First, you have clearly demonstrated a new class of anti-inflammatory agents, the NF- κ B transcriptional factor inhibitors, and have shown for the first time that 2 of those agents do inhibit inflammatory response in multiple organs in vivo. Second, this is the second study that has shown that inhibition of the NF- κ B transcriptional factor itself decreases systemic inflammatory response. Finally, in looking at 2 of these new agents you showed that one of them, ProDTC, appears to have fewer side effects and is potentially more interesting clinically.

These studies thus have broad significance not only in the field of basic immunology but in the field of clinical thoracic surgery, in which surgeons encounter many of the unfavorable side effects of the systemic inflammatory response to cardiopulmonary bypass. I think that this new class of agents, the NF- κ B inhibitors, has promise in attenuating the effects of cardiopulmonary bypass.

I have 2 main questions for you along these lines. First, you looked at a simple model of systemic inflammatory response in the rabbit. Do you have any experience regarding these agents in blocking the inflammatory response to cardiopulmonary bypass? Second, do you foresee any potential difficulties in applying this class of agents to cardiopulmonary bypass?

Mr Kovacich. Thank you for the questions. First of all, this was indeed a relatively simple model of systemic inflammation. Our main goals with this study were (1) to see whether NF- κ B was actually activated in multiple organ systems to respond to inflammatory stimuli and (2) to determine whether we could inhibit this response pharmacologically. We do not at this time have any experience in using these agents in a specific model of cardiopulmonary bypass. We do have some preliminary data from small-animal models of both global and regional myocardial ischemia-reperfusion in which we have been able to show that NF- κ B is activated. On the basis of our initial results with these myocardial ischemia-reperfusion models, it appears that these dithiocarbamate compounds are able to inhibit NF- κ B activation stimulated by ischemia-reperfusion, and this inhibition is correlated with a reduction in tissue injury related to myocardial ischemia-reperfusion. We do have plans underway to perform some large-animal porcine studies. We plan to test the ability of these agents to ameliorate the systemic inflammatory changes seen in a pig model of cardiopulmonary bypass. Specifically, we will examine the effects of these drugs on several end points of inflammation, including tissue edema and neutrophil accumulation in multiple organs, histologic

inflammatory changes, organ function, and hemodynamic parameters.

Dr Glower. This is a relatively new class of drug. Given what we do know about the inflammatory response to cardiopulmonary bypass, do you hypothesize that NF- κ B is a fairly central part of that response? Do you hope that a single agent such as an NF- κ B blocker might be all that you would need to dramatically reduce the inflammatory response to cardiopulmonary bypass, or are there other aspects of the inflammatory response that are not known to be inhibited by NF- κ B blockers and might need additional agents? For example, if we used one of these agents, would we need to use steroids in addition to block the inflammatory response?

Mr Kovacich. One of the reasons that we targeted NF- κ B

is that extensive in vitro data suggest that it is involved in the transcriptional regulation of a wide variety of known inflammatory mediators. As you know, one of the problems with the inflammatory response is that it is highly redundant and thus extremely difficult to block effectively at specific points. In our view, by targeting a common control point in the transcriptional regulation of these inflammatory genes we might more effectively attenuate dysregulation of inflammatory responses. NF- κ B activation appears to be an example of such a convergence point in the inflammatory cascade. Because NF- κ B does appear to be required for so many different genes, it is our hope that targeting this specific mechanism will allow us to attenuate the inflammatory response overall with only a single agent.

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