Experimental acute lung injury

Effects of methylprednisolone and lidocaine on histopathology and neutrophils

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ABSTRACT

Objectives: Methylprednisolone and lidocaine are commonly used in acute lung injury caused by acid aspiration. The aim of this study is to demonstrate if these 2 agents, given at an early stage, can reduce lung damage and improve oxygenation.

Methods: The study was carried out at the animal laboratories of Ataturk University, Medical Faculty, Erzurum, Turkey during the year 2002, and performed on a rabbit acid aspiration model. At an early stage, the controls were given saline solution, the second group was given lidocaine, and the third group was given methylprednisolone. The neutrophil count was determined hourly. After 6 hours of mechanical ventilation, lung biopsy was performed for histopathology.

Results: Neutrophils increased with time. The controls showed much more severe histopathological changes than the 2 treatment groups. Methylprednisolone was more effective than lidocaine at reducing lung damage.

Conclusion: Histopathology suggests that acid aspiration induced acute lung injury can be effectively treated by lidocaine and methylprednisolone, if applied early. The latter appears to be the more effective.

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Acid aspiration is secondary only to sepsis as a cause of Acute Respiratory Distress syndrome (ARDS).[1-3] The mechanism of respiratory distress due to acid aspiration is not fully understood. Cellular components and humoral mediators play a primary role in the pathogenesis of acute lung injury (ALI). Various lung models have shown that ALI can develop not only from aspiration of acid, but also of common substances, such as human breast milk or acidified soya-based samples.[4,5] Lidocaine inhibits neutrophil chemotaxis function and superoxide anion release,[6,7] while steroids diminish arachidonic acid metabolism, thus inhibiting lipoxygenase and cyclooxygenase activity. The present study, using a hydrochloric acid aspiration model, compares the effects of lidocaine and methylprednisolone on neutrophils and in preventing the histopathological changes of ALI.

Methods. The study was carried out on 30 New Zealand male rabbits (weight 2.2-3.0 kg) at the animal laboratories of the Medical Faculty of Ataturk University Medical Faculty, Erzurum, Turkey, during the year 2002, following the approval of the local ethics committee. The animals, which had never been used for any other

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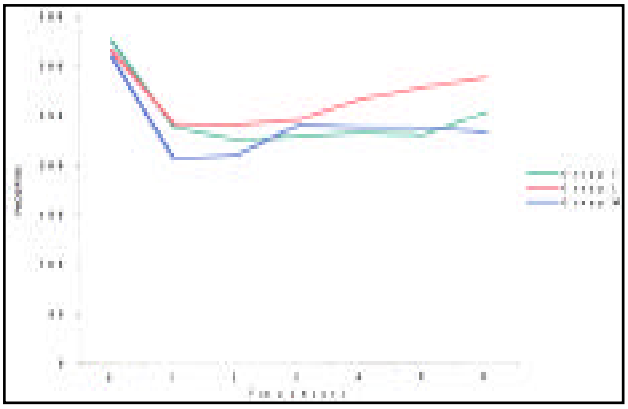

Figure 1 - PaO_2/FiO_2 rates as determined by the analysis of the arterial blood taken from the samples. PaO_2/FiO_2 - arterial oxygen to inspire oxygen concentration ratio.

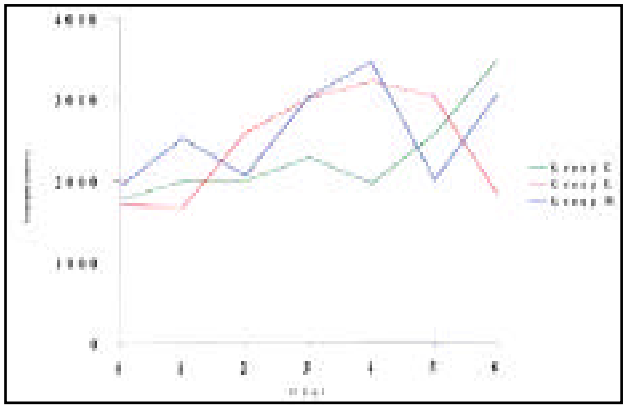

Figure 2 - Neutrophil count plotted against time.

studies, were provided by the city veterinary services, and were cared for under standard conditions.

Animals were fasted for 12 hours before the study, and then divided into 3 groups of 10: Group C (controls); Group L received lidocaine, and Group M received methylprednisolone. They were anesthetized in a glass cage filled with 3% isoflurane in equal ratios of oxygen and nitrous oxide. An open tracheostomy was created and a 3.5 mm ID tube (Rusch, Germany) was inserted; an intravenous cannula was inserted via the caudal ear vein; and the femoral artery was cannulated via a cut-down. Blood was taken for neutrophil count and arterial blood gas analysis. Lactated Ringer-dextrose, 8 ml kg^{-1} h^{-1}, was given (Ringer Laktat plus 5% Dextroz, Anonim, Turkey). Monitoring was by electrocardiogram (Lifescope 6, Nihon Kohden, Japan), acid aspiration, drug administration and mechanical ventilation. All animals were given 0.1 normal hydrochloric acid (HCl), 3 ml kg^{-1}, (pH=2) intratracheally by drops. Group C were then given saline solution, 2 ml kg^{-1} h^{-1} dose and 2 ml kg^{1} bolus; Group L were given lidocaine (Aritmal 2%, Biosel, Turkey), 2 ml kg^{-1} h^{-1} infusion, and 2 ml kg^{-1} bolus, and Group M received one dose of 30 mg kg^{-1} methylprednisolone (Prednol-L 40 mg, Mustafa Nevzat, Turkey), all intravenously. Mechanical ventilation was commenced in volume-controlled mode (Servo 900 D, Siemens, Sweden) with a tidal volume of 10 ml kg^{-1}, rate 30 $minute^{-1}$. The gas mix was isoflurane 2% in equal ratios of oxygen and nitrous oxide. positive end-expiratory pressure (PEEP) was not applied. Peak inspiratory pressure was restricted to 25 cm H_2O. The animals were ventilated for 6 hours and arterial blood samples were taken hourly for neutrophil count and PaO_2. The development of ALI was assumed as arterial oxygen to inspire oxygenconcentration ratio (PaO_2/FiO_2) changed.

Pathological preparation and laboratory analysis. After 6-hours of mechanical ventilation, all the samples were sacrificed by thiopental overdose. The neutrophil count was determined automatically by the analysis of the blood samples saved in ethylenediaminetetraacetate tubes (Coulter Stks, United States of America). The lungs were excised via sternotomy and separated from one another; the left inferior lobe was fixed in 10% formaldehyde, and buried in paraffin blocks. Sections of 5-7µm were stained with hematoxylin and eosin. The sections were evaluated under light microscopy by a blinded pathologist. Acute lung injury was scored: 1) alveolar congestion; 2) hemorrhage; 3) neutrophil infiltration and aggregation and 4) hyaline membrane formation/thickening of the alveolar wall. Each of the 4 pathological criteria was graded as a scale of 0-4; 0=minimal damage, +1=slight damage, +2=moderate damage, +3=severe damage, and +4=maximum damage.[6]

Statistical analysis. Histopathology scores were compared by Kruskal-Wallis variance analysis. The neutrophil count was compared by one-sided analysis of variance test, within and between groups. The means were subjected to Duncan's multiple comparison. The $p<0.05$ was regarded as significant, $p<0.001$ as highly significant.

Results. PaO_2/FiO_2 decreased greatly, suggesting that all animals had developed ALI (**Figure 1**). The neutrophil count varied with time, the greatest increase being in Group C (**Figure 2**). There was, however, no significant difference between Group C and the other groups. The neutrophil count increased somewhat in all groups, but the only significant increase was at 6 hours in group C ($p<0.05$).

All the scores in Group C were high. Although the alveolar wall was only mildly thickened, it was

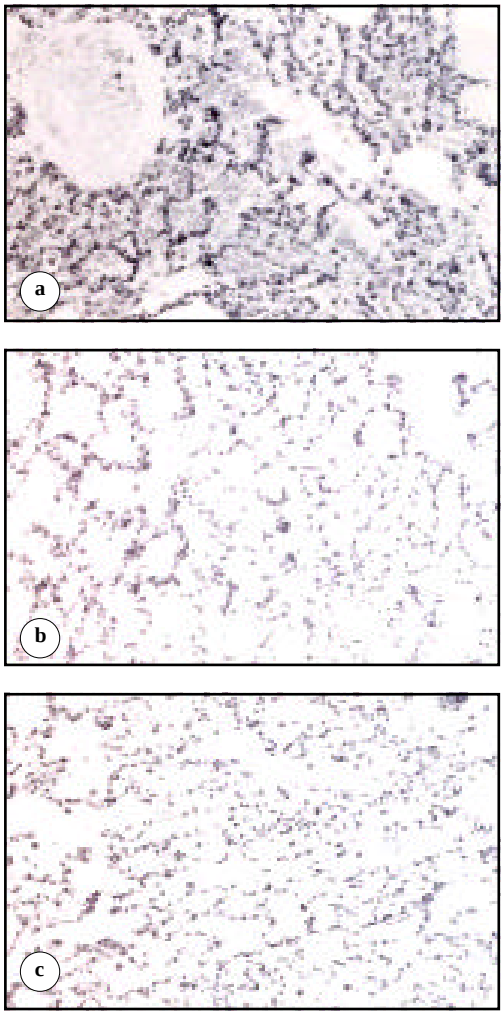

Figure 3 - Histopathologic appearances of the lungs **a)** control group histopathology, **b)** Lidocaine group histopathology and **c)** Methylprednisolone group histopathology. Hematoxylin and eosin-stain, x 200.

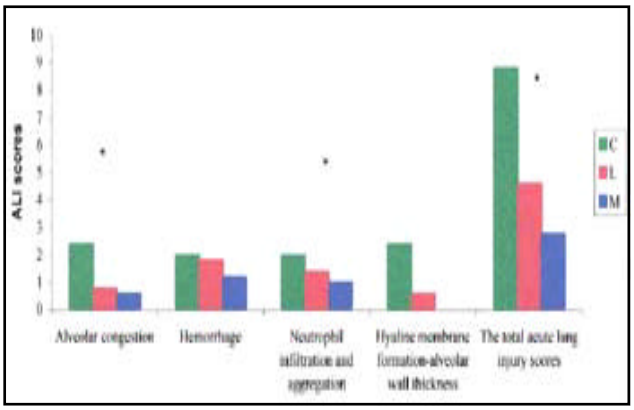

Figure 4 - Acute lung injury (ALI) scores of the groups in the histopathologic analysis. (*; $p<0.001$), (C - group control, L - group lidocaine, M - group methylprednisolone)

severely damaged (**Figure 3a**). Alveolar congestion, inflammatory cells and wall thickening were seen in Group L, although less than in Group C (**Figure 3b**). The findings in Group M were less marked than the other 2 groups, especially regarding damage to the alveolar wall (**Figure 3c**). Kruskal-Wallis variance analysis shows that the ALI scores in Groups L and M were both significantly lower than in Group C ($p<0.001$), and that they were lower in Group M than Group L ($p<0.001$). Both methylprednisolone and lidocaine greatly reduced alveolar congestion compared to control ($p<0.001$), but there was no difference between the 2 agents. Hemorrhage scores did not differ between groups. Neutrophil infiltration and aggregation scores were reduced in both treatment groups ($p<0.001$), more so by methylprednisolone ($p<0.001$). Similarly, hyaline membrane formation and alveolar wall thickening was greatly reduced by both treatments ($p<0.001$), again more so by methylprednisolone ($p<0.001$; **Figure 4**).

Discussion. Acute lung injury and ARDS are among the most serious anesthesiological problems encountered in the operating room and intensive care unit. Acid aspiration is important because, while it is not the most common cause of ALI, it is a feared complication of general anesthesia. Lung damage due to acid aspiration develops in 2 phases, early and late.[8] Early changes occur through physiochemical effects and capsaicin-sensitive afferent nerves. Tachykinins released from sensory nerves (Substance P, Neurokinin A, Neuropeptide K) cause airway mucosal edema,[9] against, which lidocaine is ineffective. In the late phase of ALI, neutrophils mediate part of the acute inflammatory response.[8] Thus, chemotaxis containing Interkleukin-8 and Thromboxane A2 accelerate neutrophil sequestration in the lungs.[10,11] Neutrophils migrate to areas exposed to acid where they adhere to the microvascular endothelium. Lidocaine reduces the consequences of the late phase of HCl aspiration; methylprednisolone inhibits the vaso- and bronchoconstriction caused by reactive oxygen products.[12] Further, in early sepsis (the most common cause of ARDS) high dose methylprednisolone significantly reduces circulatory complications and lung damage.[13]

Other studies of the use of lidocaine in the early phase of aspiration describe similar histopathological findings to ours.[14-16] However, none of these other studies included neutrophil counts, nor was HCl used to create ALI. Further, unlike them, we applied mechanical ventilation for 6 hours, a treatment, which can itself cause lung damage. In experimental ALI created by HCl acid, lidocaine, given early during mechanical ventilation applied with PEEP and 100% oxygen, gave similar histopathological findings to ours.[6] The increase in

neutrophils correlates with the degree of histopathological damage.

Histopathology suggests that methylprednisolone is effective if given early in ALI or ARDS,[13,17,18] hence we started treatment immediately after aspiration. Methylprednisolone increases the PaO_2/FiO_2 ratio and inhibits the Multiple Organ Dysfunction syndrome.[18,19] However, it is less effective in sepsis-induced ALI than in acid aspiration.[20,21] Thus the type of etiology also affects outcome. The correlation between neutrophil count and the severity of histopathological damage is one of the important points gained from animal experiments. This correlation was seen in our Group C.

In conclusion, intravenous lidocaine and methylprednisolone given early during experimental ALI diminished the lung damage scores compared to control, methylprednisolone more so than lidocaine. Although the number of neutrophils varied with time, it was greatest in the controls, which also had the greatest ALI scores. This suggests the importance of neutrophils in lung damage. We believe that our experimental study has implications for the treatment of patients with acid aspiration.

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