EFFECTS OF ENDOTOXIN (LPS) ON RATS SUBJECTED TO DECOMPRESSION

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ABSTRACT

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Introduction: Decompression sickness has been shown to produce lung permeability edema due in part to inflammatory mediator release in experimental models. Rats delivered low doses of endotoxin have been shown to have reduced lung permeability edema following toxic oxygen exposures. We evaluated the effects of low dose endotoxin administration to rats undergoing hyperbaric decompression.

Methods: Male Sprague-Dawley rats, acclimated to a 12 hour light/dark schedule were injected through a catheter inserted into the jugular vein with 0 (saline) or 5 mg/kg of the endotoxin, lipopolysaccharide from *E. Coli*. The rats were divided into 12 separate groups consisting of non-decompressed controls and those exposed to compression to 683.29 kPa for 60 minutes and decompression after pre-inoculation with endotoxin by 24, 48 or 72 hours. Post decompression, all groups were observed for gross signs of DCS, and evaluated for pulmonary edema, arterial, pleural and bronchoalveolar lavage protein, differential and white blood cell counts and inflammatory mediator levels (thromboxane B_2 and leukotriene E_4).

Statistics: ANOVA, Fisher's test.

Results: Rats pretreated with endotoxin had reduced extravascular lung water, bronchoalveolar lavage protein, arterial thromboxane B_2 and leukotriene E_4 levels (except 24 hrs post injection) and bronchoalveolar lavage Leukotriene E_4 levels (to a lesser degree thromboxane B_2) compared with controls receiving saline. Changes were also noted in the arterial and bronchoalveolar lavage white cell counts and neutrophils.

Conclusion: The results indicate some similarities with other studies showing protective effects of endotoxin against permeability edema and its causes in the lungs of affected animals.

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INTRODUCTION

We previously examined various aspects of the inflammatory process in DCS. In these studies we evaluated several preventative therapies for minimizing inflammatory responses to DCS in rats.

These therapies include:

- **Leukotriene inhibition**
- **Circadian rhythm timing**
- **Melatonin effects on Circadian timing**
- **Hyperbaric oxygen prebreathe (HBOP)**
- **Combinations of the above**

In the current study we examined the effects of infection on the bubble-induced inflammatory response to DCS in rats using an endotoxin (lipopolysaccharide from *E. Coli*) model.

BACKGROUND

When considering the growing number of individuals diving for recreational, commercial and military operations it is important to look at the health state of the individual at the time of dive. A minor illness (flu, cold, infection…) can alter white cell counts and increase the presence of a number of inflammatory mediators in the body. Such changes can alter the individual's response to additional stresses such as DCS. In these studies we examined the role of infection on bubble-induced neutrophil changes, any role infection has in DCS-induced bubble formation, and on the inflammatory response to the blood:bubble interactions using an endotoxin (lipopolysaccharide from *E. Coli* – LPS) compromised subject. Interestingly, LPS has been shown to actually reduce pulmonary edema in other models where lung injury occurs such as with oxygen toxicity.

A better understanding of these interactions will enable development of the appropriate response to increase the potential for therapeutic inflammatory inhibition.

METHODS

Protocol: 2 groups (6 sub-groups/group) of male Sprague-Dawley rats

A catheter was placed in the jugular vein of all rats. Rats were injected with either 0 or 5mg/kg lipopolysaccharide (LPS) from *E. Coli* and allowed to recover for 24, 48 or 72 hours before compression. The **C**ompression, **D**ecompression & **O**bservation (**C/D/O**) Protocol is **C**ompression to 683kPa for 1Hr, **D**ecompression to sea level, **O**bservation post dive 1Hr & euthanization/sample collection.

Group 1: 0mg/kg LPS

1) 0mg/kg LPS, 24 hr Recovery Post-LPS Injection, No C/D/O
2) 0mg/kg LPS, 24 hr Recovery Post-LPS Injection, C/D/O
3) 0mg/kg LPS, 48 hr Recovery Post-LPS Injection, No C/D/O
4) 0mg/kg LPS, 48 hr Recovery Post-LPS Injection, C/D/O
5) 0mg/kg LPS, 72 hr Recovery Post-LPS Injection, No C/D/O
6) 0mg/kg LPS, 72 hr Recovery Post-LPS Injection, C/D/O

Group 2: 5mg/kg LPS

1) 5mg/kg LPS, 24 hr Recovery Post-LPS Injection, No C/D/O
2) 5mg/kg LPS, 24 hr Recovery Post-LPS Injection, C/D/O
3) 5mg/kg LPS, 48 hr Recovery Post-LPS Injection, No C/D/O
4) 5mg/kg LPS, 48 hr Recovery Post-LPS Injection, C/D/O
5) 5mg/kg LPS, 72 hr Recovery Post-LPS Injection, No C/D/O
6) 5mg/kg LPS, 72 hr Recovery Post-LPS Injection, C/D/O

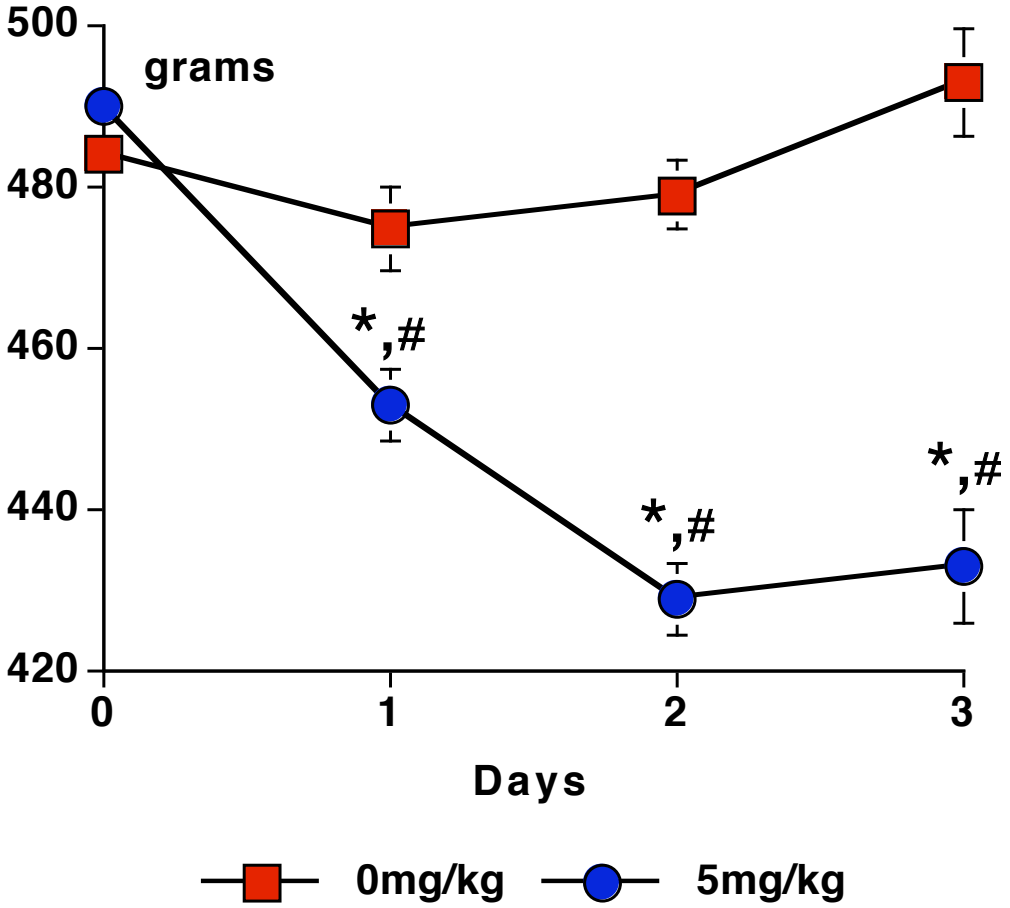
Analysis: Observe gross symptoms post compression, Collection of Bronchoalveolar lavage, pleural fluid, urine and arterial blood for protein analysis, white cell and differential cell counts and eicosanoid (thromboxane B_2 and leukotriene E_4) levels, Pulmonary Edema Assessment. Data analysis was performed using an ANOVA and Bonferroni Test

OCCURRENCES OF DCS SYMPTOMS

Groups		DAY 1 C/D/O		DAY 2 C/D/O		DAY 3 C/D/O	
		0 mg/kg	*5 mg/kg	0 mg/kg	5 mg/kg	0 mg/kg	*5 mg/kg
Symptoms Scale [1-4]	n	7	13	12	9	8	9
Tachypnea [1]		5	7	9	5	7	5
Cyanosis [1]		4	10	8	5	5	2
Dyspnea [1]		5	9	10	6	5	5
Cheyne-Stokes [1]							
Weighted Respiratory Score		*2.00*	*2.00*	*2.25*	*1.78*	*2.13*	*1.33*
PA Gas Emboli [2]			5	6	1	3	
IVC Gas Emboli [2]		3	7	8	3	1	
Thoracic Aortic Bubbles [3]			4	4		1	
Weighted Air Emboli Score		*0.86*	*2.77*	*3.33*	*0.89*	*1.38*	*0.00*
Blebs [1]		4	6	9	7	7	7
Petechiae [2]		42	4	1	4	2	4
Hematomas [2]		7	88	7	9	6	6
Perivascular Cuffing [2]		4	6	4	3	4	4
Atelectasis [2]		1		2	1	1	
Alveolar Edema [3]		1		1	1	1	
Weighted Pulmonary Score		*5.00*	*3.23*	*3.33*	*4.89*	*4.50*	*3.89*
Limb Paralysis [3]		3	5	6	3	1	1
Chokes [3]			2	1	1	1	
Seizure [3]			5	4		1	
Death [4]			4	4		1	
Weighted Neurological Score		*1.29*	*4.00*	*4.08*	*1.33*	*1.63*	*0.33*
Weighted Severity Score		***9.14***	***12.00***	***13.00***	***8.89***	***9.63***	***5.55***

*2 Rats died within 24 hours of 5 mg/kg LPS injection. They are not included in this data as they did not undergo C/D/O.

WEIGHT LOSS POST LPS INJECTION

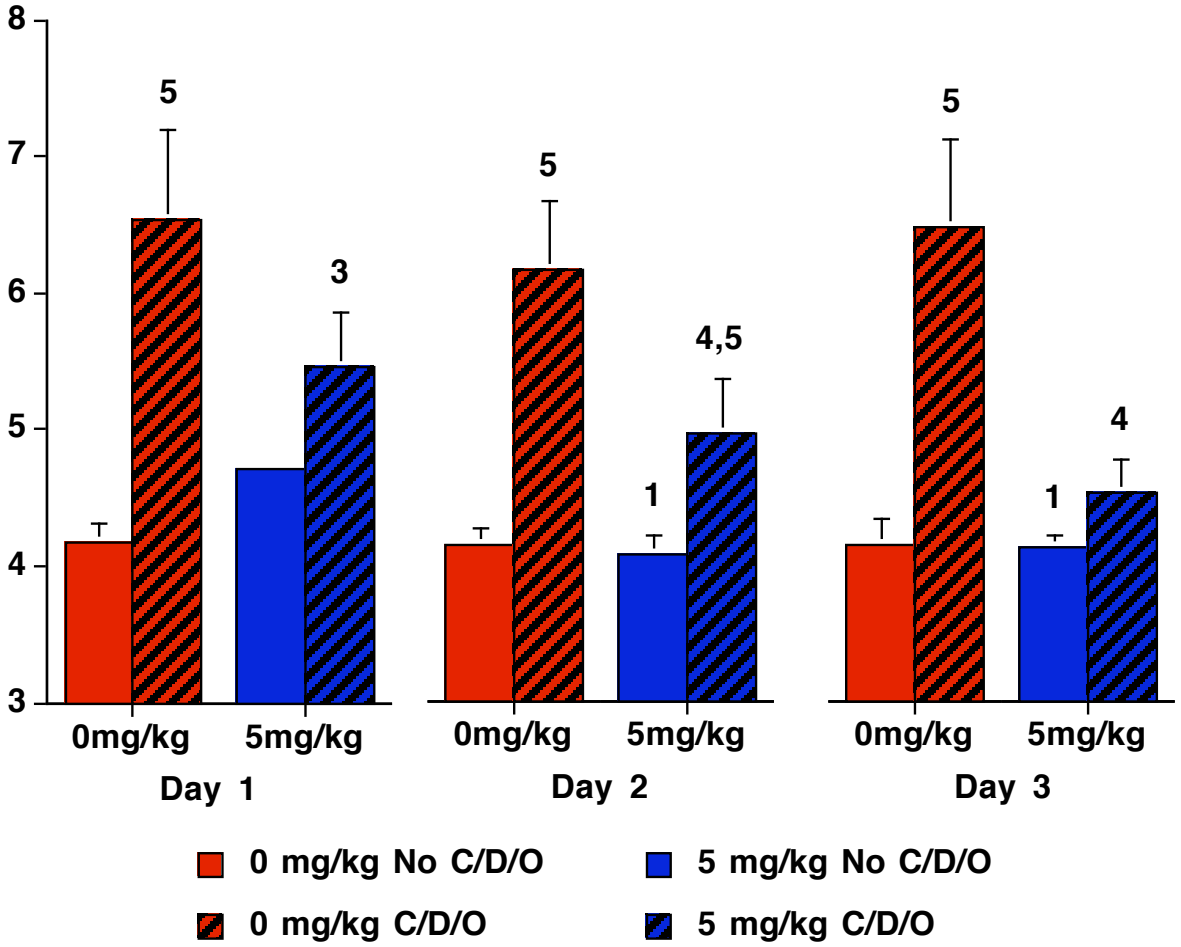


p < 0.05

* - 0 Day vs 1,2 & 3 Day

- 0 mg/kg vs 5 mg/kg

EXTRAVASCULAR LUNG WATER



p < 0.05

1 - 0 or 5mg/kg **no C/D/O** D1 vs D2, D1 vs D3 or D2 vs D3

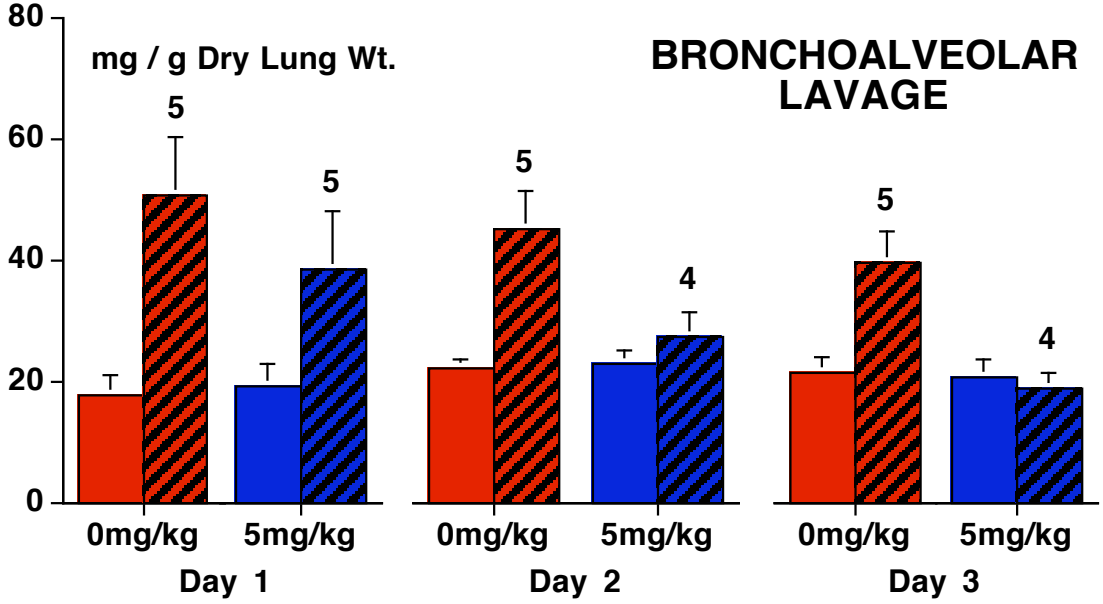
2 - 0 or 5mg/kg **C/D/O** D1 vs D2, D1 vs D3 or D2 vs D3

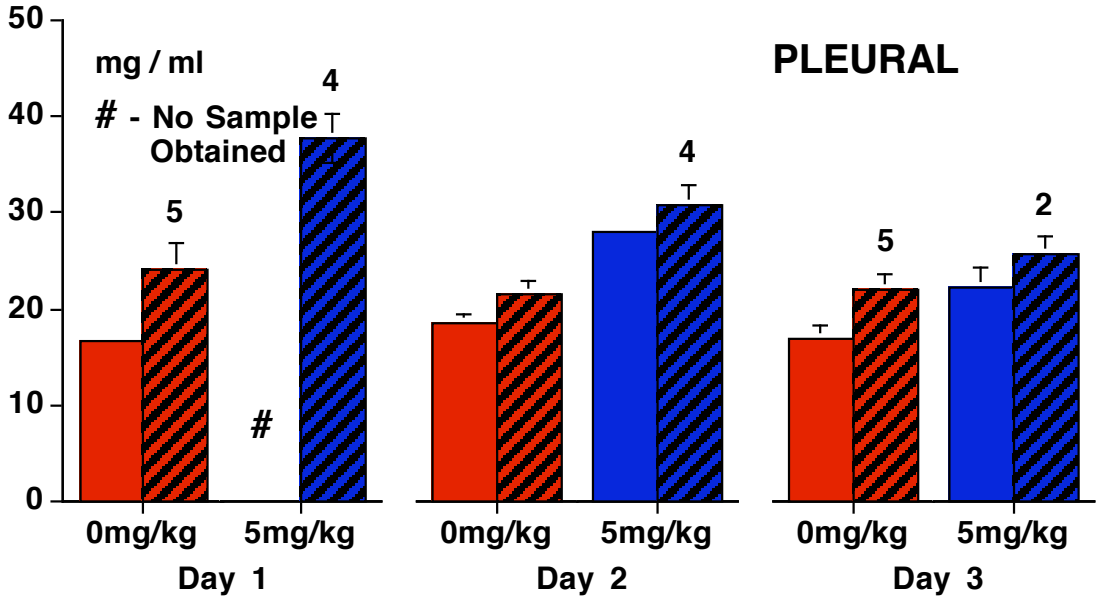
3 - 0mg/kg **No C/D/O** vs 5mg/kg **No C/D/O** for D1, D2 or D3

4 - 0mg/kg **C/D/O** vs 5mg/kg **C/D/O** for D1, D2 or D3

5 - **No C/D/O** vs **C/D/O**

PROTEIN





0 mg/kg No C/D/O 0 mg/kg C/D/O

5 mg/kg No C/D/O 5 mg/kg C/D/O

p < 0.05

1 - 0 or 5mg/kg **no C/D/O** D1 vs D2, D1 vs D3 or D2 vs D3

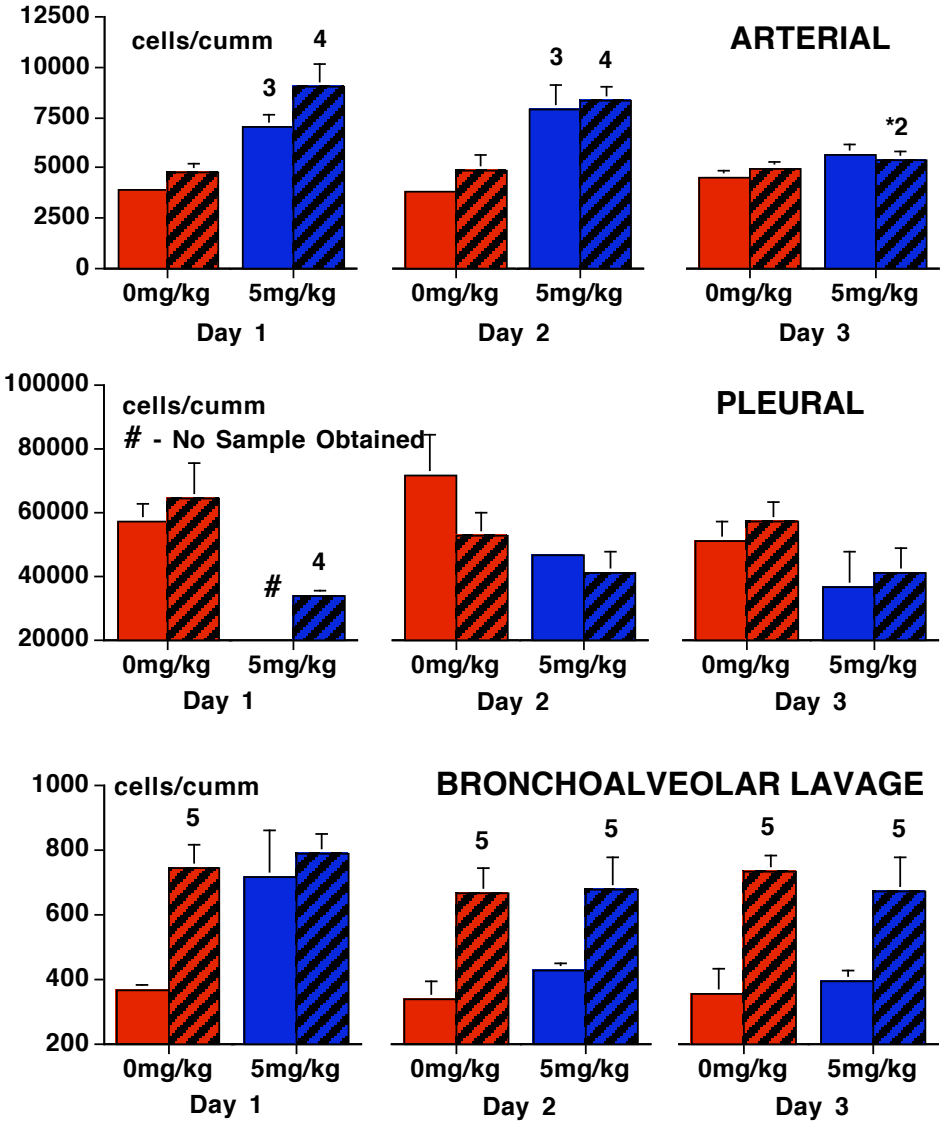
2 - 0 or 5mg/kg **C/D/O** D1 vs D2, D1 vs D3 or D2 vs D3

3 - 0mg/kg **No C/D/O** vs 5mg/kg **No C/D/O** for D1, D2 or D3

4 - 0mg/kg **C/D/O** vs 5mg/kg **C/D/O** for D1, D2 or D3

5 - **No C/D/O** vs **C/D/O**

WHITE BLOOD CELLS



0 mg/kg No C/D/O 0 mg/kg C/D/O

5 mg/kg No C/D/O 5 mg/kg C/D/O

p < 0.05

1 - 0 or 5mg/kg **no C/D/O** D1 vs D2, D1 vs D3 or D2 vs D3

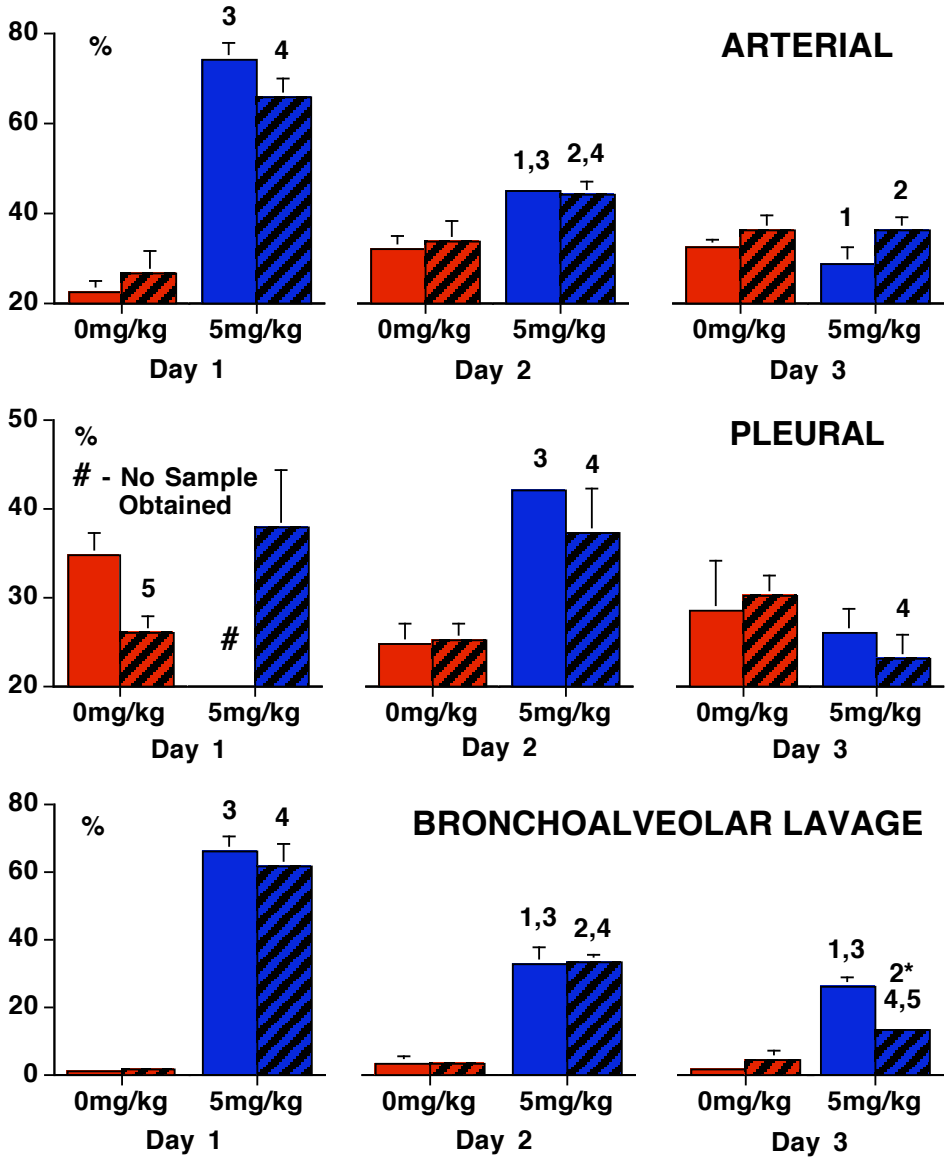
2 - 0 or 5mg/kg **C/D/O** D1 vs D2, D1 vs D3 or D2 vs D3

3 - 0mg/kg **No C/D/O** vs 5mg/kg **No C/D/O** for D1, D2 or D3

4 - 0mg/kg **C/D/O** vs 5mg/kg **C/D/O** for D1, D2 or D3

5 - **No C/D/O** vs **C/D/O**

NEUTROPHILS



0 mg/kg No C/D/O 0 mg/kg C/D/O

5 mg/kg No C/D/O 5 mg/kg C/D/O

p < 0.05

1 - 0 or 5mg/kg **no C/D/O** D1 vs D2, D1 vs D3 or D2 vs D3

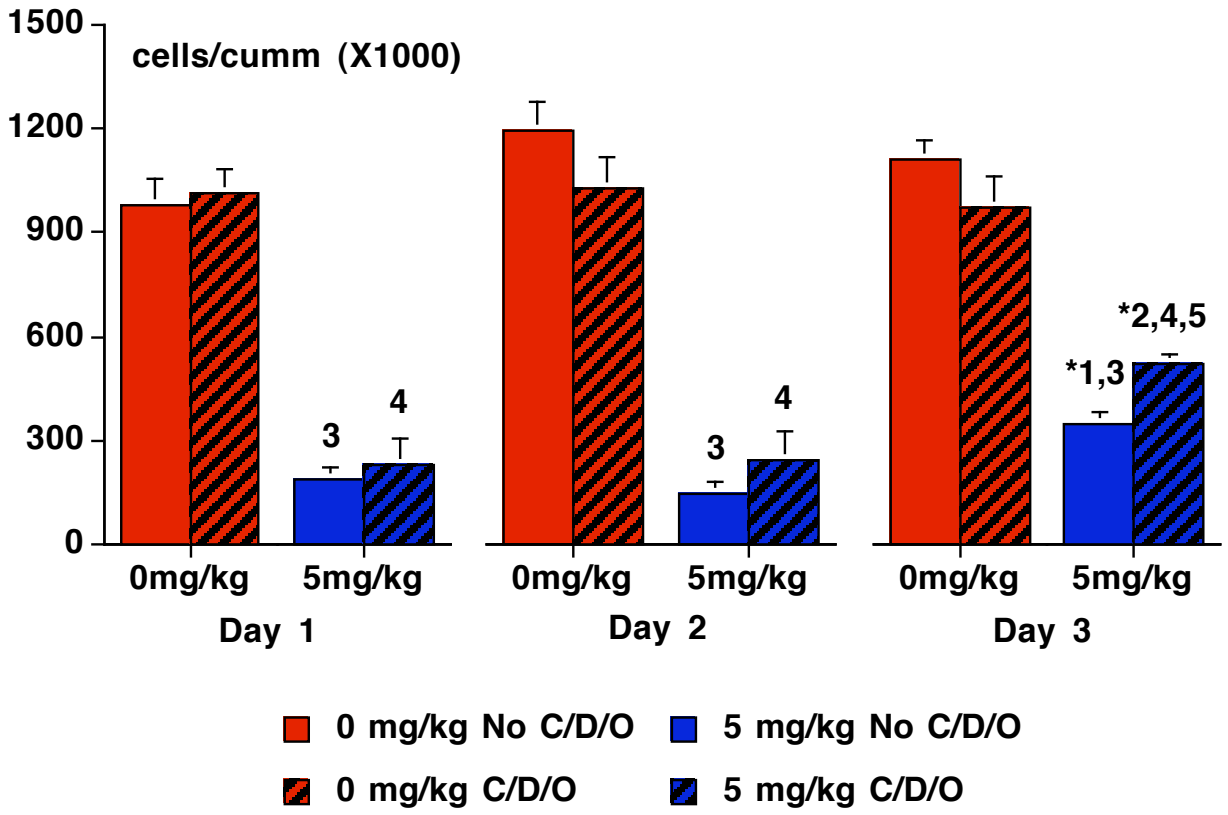
2 - 0 or 5mg/kg **C/D/O** D1 vs D2, D1 vs D3 or D2 vs D3

3 - 0mg/kg **No C/D/O** vs 5mg/kg **No C/D/O** for D1, D2 or D3

4 - 0mg/kg **C/D/O** vs 5mg/kg **C/D/O** for D1, D2 or D3

5 - **No C/D/O** vs **C/D/O**

ARTERIAL PLATELETS



p < 0.05

1 - 0 or 5mg/kg **no C/D/O** D1 vs D2, D1 vs D3 or D2 vs D3

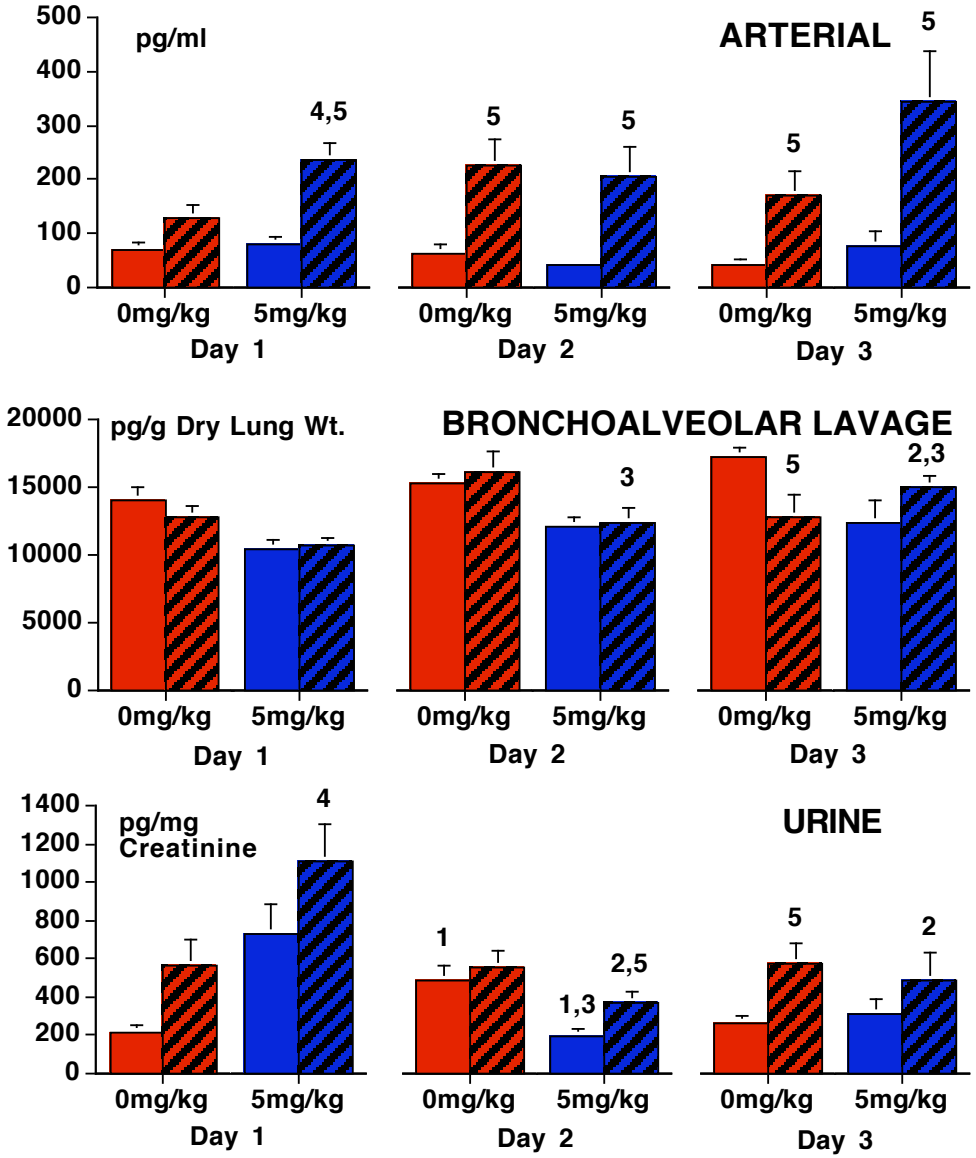
2 - 0 or 5mg/kg **C/D/O** D1 vs D2, D1 vs D3 or D2 vs D3

3 - 0mg/kg **No C/D/O** vs 5mg/kg **No C/D/O** for D1, D2 or D3

4 - 0mg/kg **C/D/O** vs 5mg/kg **C/D/O** for D1, D2 or D3

5 - **No C/D/O** vs **C/D/O**

THROMBOXANE B_2



0 mg/kg No C/D/O 0 mg/kg C/D/O

5 mg/kg No C/D/O 5 mg/kg C/D/O

p < 0.05

1 - 0 or 5mg/kg **no C/D/O** D1 vs D2, D1 vs D3 or D2 vs D3

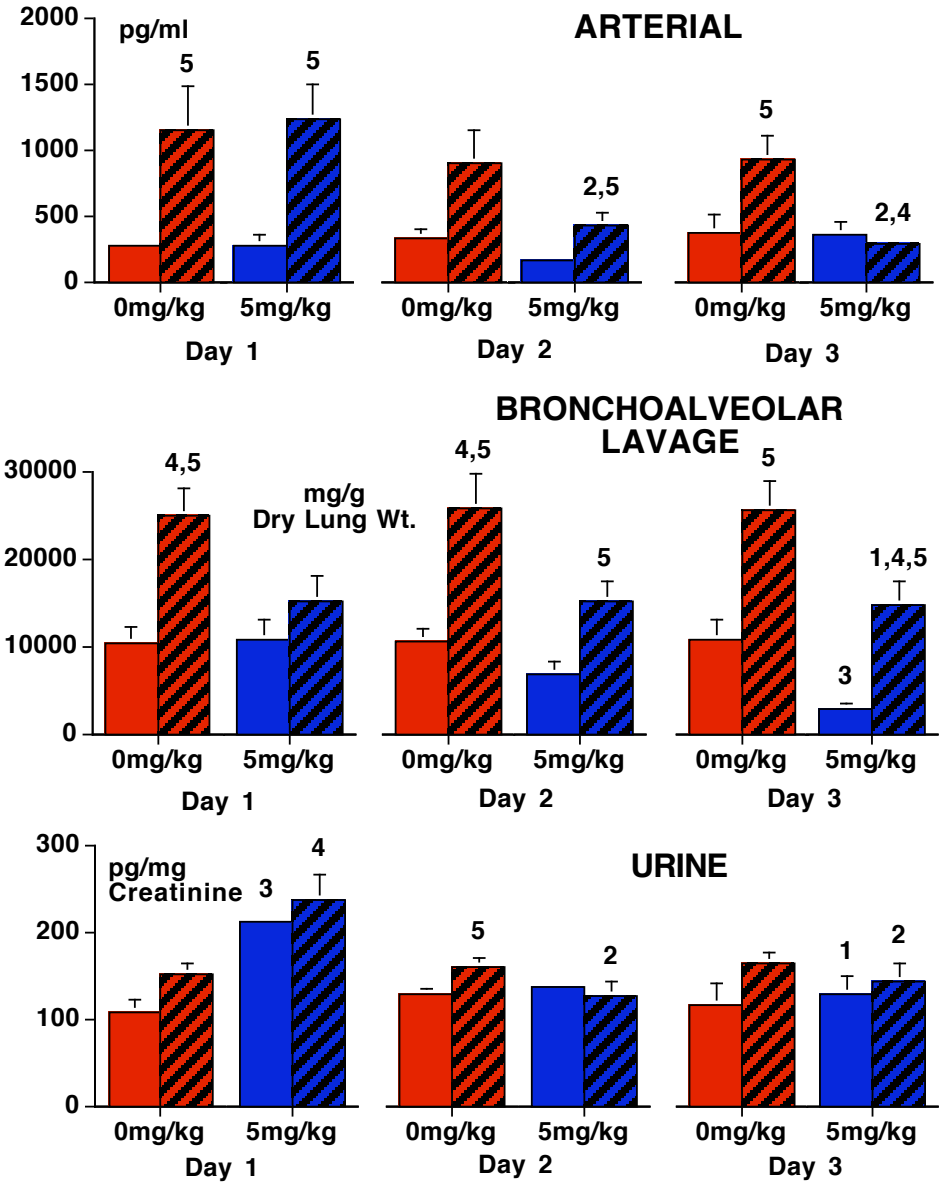
2 - 0 or 5mg/kg **C/D/O** D1 vs D2, D1 vs D3 or D2 vs D3

3 - 0mg/kg **No C/D/O** vs 5mg/kg **No C/D/O** for D1, D2 or D3

4 - 0mg/kg **C/D/O** vs 5mg/kg **C/D/O** for D1, D2 or D3

5 - **No C/D/O** vs **C/D/O**

LEUKOTRIENE E_4



0 mg/kg No C/D/O 0 mg/kg C/D/O

5 mg/kg No C/D/O 5 mg/kg C/D/O

p < 0.05

1 - 0 or 5mg/kg **no C/D/O** D1 vs D2, D1 vs D3 or D2 vs D3

2 - 0 or 5mg/kg **C/D/O** D1 vs D2, D1 vs D3 or D2 vs D3

3 - 0mg/kg **No C/D/O** vs 5mg/kg **No C/D/O** for D1, D2 or D3

4 - 0mg/kg **C/D/O** vs 5mg/kg **C/D/O** for D1, D2 or D3

5 - **No C/D/O** vs **C/D/O**

CONCLUSION

- **PULMONARY EDEMA: LPS reduced the extravascular water with or without decompression, compared with saline controls. Reductions decreased in a dose linear fashion over days 1-3.**

- **PROTEIN LEVELS: LPS reduced BAL protein levels compared with saline controls. Reductions decreased in a dose linear fashion over days 1-3.**

- **NEUTROPHILS: LPS increased neutrophil counts compared with saline controls. Reductions decreased in a dose linear fashion over days 1-3.**

- **ARTERIAL PLATELETS: LPS reduced platelet levels compared with saline controls.**

- **LEUKOTRIENES: LPS reduced arterial and BAL leukotriene levels compared with saline controls. Arterial reductions decreased in a dose linear fashion over days 1-3.**

SUPPORT: Office of Naval Research (ONR), U.S. Navy NRD-311 US Navy ONR N00014-04-1-0162