The Effects of High and Standard Doses of Prednisolone in the Puppies Experimentally Induced Meconium Aspiration Syndrome

DENEYSEL OLARAK MEKONYUM ASPİRASYON SENDROMU OLUŞTURULMUŞ KÖPEK YAVRULARINDA YÜKSEK VE STANDART DOZ PREDNİZOLONUN ETKİLERİ

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.Summary-

Objective: The aim of this study is to investigate the effects of different doses of prednisolone in the puppies having experimentally induced meconium aspiration syndrome.

Institution: This study was carried out in The Animal Laboratory of Yüzüncü Yıl University, Medical School.

Materials and Methods: Eleven puppies in their first 24 h of life were included in the study. Meconium used in the study was obtained from human babies in the first day of their life, and was released slowly to the trachea of puppies at the dose of 3 ml/kg. Two mg per kg of standard prednisolone to three puppies and 30 mg per kg of megadose prednisolone to other three puppies were given intravenously. Blood gases and vital functions of the animals were monitored, and chest X-rays were obtained. The study was over at the 20th hour after aspiration and the lungs were scored by histopathologically.

Results: While the findings were similar in all groups at the beginning and early times after meconium application, animals not treated with prednisolone got worse especially after the 8th hour. Respiration rate, oxygenation, pH, partial pressure of carbon dioxide values were better in megadose prednisolone treated group and than in standard dose prednisolone treated group ($p<0.05$). Histopathological scores of five puppies with meconium aspiration syndrome and untreated with prednisolone were worse than six puppies treated with different doses of prednisolone. Histopathological scores of three puppies treated with standard dose prednisolone were worse significantly ($p<0.05$) than three puppies treated with megadose prednisolone.

Conclusion: It was determined that prednisolone application had better contributions on physiological and histological alterations in puppies with meconium aspiration syndrome. These better effects were more pronounced in megadose treated animals.

Key Words: Meconium Aspiration Syndrome, Prednisolone, Puppy

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Ozet

Amaç: Bu çalışmada deneysel olarak mekonyum aspirasyon sendromu oluşturulan köpek yavrularında intravenöz yolla uygulanan farklı dozlardaki prednisolonun etkinliğinin araştırılması amaçlanmıştır.

Kurum: Çalışma Yüzüncü Yıl Üniversitesi Tıp Fakültesi Hayvan Laboratuvarında gerçekleştirildi.

Gereç ve Yöntem: Çalışmaya yaşamın ilk 24 saatindeki 11 köpek yavrusu alındı. Yaşamın ilk gününde sağlıklı bebeklerden alınmış insan mekonyumu 3 ml/kg dozunda köpek yavrularına aspire ettirildi. Aynı anda üç yavruya 2 mg/kg/doz ve diğer üç yavruya 30 mg/kg/doz Prednisolon intravenöz olarak uygulandı. Hayvanların kan gazı ve vital fonksiyonları takip edildi, göğüs radyogramları çekildi. Aspirasyondan sonra ortalama 20. saatte çalışma sonlandırıldı ve akciğerler histolojik olarak incelenerek skorlama yapıldı.

Sonuçlar: İlk saatlerde tüm hayvanların fizyolojik değişiklikleri birbirine benzer bulunurken, 8. saatten sonra steriod verilmeyen hayvanların genel durumu hızla bozulmaya başladı. Solunum hızı, oksijenasyon, pH ve parsiyel karbondioksit değerleri en iyi yüksek doz Prednisolon verilenlerde daha sonra standart doz Prednisolon verilenlerde iyi olarak saptandı ($p<0.05$). Prednisolon uygulanmayan ve belirgin mekonyum aspirasyon sendromu gelişen 5 yavrunun histopatolojik skorlamaları farklı dozlarda Prednisolon uygulanan 6 köpek yavrusundan daha kötü bulundu. Düşük doz Prednisolon uygulanan üç yavrunun histopatolojik skorlamaları da yüksek doz Prednisolon uygulanan üç yavruyla karşılaştırıldığında anlamlı olarak daha kötüydü ($p<0.05$).

Tartışma: Deneysel mekonyum aspirasyon sendromu oluşturulan köpek yavrularına Prednisolon uygulanmasının fizyolojik ve histolojik değişikliklere etkisi faydalı bulunmuştur. Bu olumlu etkiler, özellikle yüksek doz Prednisolon ile tedavi edilenlerde daha iyi ortaya çıkmıştır.

Anahtar Kelimeler: Mekonyum Aspirasyon Sendromu, Prednizolon, Köpek yavrusu

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Meconium stained amniotic fluid occurs in 9-20% of all deliveries. Meconium aspiration syndrome (MAS) is a disease with high morbidity and mortality, although seen in 3-5% of infants born through meconium stained amniotic fluid. The main problems are impairment in gas exchange, edema and development of onset hypoxemia, hypercarbia and pulmonary hypertension (1-3). Pathogenesis of MAS has not been elucidated yet. Recent studies in experimental animal models showed that pulmonary inflammation during the early phase of disorder played important role in respiratory distress. Polymorphonuclear leukocyte accumulation and cytokine release have been considered responsible for acute lung injury. Because of this inflammatory reaction, pulmonary vascular permeability increases, and this situation caused proteinous exudative liquid to be accumulated in alveoli and inhibition of surfactant (4,5).

Because of powerful anti-inflammatorial properties, steroids regulate granulocyte activation, reduce cytokine release and reduce lung injury in MAS by preventing lung edema (6,7). Although steroids are used in the treatment of some diseases such as chronic lung disease in newborns, their use in MAS is debate and routine application is not recommended. However, studies with steroids have been increased nowadays and new findings are obtained (8,9).

The aim of this study is to investigate the effects of different doses of prednisolone in the puppies having experimentally induced MAS and thus, whether prednisolone is effective in improving the severity of MAS.

Materials and Methods

Animals: Fifteen puppies in their first 24 hour of life were included in the study. Four puppies were excluded because of the following reasons: in one MAS finding could not be detected, in one arterial catheter could not be placed and two died unexpectantly. Therefore, 11 puppies weighing 279±23 g (range 265-352 g) were used. All puppies were placed with 2.5 Fr catheter (Sherwood Inc., USA) via umbilical artery and 2.5 Fr venous catheter via the cephalic vein. Puppies were not fed orally, instead were given intravenously 60 ml/kg/d 5%D-0.2% saline. Animals were sedated with 0.05 mg/kg midazolam intravenously prior to intubation. Immediately before intubation respiratory rate, heart rate, arterial blood gas and invasive blood pressure taken from abdominal aorta via umblical artery were determined. A standard dose (2 mg per kg) of prednisolone (Prednisolone®, 25 mg/2ml Fako, Turkey) was given intravenously to three puppies and a megadose (30 mg per kg) of prednisolone (Prednisolone®, 25 mg/2ml Fako, Turkey) was given to another randomly assigned three puppies in the treatment groups. The rest five animals were not treated with prednisolone instead a 0.9% saline was given as placebo. For intubation, animals were lied in supine position, and a laryngoscope with plain blade was used to visualize epiglottis and vocal cords. Intubation was mediated with sterile, disposible 2.0 gauge endotracheal tube (Portex Sims, UK). The study was approved by the Animal Ethics Committee of the Medical School of Yüzüncü Yıl University.

Preparation of meconium: Meconium used in the study was obtained from human babies in the first day of their life, who were healthy, bom in term. Meconium is lyophilized, sterilized and diluted five times using sterile distilled water in order to obtain 65 mg/ml meconium slurry. This solution was released slowly to the trachea of puppies using endotracheal tube at the dose of 3 ml/kg. After meconium instillation, the endotracheal tube was removed and animals were allowed to their spontaneous breathing.

Laboratory Analysis: Blood pH, partial pressure of carbon dioxide ($pC0_2$), partial pressure of oxygen ($p0_2$) and oxygen saturation were measured by an automatic blood gas analyser (Radiometer ABL 500, Copenhagen, Denmark) just after obtaining the samples from the umbilical artery. Samples for blood gas were taken every 6 hours and at the end of the study. Oxygen was provided to the puppies by the hood so that arterial $p0_2$ was found to be about 60 mmHg. Invasive blood pressure was automatically measured (Millenium® In Vivo Research, USA). Internal hood oxygen concentration was determined by an oxymeter (SLE

Oxymeter, UK). At the end of the experiment, the animals were killed with an overdose of potassium chloride.

Histological Examination: For histological analysis of the lungs, a 3x3x3 cm piece of pulmonary tissue from the right lower lobe was fixed in buffered formalin. The tissue samples were dehydrated, cleared and embedded to paraffin according to a routine process. Five micrometer sections were stained with hematoxylin and eosin for light microscopic analysis. To deteiTnine the extension and severity of the lung tissue injury, the histologic samples were assessed by a pathologist blinded to the grouping of the puppies.

Pathologic properties were classified as follows:

1) Extent of leukocyte infiltration

2) Amount of intra-alveolar leukocytes

3) Extent of exudative debris

4) Amount of intra-alveolar exudative debris.

Within each class a scoring was applied. Total injury score was determined by adding obtained scores from each class.

1) Extent of leukocyte infiltration:

0: Leukocytic infiltration is absent.

1: Leukocyte infiltration below 10% of tissue samples.

2: Leukocyte infiltration between 10-25% of tissue samples.

3: Leukocyte infiltration between 26-50% of tissue samples.

4: Leukocyte infiltration above 50% of tissue samples.

2) Amount of intra-alveolar leukocytes

0: Intra-alveolar leukocyte is absent

1: Leukocytes below 10% of alveolar surface.

2: Leukocytes between 10-25% of alveolar surface.

3: Leukocytes between 26-50% of alveolar surface.

4: Leukocytes above 50% of alveolar surface.

3) Extent of exudative debris:

0: Exudative debris is absent.

1: Exudative debris below 10% of tissue samples.

2: Exudative debris between 10-25% of tissue samples.

3: Exudative debris between 26-50% of tissue samples.

4: Exudative debris above 50% of tissue samples.

4) Amount of intra-alveolar exudative debris

0: Intra-alveolar exudative debris is absent.

1: Exudative debris below 10% of alveol surface.

2: Exudative debris between 10-25% of alveol surface.

3: Exudative debris between 26-50% of alveol surface.

4: Exudative debris above 50% of alveol surface.

Statistical methods. The physical, laboratory and histologic findings of animals were computerized with an instant packet statistical programme (SPSS vlO.0) in the computer environment and the due processings were conducted. Histograms, means and standard deviations were processed. One-way ANOVA, Mann Whitney-U test and chi-square test were used in the evaluation of the results. Statistically significant differences were determined when the p-value was smaller than 0.05.

Results

Physiological changes: The findings were similar in all groups at the beginning and early times after meconium instillation. However respiratory distress and oxygen requirement were appearent in animals not treated with prednisolone especially after the 8th hour. $P0_2/Fi0_2$ ratio at 8th hour was higher in megadose prednisolone treated animals then untreated animals ($p<0.05$) (Figure 1). This ratio was higher in both prednisolone treated groups then untreated group 12 h after treatment. While mean arterial blood pressure

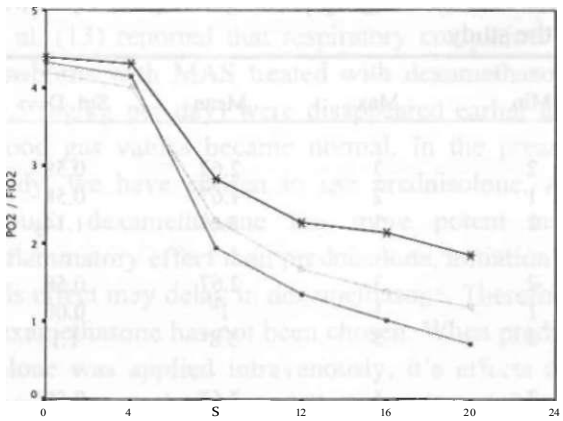

Figure 1. $P0_2/Fi0_2$ values of untreated (solid square) or treated puppies with standard (open square) or megadose prednisolone (asterix).

(mBP) values did not different among groups, pH, respiration rate and pC02 values were better in megadose prednisolone treated group and than in standart dose prednisolone treated group (Table 1).

Histological Changes: Histopathological changes in puppies treated with megadose prednisolone were minimal. Lung histopathological examination showed that leukocyte infiltration extent and intraalveoler leukocyte numbers were low in megadose prednisolone treated puppies compared to untreated ones ($p<0.05$). Exudative debris extent and intraalveolar exudative debris amount were low in megadose treated animals; however, these differences were not significant ($p>0.05$). Histological changes of megadose prednisolone treated puppies were better than standard dose prednisolone treated animals. Total score was lower in megadose prednisolone treated animals than both standard dose prednisolone treated and untreated animals (Table 2). Grade IV alveolar leukocytic infiltration of a puppy among untreated group were shown in Figure 2.

Discussion

Beside direct alveolo-capillary blocking effect of meconium, lung inflammation during the early period, as well, play important role in the pathogenesis of MAS. In early period, intrapulmonary neutrophil accumulation occurs and then cytokines

Table 1. Physiological changes of untreated or treated puppies with standard (Std. Pred.) or megadose(Mega Pred.) prednisolone

	0	4 h	8h	12 h	16 h	20 h
Breaths per minute						
Std. Pred.	42.2(2.5)	46.6(1.5)	53.6(5.6)	62.6(6.4)	67.3(10)[b]	76(7.2)[b]
Mega Pred.	41(1)	46.3(1.5)	56.3(3.2)	62.6(12)	72(6.9)	63.3(4.1)
Untreated	41.4(2.7)	48.4(3.2)	54(5.8)	91.2(9.7)	92(12)"	107(9.4)
$P0_2/Fi0_2$						
Std. Pred.	4.2(0.09)	4(0.2)	2.3(0.3)	1.67(0.2)[b]	1.3(0.03)[b]	1.1(0.1)[b]
Mega Pred.	4.3(0.09)	4.3(0.1)	2.8(0.2)	2.2(0.1)	2.1(0.3)[c]	1.8(0.1)°
Untreated	4.3(0.1)	4.1(0.2)	1.9(0.3)"	1.3(0.05)"	1(0.09)"	0.7(0.05)"
pn rn						
Std. Pred.	7.45(0.01)	7.33(0.2)	7.37(0.06)	7.3(0.02)[b]	7.25(0.04)	7.16(0.07)
Mega Pred.	7.43(0.06)	7.35(0.01)	7.4(0.02)	7.32(0.04)	7.37(0.02)	7.32(0.04)[c]
Untreated	7.42(0.05)	7.35(0.03)	7.31(0.05)	7.18(0.05)[a]	7.19(0.08)"	7.14(0.06)"
$_pCO_2$						
Std. Pred.	37(6)	35.6(8.7)	42.3(2.5)	37.6(3.7)	64.6(5)	67.6(2.5)[b]
Mega Pred.	35.6(2)	38.3(2.8)	44.3(2)	37(2.6)	49(3.6)[c]	53.3(6.1)[c]
Untreated	35.6(4.8)	38(7)	46.2(4.3)	39.8(3.5)	70.4(11)"	78.8(4.1)"
MBP						
Std. Pred.	31.3(2.3)	38(2.6)	37.3(2)	38.6(3.59	39.6(4.7)	37.3(3)
Mega Pred.	33.6(0.5)	38.3(2.5)	37.6(1.5)	42.3(2.5)	39.3(3)	36(2)
Untreated	32.6(1.9)	37.2(1.9)	37.2(1.6)	38(4)	41(3.3)	34.6(3.8)

' compared with megadose Prednisolon group $p<0.05$
[b] compared with untreated group $p<0.05$
[c] compared with standard dose Prednisolon group $p<0.05$

Table 2. The histological scores of puppies receiving at the dose of 2 mg/kg Prednisolon (Std. Pred.), 30 mg/kg Prednisolon (Mega Pred.) and untreated included in the study

	n	Min	Max	Mean	Std. Dev.
Extent ofleukocyte infiltration					
Std. Pred.	3	2	3	2.67	0.58
Mega Pred.	3	1	2	1.67	0.58
Untreated	5	2	5	3.6[c]	1.14
Amount of intra-alveolar leukocytes					
Std. Pred.	3	2	3	2.67	0.58
Mega Pred.	3	1	1	1[b]	0.00
Untreated	5	2	5	3.8[c]	1.1
Extent of exudative debris					
Std. Pred.	3	3	4	3.67	0.58
Mega Pred.	3	1	3	1.67	1.15
Untreated	5	1	5	3.8	1.64
Amount of intra-alveolar exudative debris					
Std. Pred.	3	2	3	2.67	0.58
Mega Pred.	3	1	2	I. 33	0.58
Untreated	5	1	5	3.8	1.64
Total score					
Std. Pred.	3	9	13	II. 67	2.31
Mega Pred.	3	5	7	5.67[b]	1.15
Untreated	5	10	19	15[c]	4.06

[b] **compared with megadose Prednisolon group p<0.05**
[c] **compared with standard dose Prednisolon group p<0.05**

appear. Because of these cytokines neutrophil chemotaxis continues and consequently appearent lung inflammation and edema form (10,11). This pathogenic process was demonstrated in some of studies done recently (7,11-14). Histological examination and even scoring were done in the present study. It was clearly observed that four histopathological findings, indications of lung inflammation, increased in puppies having MAS. This inflammation was more appearent in untreated animals (Table 2). In the study of Holopainen et al. (14), fourteen piglets were studied and dexamethasone (0.5 mg/kg) was given. For histologic analysis, they showed that prophylactic dexamethasone treatment tended to decrease the severity of the pulmonary inflammatory changes. Consequently, the total injury score was at a borderline (p=0.058) lower in dexamethasone group than in the controls. Results of this study were similar to ours.

Attempts to prevent inflammation develop in lungs in MAS will provide significant contributions to the treatment. Because of anti-

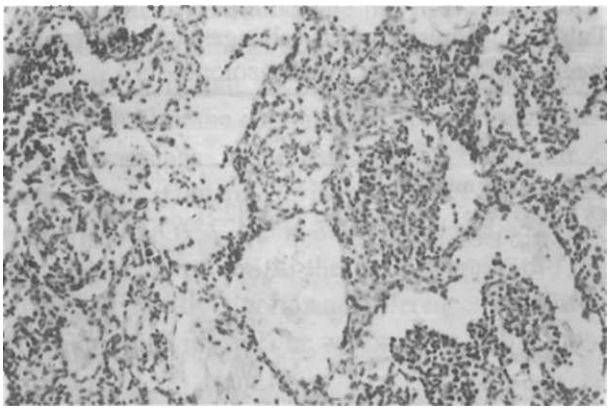

Figure 2. Grade IV alveolar leukocytic infiltration of a puppy among untreated group. Clusters of leukocytes and aspirated keratinized material in alveolar spaces and intersititial congestion (H-EX125).

inflammatory properties, steroids are being used in recent years in experimental and clinical studies related to MAS (11-14). Despite the studies done using low dose hydrocortisone and resulted unsuccesfull in the past, the studies done using dexamethasone or megadose methylprednisolone in

nowadays are getting important (15,16). Da Costa et al. (13) reported that respiratory complaints of newborns with MAS treated with dexamethasone (0.5 mg/kg per day) were disappeared earlier and blood gas values became normal. In the present study, we have chosen to use prednisolone. Although dexamethasone has more potent anti-inflammatory effect than prednisolone, initiation of this effect may delay in dexamethasone. Therefore, dexamethasone has not been chosen. When prednisolone was applied intravenously, it's effects appear in a short time, especially in newborn infants.

Studies emphasize that steroid should be applied prior to pathological process in MAS. Treatment should be initiated before neutrophil accumulation and cytokine release in lung (11,12). In MAS developed piglets, Wu et al. (12) demonstrated that cytokine amount in tracheal aspirates decreased significantly when dexamethasone was given in an early period. In another study, 11 piglets were evaluated before development of MAS or giving dexamethasone. They showed that the lung structures were better in animals after given prophylactic dexamethasone (0.5 mg/kg q 12h). At the end of the study, they concluded that steroid should be given in a very early period (11). In the present study, contrary to the other studies, puppies in their first hours of lives were evaluated. It was though that this model would be an ideal model to monitor animals' response before their postnatal lung maturation completed. Prednisolone was applied to puppies just after meconium was aspirated. The findings of steroid treated animals were similar to untreated animals during the early periods. However, especially after the 8th hour overall conditions of treated animals remain stable while overall conditions of untreated animals got worse rapidly. The most remarkable finding was that the O_2 requirement was lower in steroid treated animals. Since oxygen-dependent free radical formation has a role in tissue injury, less lung injury is expected in low oxygen environment. Beside oxygenation, pH, pCO_2, and PO_2/FiO_2 values were better in steroid treated animals. These findings demonstrated that steroid treatment in MAS could prevent physiological changes to be worsened appearently in puppies.

As observed in recent studies, the present study demonstrated that steroid treatment decreased the severity of MAS but could not prevent it completely. Thus, megadose steroid treatment get intentions. Soukka et al. (11) reported that better oxygenation, less developed lung inflammation and edema were seen in ten weeks piglets when 30 mg/kg methylprednizolone was given prior to MAS development. In the present study, megadose prednisolone (30 mg/kg) was given to three puppies. Severity of both physiological and histological changes were lower in this group compared to steroid treated and untreated puppies. Side effects of megadose prednisolone such as elevated blood sugar levels (data not shown) or high arterial tension values were not observed. Megadose steroid treatment has been used in treatment of many pediatric diseases successfully. We believe that if better results are obtained from steroid based MAS treatment studies and proved in detailed trials, these results may be used in the future for treatment purposes.

In conclusion, it was determined that prednisolone application had better contributions on physiological and histological alterations in puppies with MAS. These better effects were more pronounced in megadose treated animals. Steroid application in MAS treatment should be taken into consideration.

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