CARDIOPULMONARY SUPPORT AND PHYSIOLOGY

VESNARINONE AND AMRINONE REDUCE THE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME

Koh Takeuchi, MD,
Pedro J. del Nido, MD
Andra E. Ibrahim, MD
Hung Cao-Danh, PhD
Ingeborg Friehs, MD
Paul Glynn, PhD
Dimitrios Poutias, BS
Douglas B. Cowan, PhD
Francis X. McGowan, Jr, MD

Objective: The systemic inflammatory response is an important cause of organ dysfunction. The present study tested the hypothesis that 2 clinically used agents, amrinone and vesnarinone, would decrease inflammation and cardiac dysfunction in a relevant model of systemic inflammatory response activation. **Methods:** Rabbits received intravenous endotoxin, alone or in conjunction with amrinone or vesnarinone. Systemic effects were assessed by death, fever, behavior, and acidosis. Measures of inflammatory signaling were (1) plasma tumor necrosis factor- α and interleukin-1 β production, (2) lung tissue myeloperoxidase activity, and (3) myocardial inducible nitric oxide synthase activity. Indices of systolic and diastolic myocardial function were measured in Langendorff-perfused hearts. **Results:** Vesnarinone, in particular, reduced mortality rates (19% vs 61% for lipopolysaccharide alone, $P = .01$) and acidosis in lipopolysaccharide-treated rabbits. Both agents markedly reduced systemic tumor necrosis factor and interleukin-1 concentrations, lipopolysaccharide-mediated effects on myocardial systolic and diastolic function and on myocardial inducible nitric oxide synthase activity. Vesnarinone, but not amrinone, (1) decreased fever and lethargy, consistent with decreased central nervous system effects of endotoxin, and (2) decreased lung leukocyte infiltration. **Conclusions:** Vesnarinone and amrinone, which are used clinically for their inotropic and vasodilating properties, may be useful to limit inflammatory activation and consequent organ dysfunction. Structure-activity and/or pharmacokinetic between the compounds may be important, particularly in preventing inflammatory signaling within certain tissues. (J Thorac Cardiovasc Surg 1999;117:375-82)

Cardiopulmonary bypass (CPB) and cardiac operation stimulate inflammatory responses because of several factors, including blood contact with the extra-

corporeal circuit, ischemia-reperfusion injury, and tissue trauma. These events are associated with activation of a number of complex and interrelated pathways that include the coagulation, fibrinolytic, kallikrein, and complement systems; endotoxin release and cytokine production; white blood cell and platelet activation; endothelial activation and expression of leukocyte adhesion molecules; and production of nitric oxide, oxygen radicals, and proteolytic enzymes.¹⁻⁴ Thus therapies to modulate inflammatory signaling are desirable. Unfortunately, the effect of interventions directed against the systemic inflammatory response (eg, specific receptor or enzyme blockade) in models of inflammation or CPB has been modest, at least in part because of the diverse and interconnected nature of the pathways involved. More general therapies, such as steroids, have widely known undesirable side effects.

From the Department of Cardiac Surgery and the Anesthesiology/Critical Care Medicine Laboratory, Children's Hospital and Harvard Medical School, Boston, Mass.

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Address for reprints: Francis X. McGowan, Jr, MD, Cardiac Anesthesia Service, Children's Hospital, 300 Longwood Ave, Boston, MA 02115.

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Phosphodiesterase inhibitors, such as amrinone and milrinone, have been used for their inotropic and vasodilating properties to treat heart failure. Recently, diverse immunomodulating properties have been attributed to various members of this family of compounds, including decreased myocardial inflammation and T-cell activity in experimental myocarditis⁵⁻⁷ and decreased proinflammatory cytokine production in both humans⁸ and experimental models.⁹⁻¹¹ These results and reports that increased cytokine production occurs in human heart failure¹² have led to speculation that the anti-inflammatory actions of these compounds may be responsible for the clinical improvements in cardiac function that have been observed at concentrations that are not inotropic.⁸ Thus the purpose of the present study was to test the hypothesis that 2 clinically used agents, amrinone and vesnarinone, would significantly reduce inflammatory signaling in a model of severe systemic inflammatory response produced by intravenous endotoxin administration.

Methods

Reagents. The vesnarinone was a gift from Otsuka Pharmaceutical Co (Tokushima, Japan). All other chemicals were purchased from Sigma Chemical Company (St Louis, Mo), unless otherwise noted and were of the highest grade available. *Salmonella typhimurium* endotoxin (lipopolysaccharide; lot 121H4025) was also purchased from Sigma Chemical Company. Cell culture reagents were from Life Technologies (Gaithersburg, Md).

Experimental protocol. Animal procedures received institutional approval and were conducted in conformity with the "Guiding Principles in the Care and Use of Animals" of the American Physiology Society and the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication No. 85-23, revised, 1985). New Zealand White rabbits (2.5-3.0 kg) were given either intravenous endotoxin (0.2 mg/kg) dissolved in 1 mL/kg sterile saline solution (lipopolysaccharide-treated group) or sterile saline solution alone (1 mL/kg; control group). Two other groups received either intravenous vesnarinone (3 mg/kg as a single bolus) or amrinone (1 mg/kg bolus plus 10 µg/kg/min continuous infusion) concomitant with the lipopolysaccharide infusion. Mean arterial blood pressure was recorded continuously from an indwelling catheter in an ear artery. Hearts from animals thus treated were either used for contractility studies or prepared for measurements of inducible nitric oxide synthase (iNOS) activity. All animals received intravenous lactated Ringer's solution at a rate of 10 mL/kg/h for the duration of study.

Clinical effects of endotoxemia. Temperature (tympanic membrane) and clinical appearance were assessed hourly. Animals were given a behavioral lethargy score by an observer,

blinded to treatment status, as follows: 0 = normal activity; 1 = quiet, spontaneous activity; 2 = no spontaneous movement, but responsive (withdrawal) to touch; 3 = responds only to noxious stimuli; 4 = unresponsive to noxious stimuli.

Plasma tumor necrosis factor- α and interleukin-1 β concentrations. Approximately 1 mL of arterial blood was collected from at 0, 1, 2, and 6 hours. The blood was rapidly centrifuged (3000g for 5 min at 4°C) and frozen at -80°C. Plasma tumor necrosis factor- α (TNF- α) concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) with polygonal goat anti-rabbit TNF capture antibody (4-8 µg/mL), biotin-conjugated goat anti-rabbit TNF detection antibody (2 µg/mL), and streptavidin horseradish peroxidase conjugate (all from Pharmingen, San Diego, Calif) as per the manufacturer's instructions. TNF from activated rabbit peritoneal macrophages (Pharmingen) was used as standard. The lower limit of detection was 10 pg/mL; the assay was linear between 25 to 2000 pg/mL; the assay coefficient of variation was 9%. Plasma interleukin-1 β (IL-1 β) was measured with a commercially available solid-phase ELISA according to the manufacturer's instructions (Biotrak, Amersham, Buckinghamshire, England). The IL-1 β assay was linear between 15 and 1000 pg/mL, with a lower limit of detection of 10 pg/mL and assay a coefficient of variation of 6%.

Pulmonary inflammation. Samples of lung tissue were rapidly excised, rinsed free of blood in ice-cold phosphate-buffered saline solution, and immediately frozen in liquid nitrogen. Lung myeloperoxidase activity was measured as previously described.¹³ Lung tissue (approximately 5 g) was homogenized and then briefly sonicated in 50 mmol/L potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide. After centrifugation at 5000g at 4°C for 10 minutes, the supernatant (0.1 mL) was mixed with 2.9 mL of potassium phosphate buffer containing 0.167 mg/mL *o*-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The reaction occurred at room temperature with the absorbance change measured at 460 nm over 3 minutes. One unit of myeloperoxidase activity was defined as that degrading 1 µmol peroxide per minute.

Isolated heart preparation. Six hours after lipopolysaccharide, lipopolysaccharide + vesnarinone, or lipopolysaccharide + amrinone, animals received ketamine, xylazine, and heparin (1000 units) intravenously; their hearts were then rapidly excised and placed in ice-cold Krebs-Henseleit buffer. The composition of the buffer was NaCl, 140 mmol/L; CaCl₂, 1.25 mmol/L; KCl, 4.7 mmol/L; glucose, 11.0 mmol/L; MgSO₄, 1.2 mmol/L; NaHCO₃, 25 mmol/L; NaH₂PO₄, 0.5 mmol/L; bovine regular insulin, 10 U/L, was also included. Hearts were then rapidly perfused retrograde through the aorta in the Langendorff mode at 80 mm Hg constant pressure with 37°C buffer that had been equilibrated with a 95% oxygen and 5% carbon dioxide gas mixture and passed through a 0.2-µm filter. The final buffer pH was 7.35 to 7.45; the PO₂ was 500 to 600 mm Hg, and the PCO₂ was 30 to 40 mm Hg. The pulmonary outflow tract was incised and cannulated. A latex fluid-filled balloon connected to a micro-

Table I. Death, fever, and behavior after endotoxin

	Time (h)	LPS	LPS+AMR	P value vs LPS	LPS+VES	P value vs LPS
Alive (No.)	0	18	12		16	
	2	15	12		15	
	6	7	9	.07*	13	.01*
Temperature (°C)	0	37.6 ± 0.3	37.8 ± 0.4		37.5 ± 0.3	
	2	38.9 ± 0.6	39.9 ± 1.0	.20	38.1 ± 0.5	.04†
	6	39.1 ± 0.5	39.0 ± 0.6	NS	38.0 ± 0.4	.01†
Lethargy score (0-4)	0	0	0		0	
	2	2.5 ± 0.5	2.5 ± 0.5	NS	1.0 ± 0.5	.03†
	6	3.0 ± 0.5	2.0 ± 0.5	.15	0.5 ± 0.5	.01†

LPS, Lipopolysaccharide; AMR, amrinone; VES, vesnarinone; NS, not significant. Data are mean ± SD.

*Fisher's exact test, 2-tailed.

†Kruskal-Wallis.

manometry catheter (Millar Instruments, Houston, Texas) was used for isovolumic left ventricular (LV) function measurements. The balloon was inserted into the left ventricle and sewn to the mitral annulus to prevent herniation; balloon volume was varied with a calibrated, fluid-filled syringe. Hearts were enclosed in a water-jacketed chamber; myocardial temperature was monitored continuously with a thermistor placed in the right ventricle and maintained between 36°C to 37°C.

Peak developed LV pressure and LV end-diastolic pressure were measured at 5 different LV balloon volumes. For the purposes of statistical comparison of diastolic function, pressure-volume data from each heart was entered into the equation $P = be^{kv} + c$, as described by Glantz and Parmely,¹⁴ which we have used previously.¹⁵ Alterations in compliance were assessed as (1) chamber stiffness, calculated from the slope of this relationship as dP/dV at end-diastolic pressure (EDP) = 10 cm H₂O, and (2) chamber volume, estimated from the balloon volume necessary at EDP = 0 (ΔV_0). Coronary flow was measured by timed collection of the coronary effluent. Oxygen content was calculated from the measured oxygen tension (ABL-3 Acid-Base Laboratory, Radiometer, A/S, Copenhagen, Denmark) of aortic perfusate and coronary venous effluent samples; myocardial oxygen consumption was calculated as the measured arteriovenous oxygen difference multiplied by coronary flow.

iNOS activity. Calcium-independent nitric oxide enzyme activity (corresponding to iNOS, a key enzyme induced by proinflammatory cytokine signaling) was determined in the crude cytosolic fraction of LV myocardium by the conversion of radiolabeled arginine to citrulline, as we have previously described.¹⁶ Hearts were rapidly isolated and flushed with ice-cold homogenization buffer, which was comprised of 25 mmol/L Tris HCl, 2 mmol/L EGTA, 2 mmol/L EDTA, 2 mmol/L phenylmethylsulfonyl fluoride, 25 µg/mL leupeptin, and 0.3 mol/L sucrose, pH 7.5; the hearts were then snap frozen in liquid nitrogen.

Approximately 2 g of left ventricular myocardium was homogenized at 4°C with a polytron homogenizer and centrifuged at 1000g for 10 minutes. The supernatant was then

centrifuged at 100,000g for 1 hour at 4°C. The supernatant was saved as crude cytosol. The reaction buffer consisted of 20 mmol/L N-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid, 2 mmol/L dithiothreitol, 10% glycerol, and the aforementioned protease inhibitors. This was supplemented with 5 mmol/L tetrahydrobiopterin, 5 µmol/L flavin mononucleotide, 2.5 mmol/L NADPH, 25 mmol/L L-arginine, 1.0 mmol/L EGTA, 50 mmol/L valine (to inhibit arginase activity), and [2,3,4,5-³H]-L-arginine hydrochloride (final specific activity 0.1 mCi/mL). The reaction was initiated by the addition of crude cytosol (in a 1:1 ratio of cytosol/reaction mixture), proceeded for 1 hour at room temperature, and was terminated by the addition of 20 mmol/L citric acid, pH 4.0, in a ratio of 4:1 (citric acid/reaction mixture). Arginine was separated from citrulline by slowly passing the samples over a cation exchange column (Polypore Sulfopropyl 10 µm, 2.1 × 30 mm; Applied Biosystems, Foster City, Calif) for 8 minutes; citrulline was eluted with 0.02 mmol/L citric acid, pH 2.0. Enzyme activity was expressed as picomoles of arginine converted to citrulline per hour per milligram of protein.

Protein content was measured by the bicinchonic acid method (Pierce, Rockford, Ill) with bovine serum albumin used as standard.

Statistical analyses. All data are presented as mean ± SD. Differences in mortality rates between groups were compared with 2 × 2 contingency tables and Fisher's exact test (2-tailed). Multiple group comparisons were made with the Kruskal-Wallis test or analysis of variance followed by the Bonferroni procedure. For the purposes of statistical analysis, cytokine concentrations of 10 pg/mL or less (the lower limit of detection of the ELISA) were given a value of 0 pg/mL.

Results

Endotoxemia model. The mortality rate after lipopolysaccharide in the various groups is shown in Table I. An increase in body temperature was seen in lipopolysaccharide-treated animals but not in those that received lipopolysaccharide with vesnarinone; interest-

Fig 1. Cytokine production in response to endotoxin. Data are mean $\pm$ SD; $n = 8$ each. **A**, Plasma TNF- α in animals treated with lipopolysaccharide (LPS) alone, lipopolysaccharide + vesnarinone (VES), or lipopolysaccharide + amrinone (AMR). **B**, Plasma IL-1 β concentrations. Both vesnarinone and amrinone significantly suppressed lipopolysaccharide-stimulated TNF and IL-1 production compared with lipopolysaccharide alone (analysis of variance/Bonferroni).

ingly, fever was also sustained in animals that received lipopolysaccharide + amrinone (Table I). Endotoxemic animals and those that received lipopolysaccharide + amrinone were also more lethargic than the animals that received vesnarinone (Table I). No endotoxemic animal that received vesnarinone achieved a lethargy score of 3 or greater at any point during the 6-hour study period, whereas 50% of those that received lipopolysaccharide alone did so.

Mean arterial blood pressure in surviving lipopolysaccharide-treated animals was 71 ± 3 mm Hg, 66 ± 3 mm Hg, 63 ± 3 mm Hg, 59 ± 3 mm Hg, and 62 ± 4 mm Hg at 0, 1, 2, 4, and 6 hours after lipopolysaccharide administration, respectively. These values were not different at any of the time points in the lipopolysaccharide + amrinone or lipopolysaccharide + vesnarinone animals. Thus mean blood pressure did not appear to be affected by the drug treatments, and hypotension was not present before the hearts were isolated for Langendorff perfusion.

Serum bicarbonate was consistently lower in lipopolysaccharide-treated animals beginning 1 to 2 hours after lipopolysaccharide administration. The nadir value was 10 ± 1 mmol/L (vs 21 ± 2 mmol/L in control animals; $P = .02$). This abnormality was corrected somewhat by amrinone (14 ± 3 mmol/L) and more so by vesnarinone (18 ± 2 ; $P = .05$ vs lipopolysaccharide alone).

Markers of inflammatory activation. The effects of the different treatments on plasma TNF- α and IL-1 β in endotoxemic animals are shown in Fig 1, A and B, respectively.

Lipopolysaccharide significantly increased lung myeloperoxidase activity 6 hours after exposure; this change was prevented by vesnarinone but not by amrinone (Fig 2).

Differences in the calcium-independent conversion of radiolabeled L-arginine (the nitric oxide precursor) to L-citrulline are shown in Fig 3. Lipopolysaccharide caused a significant increase in myocardial iNOS activity that was markedly attenuated in lipopolysaccharide-treated animals who had also received vesnarinone and, to a somewhat lesser extent, amrinone.

Cardiac function. The effects on rate $\times$ pressure product (RPP), myocardial oxygen consumption, and derived indices of diastolic function (dP/dV , ΔV_0) in the different treatment groups are shown in Table II. As can be seen, both amrinone and vesnarinone prevented lipopolysaccharide-induced reductions in RPP. Interestingly, vesnarinone-treated lipopolysaccharide-treated hearts were substantially more efficient, using significantly less oxygen per unit work (RPP/myocardial oxygen consumption ratio). Diastolic function was significantly impaired by lipopolysaccharide and improved in animals that received either amrinone or vesnarinone.

Discussion

There has been increasing recognition of the importance of various effectors and mechanisms of inflammation in the pathogenesis of sepsis, ischemia-reperfusion, and rejection. The multiple mechanisms of

Fig 2. Lung myeloperoxidase activity was reduced by vesnarinone (VES) treatment ($P = .03$) compared with either lipopolysaccharide (LPS) alone or lipopolysaccharide + amrinone (AMR). Data are mean $\pm$ SD; $n = 4$ each.

Fig 3. Calcium-independent nitric oxide synthase activity in myocardial extracts from animals treated with lipopolysaccharide (LPS) alone, lipopolysaccharide + vesnarinone (VES), or lipopolysaccharide + amrinone (AMR). Data are mean $\pm$ SD; $n = 6$ each. Both vesnarinone and amrinone reduced iNOS activity resulting from lipopolysaccharide treatment (analysis of variance/Bonferroni, $P = .04$).

Table II. Myocardial function in Langendorff-perfused, endotoxin hearts

	Sham	LPS	P value vs control*	LPS+AMR	P value vs LPS*	LPS+VES	P value vs LPS*
Rate pressure product (mm Hg/s $\times 10^3$)	16.2 $\pm$ 2.2	10.1 $\pm$ 3.0	.03	14.8 $\pm$ 3.2	.1	15.9 $\pm$ 2.0	.04
Oxygen consumption (mL/min/g)	48 $\pm$ 7	65 $\pm$ 8	.06	69 $\pm$ 12	.4	42 $\pm$ 10	.04
Chamber stiffness (dP/dV @ EDP = 10)	11.4 $\pm$ 2.3	17.1 $\pm$ 3.1	.04	12.8 $\pm$ 3.1	.05	12.1 $\pm$ 2.5	.05
Chamber volume at EDP = 0 (ΔV_0)	0.7 $\pm$ 0.1	0.3 $\pm$ 0.2	.03	0.6 $\pm$ 0.1	.05	0.8 $\pm$ 0.1	.01

LPS, Lipopolysaccharide; AMR, amrinone; VES, vesnarinone; EDP, end-diastolic pressure. Data are mean $\pm$ SD.

*Analysis of variance/Bonferroni.

inflammatory activation associated with CPB (eg, trauma, ischemia-reperfusion, contact activation, coagulation/fibrinolysis) make it in many ways the most significant and diffuse of clinical models. This study did not use CPB. Nonetheless, the findings are also relevant to CPB. Most directly, endotoxemia has been frequently (although not universally) found to occur during CPB.¹ Ischemia-reperfusion can rapidly promote intramyocardial TNF production.¹⁷ Endotoxemia activates cytokine, complement, white cell, and adhesion molecule pathways, all of which are also activated by CPB. Indeed, many of the deleterious effects of CPB have been attributed to these interrelated inflammatory cascades. Thus many of the mechanisms of organ dys-

function in endotoxemia and CPB (eg, ischemia-reperfusion, white cell infiltration, nitric oxide, and oxyradical production) are qualitatively similar.

The most important finding of this study was that vesnarinone and amrinone, to somewhat different degrees, prevented or reduced many different features of the acute endotoxemic response. These included lipopolysaccharide-induced death, fever, acidosis, cardiac dysfunction, and elevated plasma cytokine concentrations. Because vesnarinone has unique pharmacologic effects unrelated to type III phosphodiesterase inhibition, including Na⁺ channel opening, decreased inward and outward K⁺ currents, and prolonged action potential duration in the heart,¹⁸⁻²⁰ we studied the effects of amri-

none in the same model. Previous studies have demonstrated varying potency of type III and IV phosphodiesterase inhibitors to decrease production of cytokines and other markers of inflammation in response to proinflammatory stimuli.²¹⁻²³ The exact mechanism of action is uncertain but is likely to include increased intracellular cyclic adenosine monophosphate (cAMP) concentrations⁹; for example, increased cAMP blocks lipopolysaccharide-mediated TNF gene transcription.^{9,22-24} This effect may be specific for TNF, however, because increased cAMP does not prevent production of IL-6 under similar circumstances.²² Furthermore, vesnarinone but not amrinone prevented natural killer cell activity and TNF- α production in a murine model of acute viral myocarditis,⁶ which also suggests that some of vesnarinone's anti-inflammatory effects may not be common to all phosphodiesterase inhibitors.

Other reports have documented inhibition of second messenger signaling in response to proinflammatory and membrane-damaging stimuli, particularly by means of phosphatidic acid release from membrane lipids.^{25,26} The ability to prevent "stress-induced" cellular activation in response to the activation of specific membrane receptors or membrane damage may underlie the wide range of effects on cytokine production, neutrophil activation, and organ function. Because the primary role of phosphatidic acid signaling appears to be to respond to various forms of cellular stress, its inhibition may preferentially target cells exposed to activating events while minimally affecting normal cells; this may offer a potential advantage compared with other agents such as corticosteroids.²⁵

There were some important differences between amrinone and vesnarinone in this model. Amrinone demonstrated a minimal ability to decrease pulmonary leukocyte infiltration as measured by lung myeloperoxidase activity. Whether this was due to less potent effects on leukocyte signaling mechanisms (eg, IL-8, adhesion molecule production) or other mechanisms requires further delineation. Intriguing was the suggestion that vesnarinone had greater effect on fever and "sickness behavior" despite equivalent effects on systemic cytokine concentrations. Fever and behavioral changes caused by lipopolysaccharide can be attributed to specific areas within the central nervous system.²⁷ Endotoxemia and cytokines are believed to affect the brain indirectly by stimulating meningeal macrophages and perivascular microglia to produce prostaglandins and other signaling compounds²⁷; whether vesnarinone has greater inhibitory effect on these pathways remains to be determined. However, it should also be empha-

sized that plasma (or brain) vesnarinone and amrinone concentrations were not measured and dose-response effects not studied. Important differences exist in different cell types with regard to phosphodiesterase isoform content (eg, phosphodiesterase III or IV) and the sensitivity of these isoforms to phosphodiesterase inhibitors. For example, vesnarinone may display some degree of selectivity for the cardiac phosphodiesterase III isoform, inhibiting neutrophil and monocyte phosphodiesterase IV at significantly higher concentrations.²⁸ Thus pharmacokinetic or pharmacodynamic explanations for any observed differences between the 2 drugs cannot be excluded.

The significance of the present study is that it suggests that agents such as vesnarinone or amrinone may be useful to modulate several aspects of the inflammatory response. Although vesnarinone has been found to improve myocardial performance in patients with heart failure, prolonged use of high-dose oral vesnarinone increased mortality rates.²⁰ The responsible mechanisms were uncertain, but it is unlikely that a similar result would occur given the much shorter duration (12-24 hours) of therapy that would be needed in the setting of CPB. It is likely, however, that this intervention would need to be combined with agents affecting other pathways for optimal effects.

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Discussion

Dr Edward D. Verrier (*Seattle, Wash*). Most of the time we would use phosphodiesterase inhibitors after the event. I have 2 questions.

Do you think that ischemia-reperfusion would precipitate similar responses or is this more consistent with an inflammatory stimuli like TNF or IL-1 or lipopolysaccharide?

Have you done any studies where the event occurs and then you give vesnarinone and then look at some of the markers of inflammation? Can you give it later or does this have to be something that is on board before?

Dr Takeuchi. I think I will try to answer the second question first. We tried vesnarinone treatment after a couple of hours after injection of lipopolysaccharide, but the effect we got was not so dramatic, so that is why we administered the vesnarinone simultaneously.

Regarding your first question, I think that the next model to study probably is the CPB model. We just focused on this simple, severe model to test the effect of this drug, to see if it is effective or not; so the results are actually positive. I think the next step will be to try to do the CPB model.

Dr del Nido. I would like to add a comment to what Dr Takeuchi said. In fact, what we are trying to address here is the inflammatory response to CPB. The interesting finding is that if you treat, in essence almost pretreat, or treat simultaneously, you get a profound reduction in the inflammatory response. We were accustomed to using these drugs as inotropes after ischemia. They do have some effect in improving function after ischemia, obviously, but there may be a role for them as a pretreatment modality.

Dr Verrier. Is there a disadvantage to treating somebody with it before bypass? Have you done the model in bypass at all?

Dr del Nido. We have not done the model in bypass. The disadvantage is the vasodilatory properties of amrinone. Vesnarinone is certainly not as potent for that. So probably vesnarinone would be the better drug to test.

Dr Christopher A. Caldarone (*Boston, Mass*). In relation to the reperfusion question, working in Dr Levitsky's laboratory in Boston, we actually investigated milrinone in a large animal model of ischemia-reperfusion injury (*Ann Thorac Surg* 1994;57:540-5). Surprisingly, in the nonischemic controls, we were not able to detect evidence of improved contractility or diastolic function in the absence of an ischemic injury.

After an ischemia-reperfusion injury with global normothermic ischemia, administration of milrinone in the reperfusion period actually had only a moderate improvement in contractility but a very dramatic improvement in early diastolic relaxation. Our impression was that the efficacy of phos-

phodiesterase inhibition was somehow limited to ischemia-reperfusion injury. Your data would suggest that we should reinterpret our results and perhaps what we were really seeing was an amelioration of an inflammatory response after the reperfusion injury.

My one question is that the end-diastolic pressure/volume relationship tends to be a better index of the extent of late diastolic relaxation. In contrast, early diastolic relaxation is

commonly estimated with tau, the constant of isovolumic relaxation, which more accurately reflects calcium handling events occurring in early diastole. Did you find a difference in the early diastolic function?

Dr Takeuchi. Actually I did measure diastolic pressure/volume in these groups, but there was no difference between those groups. In terms of the diastolic constant tau, it is significantly different.