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**Original Article*****Matrix Metalloproteinase Inhibitor: Differential Effects on Pulmonary Neutrophil and Monocyte Sequestration Following Cardiopulmonary Bypass***

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**ABSTRACT**

Acute respiratory distress syndrome (ARDS) following cardiopulmonary bypass (CPB), also known as “post-pump” or “post-perfusion syndrome” (PPS), results from sequential priming and activation of neutrophils. We hypothesized that chemically modified tetracycline (CMT-3) an inhibitor of neutrophil matrix metalloproteinase (MMP) and elastase, would prevent PPS. We performed histometric analysis of lung tissue from our porcine PPS model to correlate cellular sequestration and histologic injury with CMT-3 treatment. **Methods:** Yorkshire pigs were randomized into five groups: *Control* (n=3); *CPB* (n=5); femoral-femoral bypass 1 hour; *LPS* (n=7), *Escherichia coli* lipopolysaccharide (1µg/kg); *CPB+LPS* (n=6); and *CPB+LPS+CMT* (n=5), sequential insults and CMT-3. Protocol histometric analysis defined cellular and tissue components of lung injury. **Results:** CMT-3 decreased neutrophil sequestration in the *CPB+LPS+CMT-3* group (p<0.0001 vs. *CPB+LPS*). There were no differences in monocytes between *CPB+LPS* and *CPB+LPS+CMT* treatment groups. **Conclusions:** CMT-3 attenuates neutrophil sequestration but has no effect on mononuclear sequestration in our PPS model. This finding supports current research on leukocyte chemokines and has important implications regarding mechanisms of CMT-3. Despite lack of monocyte response to CMT-3, PPS was prevented by inhibiting neutrophils alone; confirming the primary role of neutrophils in PPS.

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## INTRODUCTION

Acute respiratory distress syndrome (ARDS) following cardiopulmonary bypass (CPB), termed "post-perfusion" or "post-pump" syndrome (PPS), is indistinguishable from other acute lung injury associated with trauma, hemorrhage, or sepsis. Many studies have described the pathophysiology of PPS (1) and concentrated effort is now being given to therapeutic strategies aimed at its reduction. We previously reported a sequential injury CPB model that is ideal for testing PPS therapies because it allows investigation of the sequence of pathophysiologic changes since the times of priming and activating stimuli are known. In that study we demonstrate that acute lung injury following CPB can develop following sequential "hits" or insults, with CPB acting as the initial inflammatory event or "first-hit"(2). A short period of CPB alone is rather innocuous. However, when combined with a sequential insult (a "second-hit" such as transient hypoxia, ischemia or endotoxemia) a systemic inflammatory response may develop resulting in endothelial injury, pulmonary edema, and PPS.

Although there is some evidence to the contrary (3–5) it is generally accepted that this systemic inflammatory response and lung injury typical of ARDS (6,7) and PPS (1,7,8) is primarily neutrophil-mediated. Our previous investigations support this concept, having shown an association between pulmonary neutrophil sequestration and physiologic lung injury (2). We found that a single insult (CPB or LPS) caused a significant increase in both lung tissue density and leukocyte sequestration compared to controls. These increases occurred without significant physiologic lung dysfunction. Sequential insults (CPB+LPS) caused a further increase in lung tissue density and leukocyte sequestration which were associated with severe lung dysfunction and PPS. It is our observation that the subsequent insult, or "second-hit," surpasses the activation threshold of sequestered leukocytes (primarily neutrophils) resulting in release of a plethora of enzymes, oxygen radicals, and cytokines. It is only after the "second-hit" triggers this neutrophil response that clinical PPS develops.

Elastase and matrix metalloproteinases are two major components of this response. Elevated levels of neutrophil elastase and matrix metalloproteinases are present in plasma of patients following CPB (9,10) and in both plasma and bronchoalveolar lavage (BAL) fluid of patients with ARDS (11). Elastase is a neutral serine protease that degrades not only elastin (12) but collagen types I, II, III, VI (13,14) fibrinogen, fibronectin (15) and proteoglycans. Matrix metalloproteinases (MMPs) are a family of enzymes secreted by numerous cells which are involved in homeostasis, remodeling and response to injury. They degrade elastin, proteoglycans, gelatin, and a number of collagens, including type IV which provides the framework for the basement membrane of pulmonary capillaries (16,17). In addition, MMPs inactivate endogenous antiproteases such as  $\alpha_1$ -antitrypsin ( $\alpha_1$ -protease inhibitor,  $\alpha_1$ -PI) allowing unre-

stricted protease activity (18). Thus, these mediators are key elements in acute lung injury caused by sequestered neutrophils following CPB.

We have previously shown that a new chemically modified tetracycline (CMT-3) prevents lung injury in our PPS model (19). CMT-3 significantly improved physiologic parameters such as  $\text{PaO}_2$ , venous admixture and ventilatory efficiency index (VEI) as well as decreasing biochemical parameters typical of PPS such as bronchoalveolar lavage (BAL) elastase and gelatinase levels and lung water. Our objective in the present study was to perform extensive histometric analysis of lung tissue from control and treatment groups to determine leukocyte response to sequential insults (CPB and LPS) and changes in that response mediated by CMT-3.

## MATERIALS AND METHODS

### SURGICAL PREPARATION AND VENTILATION

Healthy Yorkshire hybrid pigs (15–20 kg) were instrumented for hemodynamic and ventilatory monitoring as detailed in our previous report (19). Initial ventilator settings were  $\text{FiO}_2$  50%, tidal volume 12 cc/kg, and rate of 10 breaths/min. Adjustments were made in the respiratory rate to obtain a baseline  $\text{PaCO}_2 = 45\text{--}55$  mm Hg. The lung was sighed every 30 minutes by manual delivery of 150% of the set tidal volume. Preliminary experiments in our laboratory have shown that induction of PPS causes fatal elevation of arterial  $\text{PCO}_2$ . Therefore, base excesses (BE) below  $-3$  mEq/L were corrected with intravenous sodium bicarbonate and adjustments were made in ventilatory rate to maintain  $\text{PaCO}_2$  within normal range (40–45 mm Hg). Heating pads and warmed IV fluids were utilized to maintain a core temperature between  $34\text{--}38^\circ\text{C}$ . All groups received lactated Ringers solution (25 ml/kg/hr) in addition to bolus infusion of Dextran 70 to maintain cardiac output (CO) within 10% of baseline.

### CARDIOPULMONARY BYPASS

After a baseline activated clotting time (ACT)<sup>a</sup> was obtained, all animals to be placed on CPB were fully anticoagulated with heparin (300 U/kg). With verification of adequate anticoagulation (ACT > 480 seconds), a surgical cut-down was performed to facilitate placement of a 14 F arterial catheter into the right femoral artery, and a venous cannula, Medtronic 18 F percutaneous femoral arterial catheter<sup>b</sup> into the right femoral vein which was advanced into the right atrium. The cardiopulmonary bypass circuit included Cobe oxygenators (Cobe Duo flat plate membrane), tubing pack, an arterial filter (40 $\mu\text{m}$ ),<sup>c</sup>

a Hemochron 401, International Technidyne Corp., Edison, NJ

b Medtronic, Inc., Anaheim, CA

c Cobe Cardiovascular Inc., Arvada, CO

and a Sarns roller pump.<sup>d</sup> The pump prime solution consisted of lactated Ringers (1500 ml), mannitol (5 g), sodium bicarbonate (35 mEq), and porcine lung heparin (5000 IU).

Non-pulsatile CPB for one hour was initiated at a flow rate of 120 mL/kg/min. Mean arterial blood pressure was maintained (30–60 mm Hg) by adjusting blood flow rate. Oxygen and air flow to the oxygenator were titrated to maintain physiologic blood gases (pH 7.35–7.45, PCO<sub>2</sub> 35–40 mm Hg, and PO<sub>2</sub> 150–300 mm Hg). During CPB the integrated warming coils in the blood reservoir were used to decrease body temperature to 28°C over a period of 10–15 minutes. Body temperature was returned to normal over 20 minutes before terminating CPB. Thirty minutes prior to termination of CPB, intravenous infusion of isoproterenol (4 µg/min.) was initiated to facilitate weaning from the pump and maintain MAP at 30–60 mmHg. During this short weaning period when animals were not on full bypass support, isoproterenol increased effective ejection volume to eliminate cardiac distension and prevent ischemia. Five minutes before discontinuation of CPB, calcium chloride (500 mg) and magnesium sulfate (1 g) were given to avoid arrhythmias associated with electrolyte imbalance. Within 30 minutes animals had returned to a baseline status defined as: 1) blood volume of the entire circuit transfused back into the animal, 2) heparin reversed with protamine (1.3 mg/100 U heparin), and 3) pulmonary pressures, systemic pressure, and cardiac output all within 10% of baseline without assistance from inotropic agents. Animals not randomized to a group exposed to CPB received sham CPB (surgical preparation without anticoagulation or bypass).

#### ENDOTOXIN INFUSION

Animals receiving LPS were infused with 1 µg/kg of *E. Coli* lipopolysaccharide (LPS; SIGMA 111:B4) mixed in 500 ml of saline and delivered over one hour via a volumetric infusion pump. Animals randomized to a group not exposed to LPS received sham LPS (500 ml saline vehicle only).

#### CHEMICALLY MODIFIED TETRACYCLINE (CMT-3)

CMT-3 (6-demethyl-6-deoxy-4-dedimethylaminotetracycline) is a chemically modified, non-antibiotic tetracycline with a molecular weight of 371.35 g/mol. CMT-3<sup>e</sup> was obtained in powder form, dissolved in dimethyl sulfoxide (DMSO), and then administered intravenously at a dose to achieve a 25 µM (9.2 µg/mL) blood concentration. This concentration effectively inhibited elastase in prior unpublished studies using other disease models. We assumed predominantly intravascular distribution for our dosing regimen. This method of delivery achieved a serum concentration of 1.2±0.367 µg/mL at one hour as determined by high pressure liquid chromatography (HPLC).

#### PROTOCOL

- Control:** (n = 3) Animals were subjected to 1 hour of sham CPB (surgical preparation without bypass), followed by sham LPS (infusion of saline vehicle without LPS) and then monitored for 2 hours. Neither heparin nor protamine were given.
- CPB:** (n = 4) Animals were subjected to 1 hour of CPB, followed by sham LPS infusion and then monitored for 2 hours.
- LPS:** (n = 6) Animals were subjected to 1 hour of sham CPB followed by LPS infusion and monitored for 2 hours. Neither heparin nor protamine were given.
- CPB+LPS:** (n = 6) Animals were subjected to 1 hour of CPB followed by LPS infusion and monitored for 2 hours.
- CPB+LPS+CMT:** (n = 5) Animals were subjected to 1 hour of CPB followed by simultaneous infusion of LPS+CMT, and monitored for 2 hours.

It is important to note that although a protamine/heparin group was not controlled for in these experiments, prior experiments with this model have defined this group as having no effect on either physiologic or histologic parameters (2). Animals were euthanized with an overdose of pentobarbital (90 mg/kg IV). The experiments described in this study were in compliance with the "Guide for the Care and Use of Laboratory Animals," published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985). The protocol was approved by the Committee for the Humane Use of Animals (CHUA) at the SUNY Health Science Center, Syracuse, NY.

#### HISTOMETRIC ANALYSIS

At necropsy all of the animals in each treatment group had the right cardiac lobe excised and the hilar airway cannulated. Glutaraldehyde fixative (2.5%, phosphate-buffered) was slowly instilled through the cannula until air was no longer displaced from the airway. The lung was then immersed in glutaraldehyde and additional fixative was infused with a syringe while pressure was monitored with a mercury manometer. When the pressure of the fixative stabilized at 25 mm Hg the cannula was clamped and the tissue was stored in glutaraldehyde at 25 mm Hg airway pressure and room temperature for at least 24 hours.

Each lung specimen was studied according to a stratified random sampling method that assured the unbiased assessment of parenchymal structures lacking a homogeneous distribution. One tissue block from the fixed lobe of each animal was randomly chosen and processed for routine paraffin sections. The blocks were sectioned grossly until the entire profile of the tissue entered the plane of the section, usually constituting a rectangle of approximately 15 mm × 20 mm. At that point, ten serial sections made at 7 mm were individually mounted on numbered slides. Either even or odd numbered slides were then

d 3M Healthcare, Ann Arbor, MI

e CollaGenex Pharmaceuticals, Newtown, PA

randomly stained with hematoxylin and eosin. In this manner, five equidistant sections were studied in each animal. A sampling probe consisting of a vertical line traversing the height of the slide was established for each of the serial sections as follows: The sampling probe in the first section was drawn at the left edge of the tissue, the probe on the last section was drawn close to the right edge, and the middle three sections were drawn at equidistant locations. Ten sampling areas were designated 1 mm apart along each sampling probe. Thus, 50 unbiased sampling areas were established in each animal ( $n = 150$  for control,  $n = 200$  per treatment condition) prior to microscopic analysis. Each sampling area was located blindly using the x-y vernier scales of the microscope stage and then observed with a 100 $\times$  oil-immersion objective using a high-resolution video camera. The field of view was defined by a square grid consisting of 64 intersections that covered an area of 6400 mm<sup>2</sup> at the focal plane. Areas featuring bronchi, interlobular connective tissue or muscular blood vessels were discarded by advancing the stage 0.5 mm along the sampling probe thus limiting quantification to alveolar parenchyma. Tissue density was estimated for each sampling area as the percentage of intersection points falling over tissue structures (as opposed to points corresponding to spaces). Cell counts were made in all focal planes following a counting rule designed to avoid overestimation of the number of objects per unit area (20). Leukocytes were categorized (Table 1) into six groups: 1) neutrophils within tissue, 2) total neutrophils (tissue and spaces), 3) mononuclear cells within tissue, 4) total mononuclear cells (tissue and spaces), 5) total leukocytes (neutrophils and mononuclear cells), and 6) macrophages.

### STATISTICS

All values are reported as mean  $\pm$  SEM and were analyzed on a mainframe computer with Minitab (Release 9.1) statistical package. Tissue density estimates and cell counts were compared between treatment groups and between cell categories using analysis of variance (ANOVA). Differences were considered significant at a probability level of 95% ( $p < 0.05$ ).

## RESULTS

(See Table 1 and Figure 1)

**Between treatment groups:** Neutrophil infiltration into the pulmonary interstitium following CPB, LPS and CPB+LPS, significantly increased from controls (Figures 2–5). We found no evidence of neutrophil infiltration into the air spaces in any group. Treatment with CMT-3 (CPB+LPS+CMT) significantly reduced neutrophil infiltration compared to the “two-hit” group (CPB+LPS), bringing values below “single-hit” levels (LPS or CPB) but not returning them to control levels (Figure 6). Monocyte infiltration into the pulmonary interstitium and air spaces following CPB, LPS, and CPB+LPS was significantly greater than control (Figures 7 and 8). Treatment with CMT-3 had no effect on sequestration of monocytes compared to the “two-hit” group (CPB+LPS). Compared to control, macrophage sequestration was increased in alveoli following LPS, and CPB+LPS, but not following CPB alone. Treatment with CMT-3 did not reduce sequestration of alveolar macrophages compared to the “two-hit” group.

**Between neutrophils and monocytes:** The response of neutrophils to control and CPB was not significantly different from monocyte response to control and CPB. However, monocyte sequestration was significantly greater following LPS, CPB+LPS, and CPB+LPS+CMT when compared to neutrophil response to the same conditions. Furthermore, as expected from the between treatment group comparisons, CMT-3 treatment significantly affected the differences between neutrophils and monocytes; lowering numbers of sequestered neutrophils but not monocytes (Figure 9).

Histologic sections from animals that received either CPB or LPS were generally observed to have thickened alveolar walls and greater leukocyte infiltration as compared to controls. Animals exposed to both CPB+LPS exhibited even more extensive leukocyte infiltration and congested blood vessels compared to all other groups. These effects were attenuated by treatment with CMT-3.

**Table 1: Differential pulmonary leukocyte counts by treatment condition**

	CONTROL	CPB	LPS	CPB+LPS	CPB+LPS+CMT
Neutr./tissue	0.7 $\pm$ 0.1	1.5 $\pm$ 0.1 $\dagger$	1.2 $\pm$ 0.1 $\dagger$	1.8 $\pm$ 0.1 $^*$	1.1 $\pm$ 0.1 $\dagger$
Total neutr.	0.7 $\pm$ 0.1	1.5 $\pm$ 0.1 $\dagger$	1.2 $\pm$ 0.1 $\dagger$	1.8 $\pm$ 0.1 $^*$	1.1 $\pm$ 0.1 $\dagger$
Monos./tissue	0.9 $\pm$ 0.1	1.2 $\pm$ 0.1 $\dagger$	1.5 $\pm$ 0.1 $\dagger$	2.2 $\pm$ 0.2 $\dagger$	1.8 $\pm$ 0.1 $\dagger$
Total monos.	0.9 $\pm$ 0.1	1.3 $\pm$ 0.1 $\dagger$	1.6 $\pm$ 0.1 $\dagger$	2.3 $\pm$ 0.2 $\dagger$	2.1 $\pm$ 0.1 $\dagger$
Total leuko.	1.6 $\pm$ 0.1	2.8 $\pm$ 0.2 $\dagger$	2.8 $\pm$ 0.2 $\dagger$	4.2 $\pm$ 0.2 $\dagger$	3.1 $\pm$ 0.2 $\dagger$
Macrophages	0.6 $\pm$ 0.1	0.7 $\pm$ 0.1	1.1 $\pm$ 0.1 $\dagger$	1.3 $\pm$ 0.1 $\dagger$	1.3 $\pm$ 0.1 $\dagger$

Cell categories: neutrophils (*neutr.*) within tissue, total neutrophils (tissue and spaces), monocytes (*monos.*) within tissue, total monocytes (tissue and spaces), total leukocytes (*leuko.*) (neutrophils and monocytes), and macrophages. CPB = cardiopulmonary bypass, LPS = lipopolysaccharide, CMT = chemically modified tetracycline. Cells/6400  $\mu\text{m}^2$ .  $\dagger = p < 0.05$  vs. CONTROL,  $^* = p < 0.05$  vs. ALL GROUPS. All data are means  $\pm$  SEM.

### Pulmonary Leukocyte Counts

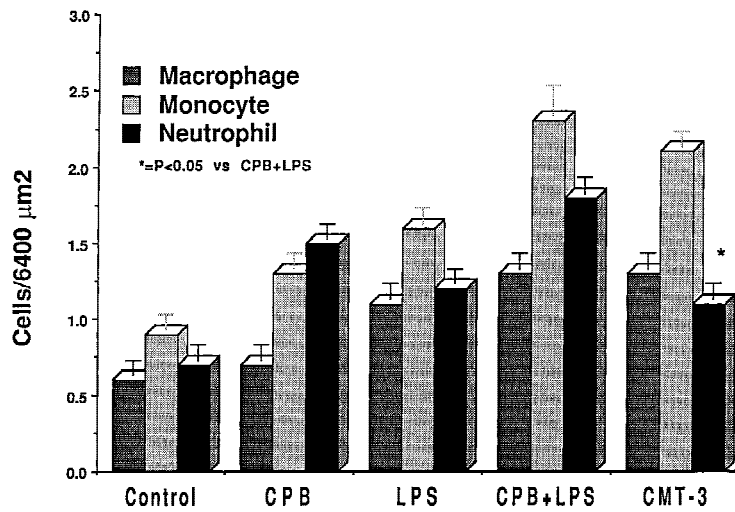


Figure 1: PULMONARY LEUKOCYTE COUNTS, number of cells per 6400 mm<sup>2</sup> field with standard errors (SEM). Cell counts from Table 1. Monocytes = total monocytes, Neutrophils = total neutrophils, and Macrophages. CPB = cardiopulmonary bypass, LPS = lipopolysaccharide, CMT-3 = chemically modified tetracycline treatment group (CPB+LPS+CMT).

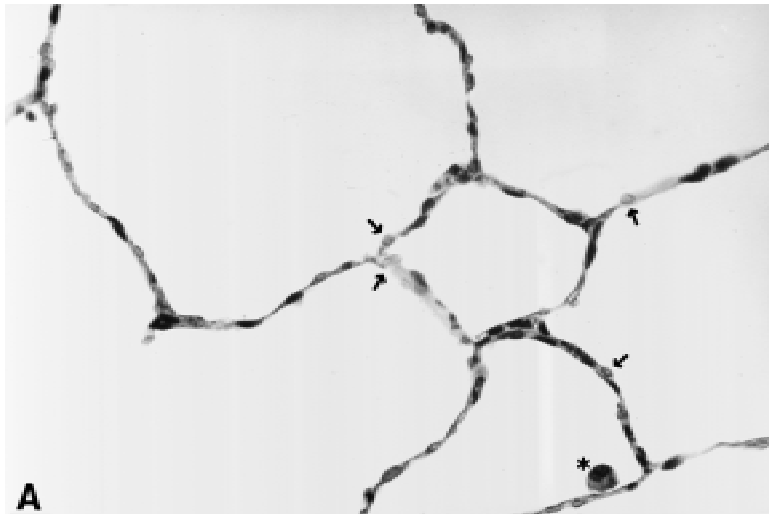


Figure 2: CONTROL (125x), the alveolar walls are slender and one cell thick. Capillaries are not conspicuous except where an erythrocyte is present (arrows). Alveolar macrophages (asterisk) are least numerous in the control.

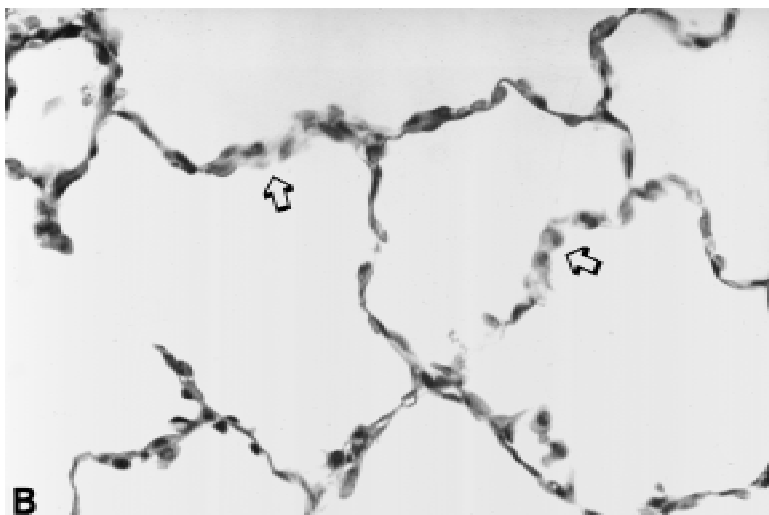


Figure 3: CPB (125x), Alveolar walls exhibit focal widening (open arrows) associated with the presence of monocytes and/or neutrophils.

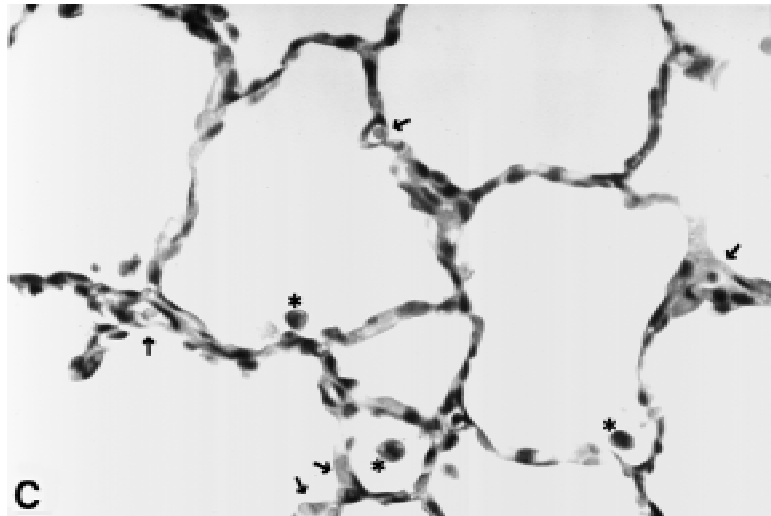


Figure 4: LPS (125 $\times$ ), the presence of cells other than pneumocytes within the alveolar wall is widespread. Blood capillaries are enlarged (arrows) and alveolar macrophages (asterisks) are larger and more vacuolated than in the CPB or control specimens.

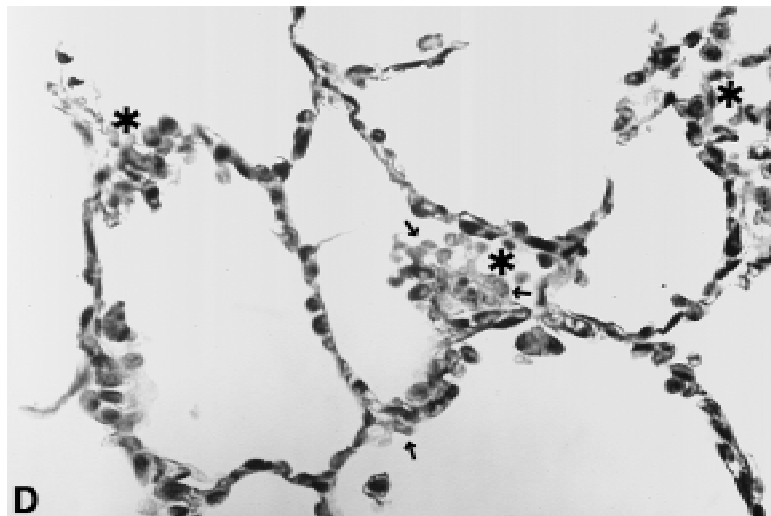


Figure 5: CPB+LPS (125 $\times$ ), the micro-circulation appears congested (arrows) and alveolar walls are heavily populated with monocytes and neutrophils. Areas of cellularity (asterisks) mark some alveolar corners and are also present in the alveolar lumen.

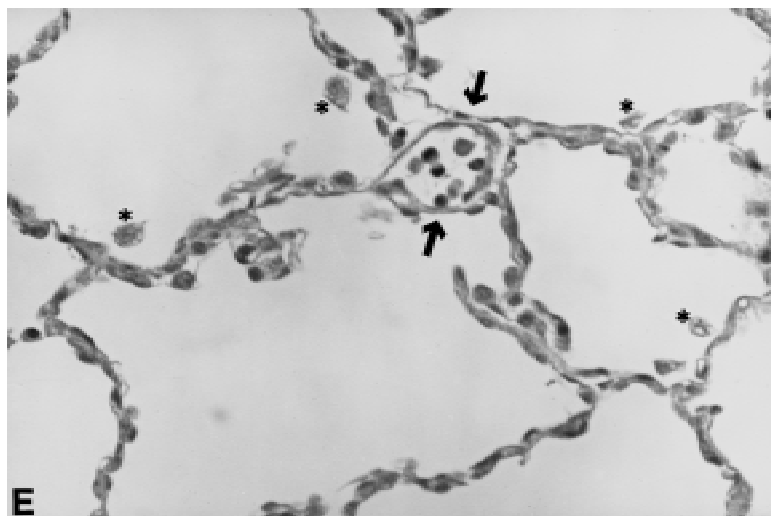
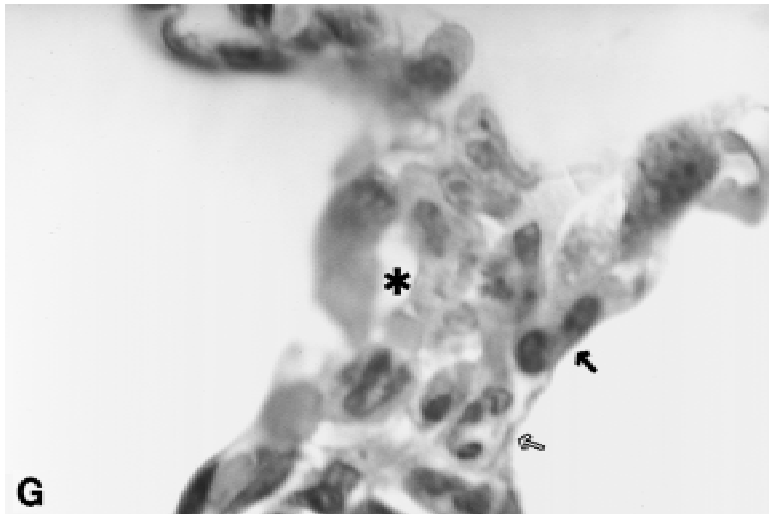


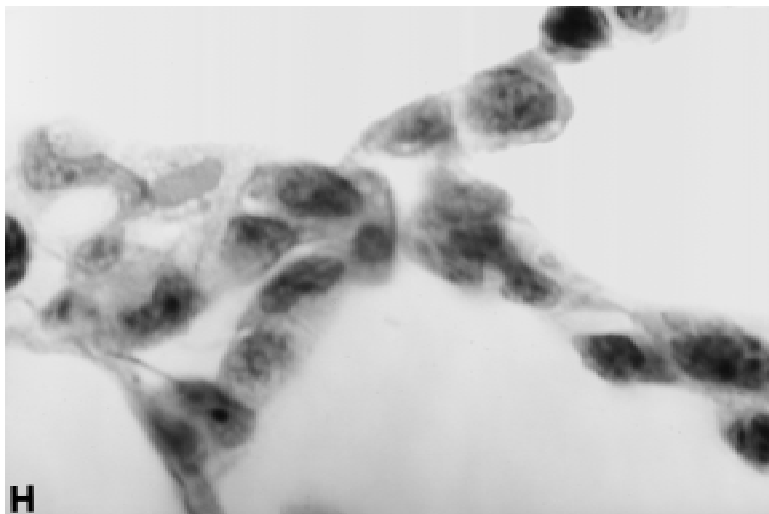
Figure 6: CPB+LPS+CMT (125 $\times$ ), monocytes and neutrophils are numerous in blood vessels (arrows) and within the interstitium, but not in the alveolar lumina. Alveolar walls are enlarged and alveolar macrophages (asterisks) are common.



**Figure 7: CONTROL (500×),** the alveolar walls of untreated animals consist mainly of pneumocytes and are, for the most part, one cell in thickness.



**Figure 8: CPB+LPS (500×),** focal widening of the alveolar wall is associated with capillary congestion (asterisk) and the appearance of monocytes (arrow) and neutrophils (open arrow).



**Figure 9: CPB+LPS+CMT (500×),** treatment with CMT-3 is followed by a sharp decrease in neutrophils, with a consistent and unchanged infiltration of monocytes.

## DISCUSSION

We demonstrated that CMT-3 reduces neutrophil sequestration in the pulmonary interstitium of our porcine PPS model. Interestingly, monocyte and macrophage sequestration in the interstitium and alveoli was not reduced by CMT-3 treatment under the same conditions. Reasons for the differential response between neutrophils and monocytes are unclear. Since CMT-3 is a relatively new drug, its mechanisms of action are still being elucidated. However, knowing CMT-3 has differential effects on neutrophil and monocyte sequestration suggests this drug must preferentially inhibit some factor leading to neutrophil sequestration and/or activation that has little or no effect on monocytes and macrophages. There are a few possibilities to explain the mechanism behind this observation.

First, differential pulmonary sequestration of neutrophils and monocytes may be a function of their ability to enzymatically degrade the basement membrane and diapedese from the vasculature into the lung interstitium. There are known differences between the MMPs produced by neutrophils and monocytes and the influence CMT-3 has on each. For instance, it has been demonstrated that monocytes, macrophages and epithelial cells produce MMP-1 (fibroblast-type collagenase), while neutrophils produce MMP-8 (neutrophil interstitial collagenase) (21). Chemically modified tetracyclines (CMTs) are able to effectively inhibit neutrophil-type collagenase (MMP-8), but not monocyte derived collagenase (MMP-1) (22). Although chemotactic activity of granular enzymes such as the MMPs has not been specifically described, these substances, or factors concomitantly released with them, may be involved in leukocyte sequestration by degrading the basement membrane, allowing increased diapedesis of leukocytes into the interstitium. Additionally, the mobilization and response time of these two collagenases may implicate their involvement. MMP-8 is released from stored neutrophilic granules in a matter of seconds (21). As a result, levels of neutrophil collagenase are effectively regulated by factors which affect degranulation or direct enzyme inhibition as with CMT-3. MMP-1, however, is constitutively synthesized and secreted in 6–12 hours without intracellular storage (21). Since monocyte derived collagenase is transcriptionally activated, levels of this collagenase are regulated by intracellular promoters of transcription such as cytokines and growth factors. CMT-3 has no known effects on this type of MMP.

Second, differential pulmonary sequestration of neutrophils and monocytes may relate to the known variability in chemoattractant cytokines among neutrophils and monocytes. Chemotactic cytokines, or chemokines, are important mediators in the inflammatory response. They are reviewed extensively elsewhere (23,24) but briefly, these low molecular weight proteins are grouped into two families based on sequence of cysteine residues at the amino terminus. Members of the so called CXC chemokine family<sup>†</sup> such as IL-8, Gro-a, b and g, GCP-2, ENA-78, LIX and NAP-2 are chemotactic for neutrophils but

not mononuclear cells (25). On the other hand, members of the CC chemokine family<sup>‡</sup> such as MCP 1–5, RANTES, MIP-1a and b, SDF-1, I-309, MDC, TECK, and fractalkine attract monocytes but not neutrophils (25). Our data demonstrates a clear dichotomous relationship between mononuclear cells and neutrophils treated with CMT-3 which coincides perfectly with published chemokine research. Furthermore, this observation points to the possibility of another mechanism by which CMT-3 exerts anti-neutrophilic activity. In addition to elastase and MMP inhibition, our data imply that CMT-3 may also work by inhibition of any or all of the CXC chemokines.

Third, differential pulmonary sequestration of neutrophils and monocytes may result from differences in adhesion receptors and cell to matrix interactions, presently thought to be one of the main mechanisms of chemokine stimulation (25). CMT-3 could affect the CXC chemokine receptors CXCR1 and CXCR2 which are expressed specifically by neutrophils, as opposed to affecting the chemokines themselves or the CC chemokine receptors.

In summary, despite the failure of CMT-3 to inhibit monocyte/macrophage sequestration, clinically measurable PPS was prevented by inhibiting neutrophils. This confirms other reports that identify neutrophils and their mediators as dominant factors in the development of acute lung injury following cardiopulmonary bypass. When combined with our previous study documenting physiologic and biochemical parameters (19), the present data show that neutrophil sequestration can be reduced by CMT-3 and that this correlates with a reduction in PPS. This implies that future strategies to alter the pathogenesis of PPS should target neutrophils and their mediators. Furthermore, our observation that mononuclear cells and neutrophils have a markedly different response to CMT-3 suggests this drug preferentially inhibits neutrophil sequestration and/or activation while remaining ineffective against the activity of monocytes and macrophages. Whether related to MMP's, chemokines or some other unknown factor awaits further research.

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<sup>†</sup> IL-8 denotes interleukin 8, GRO growth-related oncogene, GCP granulocyte chemotactic protein, ENA epithelial-cell-derived neutrophil-activating peptide, LIX lipopolysaccharide-induced CXC chemokine, NAP neutrophil activating peptide.



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