Erciyes Tıp Dergisi 10: 369-375,1988

Pathologic lung changes in cecal ligated septic rats: Effect of the zinc sulphate pretreatment[x]

Metin ŞEN[xx], Erdoğan M. SÖZÜER[xxx], Süleyman BALKANLI[xxxx], Yücel ARITAŞ[xx], Zeki YILMAZ[xxxxx], Yaşar YEŞİLKAYA[xxxxxx]

Summary: This study investigates the role of zinc sulphate pretreatment in cecal ligated septic rats. 40 rats were divided into 4 groups. Group-I: Ether anesthesia and laparotomy were done. Group-2: Ether anesthesia, laparotomy and cecal ligation were done. Group-3: 5mg/kg zinc sulphate pretreatment was given intramusculary. 2 hours later, ether anesthesia and laparotomy were done. Group-4: 5mg/kg zinc sulphate pretreatment was given intramusculary. 2 hours later ether anesthesia, laparotomy and cecal ligation were done. Intramuscular zinc sulphate treatment was repeated every 6 hours in group 3 and 4 during the study. All of the rats were killed after 72 hours and lung biopsies were taken for histologic examination. The histologic examination revealed that there were some pathologic changes in lung in group-2 which was resolved with the zinc sulphate pretreatment in group-4. There was no difference between group-I and group-3 in histologic examination. As a summary we used the cecal ligation model to produce sepsis in rats. We have demonstrated that the zinc sulphate pretreatment restores some of the pathologic changes in lungs which were observed after cecal ligation.

Key words:Zinc, sepsis, septic shock

Respiratory insufficiency is one of the major causes of death in patients with septic shock. Lysosomal enzymes released from white cells sequestered in the lung, disseminated intravascular coagulation, microembolic blockage of the pulmonary microcirculation and many other factors such as vasoactive mediators (kinins, serotonin, prostaglandin etc.) have been incriminated in the lung pathology in sepsis.

---

x *From The Departments of General Surgery and Pathology Erciyes University, Medical School Hospital, Kayseri/Turkey.*
xx *Associate Professor of mGeneral Surgery*
xxx *Instructor of General Surgery*
xxxx *Assistant Professor of Pathology*
xxxx *Asistant Professor of General Surgery*
xxxxxx *Professor of General Surgery*

The purpose of this study is to determine the effect of sepsis on pulmonary histology and the effect of zinc sulphate pretreatment on sepsis-induced pulmonary pathology.

Material and Method

Male rats, body weight between 210-260 gm (mean 220 gm) were used in this study. 40 rats were divided into 4 equal groups, and they were fasted for 24 hours before each experiment.

We preferred a surgical operation that particularly destroys the normal barrier of gastrointestinal tract to produce a natural sepsis which occurs in the clinical patients. Rats were operated on with ether anesthesia. A 2 cm midline abdominal incision was made and the cecum was ligated just below the ileocecal valve with 000 silk. Ligation at this point permitted the bowel continuity to be maintained. The antimesenteric cecal surface was punctured twice with an 18-gauge needle and the cecum was replaced into the peritoneal cavity. The abdomen was then closed in two layers. It was shown that the mortality rate was % 94 (II) within 72 hours following cecal ligation with two punctures with 18-gaugu needle.

At the end of 72 hours, all rats were killed with decapitation and autopsies were performed. The lungs were removed and rinsed with formaline. The peritoneal and blood cultures were taken. The study was performed in 4 groups:

Group-I (n= 10) (Control group - A): Laparotomy was done with ether anesthesia.
Group-2(n=10)(Septic group): Laparotomy and cecal ligation with two punctures were performed with ether anesthesia.
Group-3(n=10) (Control group-B): Zinc sulphate (5mg/kg, intramuscularly) pretreatment was applied and 2 hours later laparotomy was performed with ether anesthesia.
Group-4(n=10) (Pretreatment group):Zinc sulphate (5mg/kg, intramuscularly) pretreatmert was given and 2 hours later laparotomy and cecal ligation with two punctures were performed with ether anesthesia.
The Zinc sulphate treatment was given intramuscularly in the period of every six hours during the study in group-3 and 4.

Results

At the end of laparotomy and cecal ligation, rats were placed in the individual cages and were observed for 72 hours. All of the rats rapidly recovered from the anesthesia and appared healthy approximately 24 hours later. The Cecal ligated rats (Group-2 and 4) subsequently became ill. Their reactions were slow and were less interested with their surroundings. They became progressively lethargic.

At autopsies of the cecal ligated rats, the cecum was gangrenous and the peritoneal cavity contained 1-2 ml of cloudy fluid. Peritoneal cultures showed numerous enteric organisms (Table I). The resuls of blood cultures were similar to those seen in peritoneal cultures (Table II). Thus sepsis was shown both clinically and bacteriologically.

Table I. Microorganisms isolated from peritoneal cultures of the rats

	Group-I n= 10	Group-2 n= 10	Group-3 n= 10	Group-4 n= 10
E.Coli	-	9	1	7
Proteus	1	3	2	2
Pseudomonas	-	3	1	-
Staph.aureus	1	-	-	2
None	8	-	7	-

Table II. Microorganisms isoleted from blood cultures of the rats.

	Group-I n= 10	Group-2 n= 10	Group-3 n= 10	Group-4 n= 10
E.Coli	-	8	-	5
Proteus	-	-	-	-
Pseudomonas	-	1	-	1
Staph. aureus	-	-	-	1
None	10	1	10	4

Histologic examination of the lung in group-1 and -3 , showed minimal edema in the interstitial space which was unique pathologic findings (Figure I). In group-2; edema , hemorrhage, atelectasia and polymorphonuclear leukocyte infiltration were observed. (Figure 2). Histologic examination of the sections from group-4 showed minimal atelectasia and minimal edema of the interstitial space, in which the severity of these findings were less than group-2, but more than group-1 (Figure 3).

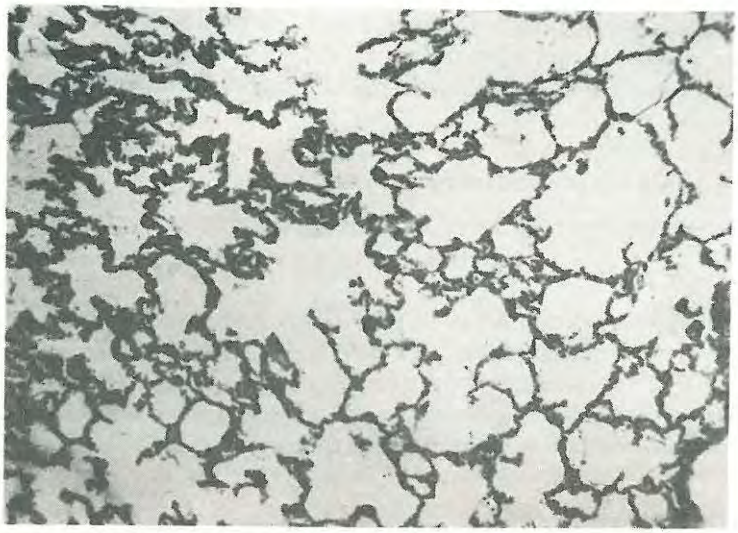

Figure 1- Normal histologic findings in the examination of Group-1 rats (Hematoxylin-Eosin X 100)

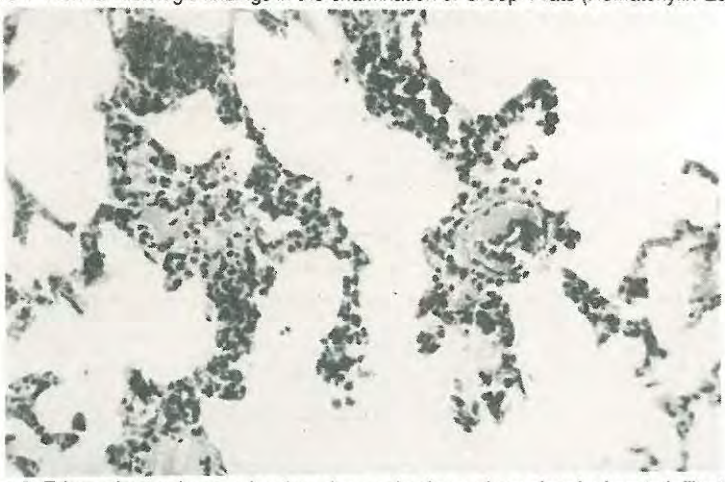

Figure 2- Edema, hemorrhage, microthrombus and polymorphonuclear leukocyte infiltration at the lung histologic examination of the rats in Group-2 (Hematoxylin-Eosin X 180).

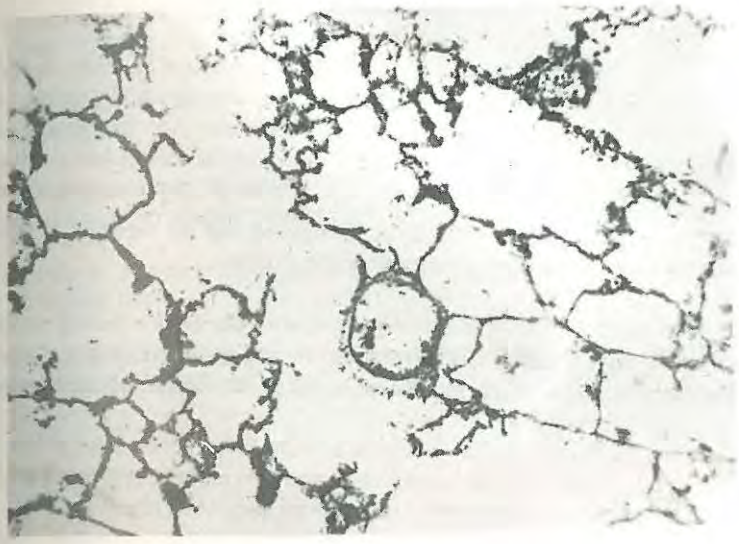

Figure 3- Minimal edema in Group-4 rats (Hematoxylin-Eosin X 100).

Discussion

Even with the invent of potent antimicrobial agents, sepsis is the major cause of death in most surgical clinics. The mechanism by which endotoxins of gram-negative bacteria produce their harmful effects on the pulmonary system is still very poorly understood. Lysosomal enzymes released from white cells sequestered in the lung, disseminated intravascular coagulation, microembolic blockage of the pulmonary circulation have been incriminated in the lung pathology in sepsis.

Demling et al. (6) reported that the lysosomal enzymes (beta glucuronidase, aryl-sulphatase-A) are released into the lung after endotoxin, probably from sequestered leukocytes, with the degree of release directly affecting the degree of vascular injury. It has been hypothesized that these enzymes alter the microvascular permeability. Lysosomal enzymes released from white cells sequestered in the lung have been reported as a possible cause of lung damage (8,9,10,12).

The Zinc is a biologically essential element and serves as a coenzyme in more than 80 enzyme systems. In cardiac and some other tissues, zinc has exerted a stabilizing effect on the lysosomal membranes and reduced the liberation of acid hydrolases in such tissues (4,5).

Chvapil et al. (I) reported that the zinc has a protective effect on the development of the injury when administered simultaneously with the noxious agent. When the injury has been established, subsequent administration of zinc is not effective. One of the principal functions of zinc is the stabilization of biomembranes (1,2,3). They showed that one of the possible mechanisms of the stabilizing effect of zinc is its interference with peroxidation of lipidis in-vitro and in-vivo systems. It is shown that the zinc protects the liver against the noxious effect of CCl_4 primarily by interference with lipid-Peroxidation-related tissue damage (4). Freeman et al. (7) reported that the exposure to nitrogen dioxide developed the lung edema in animals, probably because of lipoperoxidative changes.

If an agent causes pathologic changes by releasing lysosomal enzymes which play a role in the lung pathology, another agent which has a lysosomal membrane stabilizing effect might protect the lung against pathological changes. We chose the zinc to protect the lung against sepsis because of its potential stabilizing effect on the lysosomal membranes.

The experimental design of this study does not allow to determine the mechanism of the effect of zinc on the septic changes in lungs. Pretuced by cecal ligation there are several steps where the zinc might interfere, but we believe that the zinc pretreatment exerts beneficial effect on the septic changes in the lungs of the rats.

References

1. *Chvapil M, Ryan JN, Brada Z: Effects of selected chelating agents and metals on the stability of liver lysosomes.* ***Biochem Pharmacol*** *21:1097-1105, 1972.*

2. *Chvapil M, Ryan JN, Zukoski CF: Effect of zinc on lipid peroxidation in liver microsomes and mitochondria.* ***Proc Soc Exp Biol Med*** *141: 150-153, 1972.*

3. *Chvapil M, Ryan JN, Zukoski CF: The effect of zinc and other metals on the stability of lysosomes.* ***Proc Soc Exp Biol Med*** *140: 642-646, 1972.*

4. *Chvapil M, Ryan JN, Elias SL, Peng YM: Protective effects of zinc on carbontetrachloride-induced liver injury in rats.* ***Exp Mol Pathol*** *19: 186-196, 1973.*

5. *Chvapil M, Owen JA: Effects of zinc on acute and chronic isoproterenol induced heart injury.* ***J Mol Cell Cardiol*** *9:151-155, 1977.*

6. *Demling RH, Proctor R, Grossman J, et al: Lung injury and lysosomal enzyme release during endotoxemia.* ***J Surg Res*** *30: 135-141, 1981.*

7. *Freeman G, Crance SC, Stephans RJ: Pathogenesis of the nitrogen dioxide induced lesions in the rat lung.* ***Amer Rev Resp Dis*** *98: 429-443, 1968.*

8. Kux M, Coalson JJ, Masscon WH, Guenter CA: Pulmonary effects of E.Coli endotoxin: Role of leukocytes and platelets. **Ann Surg** 175: 26-30, 1972.

9. Myrvold HE, Svalander C: Pulmonary microembolism in early experimental shock: A morphological study in dogs. **J Surg Res** 23: 65-74, 1977.

10. Pingleton WW, Coalson Jj, Hinshaw LB, Guenteer CN: Effect of steroid pretreatment on development of shock lung. Hemodynamic, respiratory and morphological studies. **Lab Invest** 27: 445-450, 1972.

11. Wichterman KA, Baue AE, Chaudry IH: Sepsis and septic shock. A review of laboratory models and a proposal. **J Surg Res** 29: 189-201, 1980.

12. Wilson JW: Treatment and prevention of pulmonary cellulary damage with pharmacologic doses of corticosteroids. **Surg Gynecol Obstet** 134: 625-629, 1972.