# DOES ANTI-INFLAMMATORY THERAPY ATTENUATES THE LUNG INJURY CAUSED BY ISCHEMIA/REPERFUSION OF THE LOWER EXTREMITIES IN RABBIT

**A. BALTALARLI, MD*, İ. GÖKŞİN, MD*, M.H. US, MD**, R. ORTAÇ, MD***, B. BALTALARLI, MD****, O. RENDECİ, MD*, M. SAÇAR, MD*, H. ŞİRİN, MD***

**From:**
* Department of Cardiovascular Surgery, Pamukkale University, Denizli, Turkey
** Department of Cardiovascular Surgery, GATA Haydarpaşa Hospital, İstanbul, Turkey
*** Department of Pathology, İzmir Behçet Uz Hospital, İzmir, Turkey
**** Department of Radiation Oncology, Pamukkale University, Denizli, Türkiye

The study was carried out in the Department of Cardiovascular Surgery of the Medical Faculty of Pamukkale University, Denizli, Türkiye

**Adress for reprints:**
Dr. Ahmet Baltalarlı
P.K. 283, 20100
Denizli, Türkiye
Tel: +90 258 2131577
Fax: +90 258 2132016
e-mail:
ahmetbaltalarli@superonline.com

***It is known that acute transient aortic occlusion predisposes to lung injury. The aim of this study was to determine whether dexamethasone (Dxm) and tenoxicam (Tnx) could have any protective effect on lung injury in an animal model.***
***Thirty-five white rabbits were randomized into four groups. In IR group (n=10), ischemia/reperfusion (IR) injury was induced by infrarenal aortic clamping for 3 hours and reperfusion for 2 hours. Dxm group (n=10) was pretreated with 1 mg/kg dexamethasone, Tnx group (n=10) with 10 mg/kg tenoxicam, before the clamping. Five animal was sham operated without aortic occlusion.***
***Ischemia/reperfusion resulted in a significant increase in lung injury scores (mean 2.6+ in IR group and 2.5+ in Tnx group). The animals pretreated with dexamethasone had a significantly lower score (1.7+, $p<0.05$).***
***Dexamethasone can attenuate lung injury, but nonsteroidal anti-inflammatory drug tenoxicam can not.***

***Key words: Dexamethasone, tenoxicam, ischemia, reperfusion, organ preservation***

Lung injury is known to occur after temporary occlusion of the aorta and subsequent ischemia/reperfusion (IR) of the lower extremities (1,2,3). Polymorphonuclear neutrophil leucocytes (PMN) have been shown to have a central role in

the lung injury caused by IR of the lower extremities, and their depletion exerts a protective effect on the lungs under these conditions (3).

In clinical setting, temporary ischemia of the lower extremities may result in shock and acute lung injury that requires inotropic and ventilatory support (4).

Tenoxicam is a nonsteroidal anti-inflammatory (NSAI) drug that inhibits cyclooxygenase (resulting in similar inhibition of endothelial prostacyclin and platelet thromboxane production), but it also appears to have inhibitory actions on in vitro neutrophil function (5).

Dexamethasone has been shown to inhibit pulmonary inflammation in endotoxin shock and asthma by inhibiting nitric oxide synthase (INOS) (6,7).

We studied the effect of pharmacologic intervention with these two anti-inflammatory drugs on acute lung injury.

## METHOD

Thirty-five New Zealand white rabbits (2.5 to 3 kg) were used in the study. All animals received humane care in compliance with the European Convention on Animal Care. The study was approved by the Institutional Ethics Committee.

During the surgical procedures, anesthesia was induced and then maintained with intraperitoneal ketamine (30 mg/kg) and xylazin (6 mg/kg) fractionally as needed. During the surgical procedures, body temperature was maintained with a water-filled heating pad. The animals were placed in a nose cone to breathe oxygen at a rate of 0.5 L/minute. Rectal temperature was monitored and maintained close to 38°C under a warming light. A jugular venous line was established for intravenous fluid infusion through the neck incision. The animals were then given heparin (1000 units/kg) via right jugular vein.

The abdominal aorta was exposed through a midline abdominal incision, and the aorta was exposed just above the iliac bifurcation. A bulldog clamp was used for aortic occlusion. Reperfusion was confirmed visually and by Doppler assessment in femoral region.

The animals were randomized into four groups. In IR group (n=10), the aorta was cross-clamped for 3 hours, followed by 2 hours of reperfusion. In Dxm group (n=10), animals were pretreated with 1 mg/kg dexamethasone via jugular vein before aortic cross-clamping. In Tnx group (n=10), animals were pretreated with 10 mg/kg tenoxicam 3 hours before the ischemia and ischemia was followed by 2 hours of reperfusion. In Sham group (n=5), abdomen was left open during the same period and they were pretreated with equal volume of saline.

By the end of the 5th hour, both the lungs and trachea were harvested. The left main bronchus was cannulated and secured. Saline (15 ml) was then injected as 3 aliquots of 5 ml each. Each aliquot was injected quickly and then withdrawn slowly 3 times to obtain bronchoalveolar lavage (BAL) specimen. Fluid recovery was routinely 90% or more. Combined aliquots of BAL fluid were spun at 1000g for 10 minutes to remove cells. The cell pellet was resuspended in 1 ml of saline, and 100 cells were counted to detect PMN rate.

After removing the right lung, it was inflated and fixed with 10% formalin. Fixed specimens were paraffin-embedded, sectioned in 6 $\mu$m pieces, and stained with routine hematoxylin-eosin stain. The specimens were examined by the same pathologist who was blinded to the study. At least two different sections of each specimen were examined to accurately determine the degree of injury. Lung injury was rated with a semiquantitative scoring system described by Tassiopoulos et al (8), based on congestion, interstitial edema, PMN infiltration, and airspace hemorrhage, as follows: 0, no changes; 1+, focal, mild, subtle changes; 2+, multifocal mild changes; 3+, multifocal prominent changes; and 4+, extensive prominent changes.

The parametric data (the rate of PMN in BAL fluid) were expressed as mean±standard deviation, and compared with Student t test. Non-parametric values of lung injury scores were analyzed with Mann-Whitney U test. A p value of less than 0.05 was considered significant.

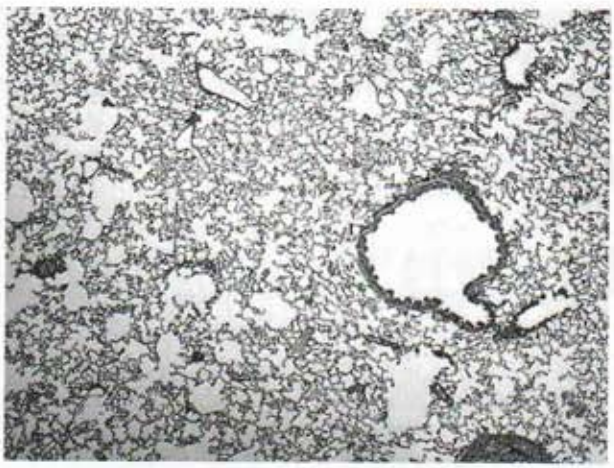

**Figure 1:** Normal histologic appearance of the rabbit lung. (Sham group, hematoxylin-eosin stain, original magnification X20, injury score:0+)

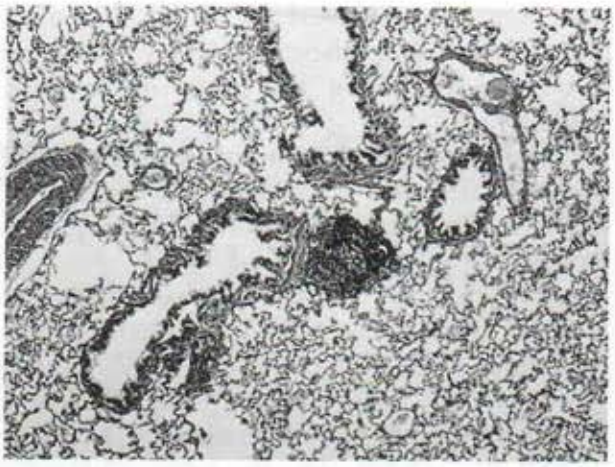

**Figure 2:** Histologic picture of the lung, minimal vascular congestion and cellularity of lung interstitium. (Dxm group, hematoxylin-eosin stain, original magnification X20, injury score:1+)

## RESULTS

In Sham operation group, no congestion and neutrophil infiltration was found in lung histology (Figure 1). In BAL cytology, no neutrophil was examined; the dominating cells were macrophages.

The groups exposed to aortic occlusion showed significant differences in the degree of lung injury (Figure 2,3,4). IR and Tnx groups had lesions ranging from 2+ to 4+, with average injury scores of 2.6+ and 2.5+, respectively ($p>0.05$). The score of Dxm group was significantly lower than the others (ranging from 1+ to 3+ with a mean of 1.7+, $p<0.05$).

In BAL cytology, also the rate of PMN was significantly lower in Dxm group (10.8±3.7%,

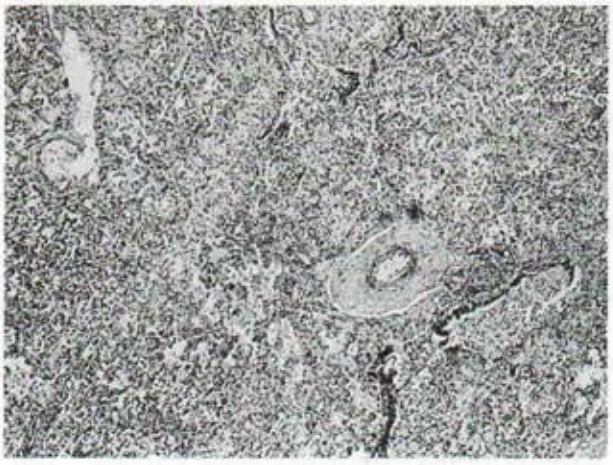

**Figure 3:** Histologic picture of the lung, increased vascular congestion and cellularity in the lung interstitium. (Tnx group, hematoxylin-eosin stain, original magnification X20, injury score:3+)

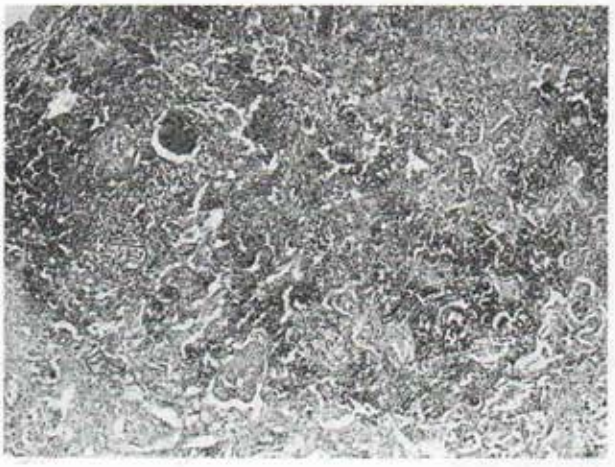

**Figure 4:** Histologic picture of severe lung injury. There is alveolar flooding, indicating pulmonary edema. (IR group, hematoxylin-eosin stain, original magnification X20, injury score:4+)

p<0.05) than Tnx (23.4±6.5%) and IR groups (27.9±5.5%).
There was no significant difference between Tnx and IR groups according to their lung injury scores and, BAL cytology (p>0.05).

## DISCUSSION

It was demonstrated that acute ischemia of the lower extremities in rats resulted in significant lung injury (1,8). According to the Stallone's work (1), changes in lung tissue were seen even in the animal that was killed with the aortic clamp still in place. The lung injury process begins once the blood supply to the lower extremities is interrupted, and aggravated during reperfusion (8). In our model, only the arterial blood flow to the lower extremities was blocked, whereas the venous and lymphatic return were maintained open during ischemia, both ischemia and reperfusion contributed to the injury process in the lung. I/R of lower extremity causes lung injury by PMN sequestration in pulmonary microvasculature, increased endothelial permeability, and interstitial edema (2). The injuries are PMN-dependent and can be attenuated by prior depletion of circulating PMNs (3,9).
It was demonstrated that acute ischemia of the lower extremities in rats results in a significant increase in serum tumor necrosis factor (TNF) concentration and a subsequent increase in nitric oxide (NO) production in the lung; and TNF and NO are significant determinants of the lung injury process that is caused by lower extremity I/R (1,2, 8,10). The administration of dexamethasone results in decreased production of NO by both a direct and an indirect way by decreasing TNF production (6,8,11). Because of lesser degree of neutrophil accumulation in Dxm group, it can be stated that pre-treatment with dexamethasone before IR is associated with lesser degrees of lung injury.
Hasçelik et al. (12) suggested that tenoxicam is a potent inhibitor of neutrophil chemotaxis. In the study of Angelis et al. (13), tenoxicam was likely to possess anti-inflammatory properties which are independent from effects on cyclooxygenase.
Hellewell (14) found that neutrophil accumulation induced by C5a was attenuated by ibuprofen in cutaneous microcirculation, but leucocyte infiltration in pulmonary airspace was increased in the same animal. Similarly, pulmonary neutrophil accumulation was not decreased by tenoxicam pretreatment, probably caused by inhibition of thromboxane production, which under normal circumstances serves to decrease local blood flow and keep the inflammatory response localized and regulated.
In conclusion, lower extremity ischemia and reperfusion causes a significant lung injury. It can be attenuated by dexamethasone pretreatment.

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