

In Vitro Action of Meconium on Bronchomotor Tonus of Newborns with Meconium Aspiration Syndrome

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Abstract

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Abbreviations: MAS - Meconium Aspiration Syndrome; IL - interleukins (IL-1 β , IL-6 and IL8); TNF-alpha - tumor necrosis factor; PLA2 - phospholipase A2; PAF - platelets activating factor; NO - Nitric Oxide; H & E - hematoxylin-eosin; X - the median value; SD - standard deviation; SEM - standard error

AIM: Here we studied the role of meconium in the respiratory system on live and exited newborns (weight 250-3000 g). Throughout this study is followed the response of tracheal rings in acetylcholine and histamine in different molar concentrations (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} mol/dm³).

METHODS: To study the smooth tracheal musculature we used 23 tracheal preparations obtained from the newborns exited from meconium aspiration.

RESULTS: Based on the functional analysis of the tracheal specimen we have concluded that the meconium aspiration did not change the smooth musculature response on acetylcholine and histamine when compared to control group, exited from lung inflammatory processes (e.g., pneumonia, bronchopneumonia, atelectasis, cerebral hemorrhage), where tracheal smooth musculature response is significant (P for other causes is not significant (P > 0.01).

CONCLUSION: The conclusions suggest that meconium did not potentiate the constrictor action of acetylcholine and histamine in the tracheobronchial system and did not cause modulation of bronchomotor tonus in case of his aspiration. Meconium causes mild relaxation of smooth tracheal musculature with a mechanism which is not mediated by cyclooxygenase products, from tracheal epithelium or proteins. Also, direct activity in the smooth musculature of several tested acids seems to have no significant impact in increasing the tonus of respiratory airway of smooth tracheal musculature.

Introduction

The mechanisms which contribute to increasing the reactivity of respiratory airways in Meconium Aspiration Syndrome (MAS) are very unclear. Meconium is a biologically active substance with very powerful contractile effect in vascular and airway smooth musculature, composed of leukotriene, PAF, ET-1 etc. Meconium contains high concentrations of fatty acids [1] and biliary acids [2] that may induce contraction of smooth musculature of respiratory airways. This contraction depends on the concentration of aspirated meconium [3]. Meconium aspiration syndrome (MAS) is an important cause of mortality and respiratory morbidity in newborns babies. Mechanic obstruction of respiratory airways,

dysfunction of pulmonary surfactant, pulmonary inflammation and vasoconstriction are pathologic mechanism associated with MAS syndrome. Damage reactivity of respiratory airways also could be associated with MAS syndrome [4] [5].

The airway obstruction could affect reflexive alteration of bronchomotor tonus associated with bronchoactive substances. Interactions between individual pathogenetic factors are not yet known. Meconium is found to be present from 12 weeks of gestation. It is a product of amniotic fluid of fetus containing plaque cells of vernix caseosa secretion and gastrointestinal cells [6]. Meconium contains 4 different fatty acids (e.g. Choline, henodeoxicholic acid and lithocholic) and minerals from witch copper, zinc, manganese, calcium, iron and phosphorus are more frequent component [7] [8]. Also contains

plasma proteins (alpha 1 antitrypsin) [9] [10] and other active substances such as interleukins IL-1 β , IL-6 and IL8, tumor necrosis factor (TNF-alpha) [11] and phospholipase A₂ (PLA₂) [12] which may induce directly or indirectly pulmonary inflammation, by increasing production of cytokines and by activating white blood cells or epithelial/endothelial cells of lungs. In vitro exposure of meconium increase release of IL-8, TNF-alpha [13], endothelin-1, platelets activating factor (PAF), leukotrienes, thromboxane A₂, induced of NO synthetase [14], NO [15], PLA₂ and other substances which affect the reactivity of respiratory airways and inflammation.

Recent studies of meconium aspiration in rats have shown the increased response of smooth musculature of respiratory airways to methacholine after seven days, and also to histamine in rabbits after 5.5 hours from meconium application. Mechanisms of increased reactivity of respiratory airways in MAS syndrome are unknown. Direct inflammatory effects of meconium on the release of broncho active substances (PAF, leukotrienes, etc.) and hypoxia accompanied by oxidative damage from oxygen therapy may be operative in this process [12].

Purpose of the study is to demonstrate the action of meconium in newborn babes with MAS syndrome in the inflexion of acetylcholine and histamine action in the smooth musculature of the tracheobronchial system in alive and the dead newborn babes.

Material and Methods

The research was conducted in cooperation with the Gynecology Obstetrics Clinic, Pathologic Anatomy Institute and Experimental Unit of Medical Faculty in Prishtina, with permission of the Ethics Commission by respecting principles of Helsinki Declaration.

Classification of tracheal preparation of newborn babies in different weeks of gestations is made by histopathological examination of tracheal preparation (in blocks of paraffin). The preparations have been stained with the standard: hematoxylin-eosin (H & E) methods.

Research has been conducted in 33 subjects of in vitro isolated tracheas of babies in different weeks of gestations (weight 250 to 3000 g). Tracheas were taken immediately after autopsies. Above bifurcation of the trachea were taken 6 tracheal rings and placed in Krebs solutions DIP (pH = 7.4). The water bath temperature was kept at 37°C, and solution in the bath is aerosolised continuously with a gas mixture (95% CO₂ and 5% O₂), which has flowed in continual mode through the bath solution. Tracheal rings are serially connected with each other. The

series consisting from 6 rings are placed in the bath for isolated organs (volume 50 ml), so that lower part of the ring is connected to the holder, while the upper part is connected to the transducer (" Force transducer" Statham UC₂). The smooth musculature response is registered in the multi-channel recorder (Watanabe HSE 6600). After 30 minutes is registered tonus of tracheal rings, then preparation is exposed to different molar concentration (10⁻¹, 10⁻², 10⁻³, 10⁻⁴, mol/dm³) of acetylcholine and histamine. Doses are changed every 15 minutes, while effects of broncho constrictor agents are followed 3 minutes, after application. Then preparation is rinsed several times with Krebs solution, before application of another substance.

Results are processed with statistic computer program GraphPad InStat III with comparing t-test for two working groups.

Results

In Table 1 and Figure 1 is showed the acetylcholine dose response on smooth tracheal musculature in newborn babies of different age groups of meconium aspiration syndrome vs control group (P < 0.01).

Table 1: Acetylcholine dose response of smooth tracheal musculature in newborn babes of different age groups of meconium aspiration syndrome MAS (Mean \pm SEM)

| Groups | Ach log ⁻⁴ | Ach log ⁻³ | Ach log ⁻² | Ach log ⁻¹ |
|---------|-----------------------|-----------------------|-----------------------|-----------------------|
| MAS | 2.91 \pm 0.98 | 4.79 \pm 1.56 | 10.75 \pm 3.33 | 19.70 \pm 4.73 |
| Control | 4.5 \pm 1.53 | 7.18 \pm 2.21 | 13.37 \pm 3.46 | 23.96 \pm 4.89 |

In Table 2 and Figure 2 is showed the cumulative response of histamine in smooth tracheal musculature in newborn babies of different age groups of meconium aspiration syndrome vs control group (P < 0.01).

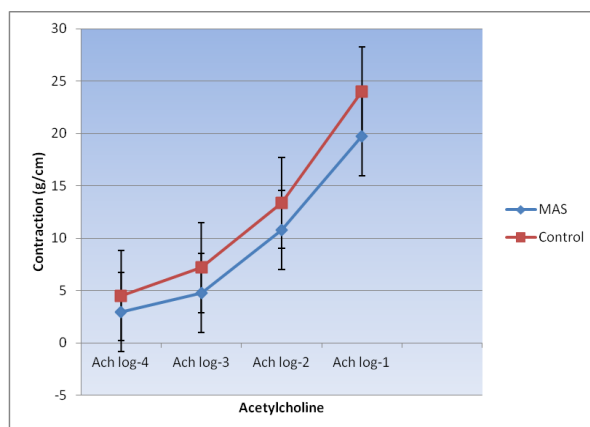


Figure 1: the Cumulative action of acetylcholine in smooth musculature in newborn babes with different age groups of Meconium Aspiration Syndrome (Mean \pm SEM)

Table 2: Histamine dose response of smooth tracheal musculature in newborn babes of different age groups of meconium aspiration syndrome MAS (Mean \pm SEM).

| Groups | Histamine log ⁻⁴ | Histamine log ⁻³ | Histamine log ⁻² | Histamine log ⁻¹ |
|---------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| MAS | 4.161 \pm 1.76 | 8.0 \pm 2.81 | 14.75 \pm 4.04 | 22.0 \pm 5.60 |
| Control | 5.25 \pm 1.68 | 10.18 \pm 2.56 | 19.06 \pm 3.72 | 27.08 \pm 5.06 |

Figure 3 is presented comparative effect of acetylcholine and histamine action in newborn babies of different age groups of meconium aspiration syndrome vs control group ($P < 0.1$).

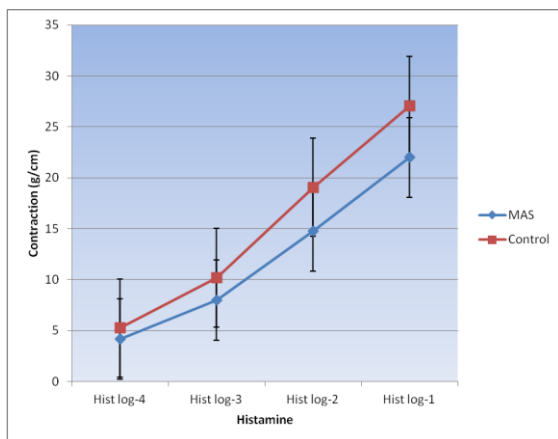


Figure 2: the Cumulative action of histamine in smooth tracheal musculature in newborn babes of different age groups of Meconium Aspiration Syndrome (Mean \pm SEM)

Discussion

The studies of newborn babies affected with MAS during neonatal period have shown the abnormality of functionally pulmonary tests, diminished obstruction of respiratory airways, an episode of bronchospasm, and need for bronchodilator therapy.

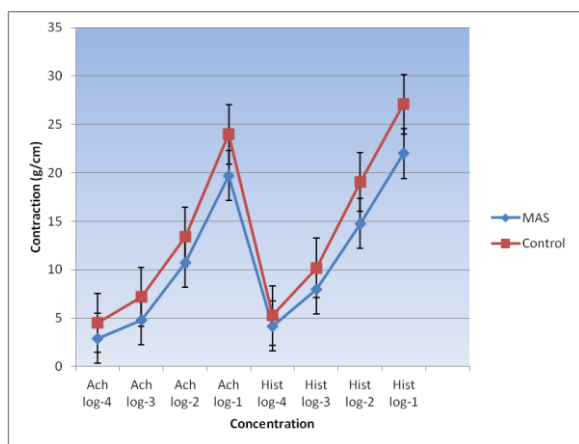


Figure 3: the Cumulative action of histamine and acetylcholine in smooth tracheal musculature in newborn babes of different age groups of Meconium Aspiration Syndrome (Mean \pm SEM)

Progressive lung inflammation may increase

the reactivity of respiratory airways. Thus the use of bronchodilators together with anti-inflammatory drugs may be useful in MAS syndrome. Meconium stasis of the amniotic fluid happens at least in 8% of all births. Incidence varies from 5-8 % before 39 weeks of gestation. Incidence could increase by 12 % after 39 weeks of gestation [15]. Prematurity is not a risk factor. MAS is very rare in newborn babes before 34 weeks of gestation. Meconium stasis presents a risk for newborn babes; MAS proceed in 10-30% and 19-34% cause mortality. Risk factors in MAS syndrome include newborn babes 1-3 weeks after birth date period, maternal diabetes, hypertension in gestation, difficult births, respiratory distress syndrome (RDS) in newborn babes, intrauterine hypoxia [16].

Symptoms and signs of MAS syndrome include tachypnea, nasal secretion, retraction, cyanosis or increased desaturation, also heavy stasis in umbilical cordon with skin spots. Meconium stasis can be noticed in oropharynx, larynx and trachea. The prophylactic suction of nose and mouth before the birth of body of the babes decreases the risk of MAS [17].

Recently, new therapeutic strategy for the treatment of MAS is suggested [18] including anti-inflammatory drugs, such as antagonists of prostaglandins, high-frequency ventilation, exogenous surfactant, nitric oxide and water inhalation [18].

The rings of tracheal tissue and lungs of guinea pigs are incubated for one hour in a water bath for isolated organs, in three different concentrations with human meconium to investigate whether there is a connection between the cumulative dosages of acetylcholine, histamine and meconium concentrations and contraction response [19]. Current studies show that the contractile response of the rings of tracheal and lungs tissue gradually increases with cumulative doses of acetylcholine and histamine in different concentrations of meconium and control condition [19]. However, there is no complete response in decreased concentration of meconium, which has lower tracheal reactivity to histamine and acetylcholine [19]. High concentration of meconium tends to increase tracheal reactivity. Incubation in a lower concentration of meconium 1 mg/ml in rabbit trachea act by lowering reactivity in vitro of acetylcholine and histamine [19]. In vitro relaxation of smooth tracheal musculature has been demonstrated in rats [20], but relaxation response increases with increasing of meconium concentration. Presence of reduced concentration of meconium in amniotic fluid may present a sign of physiologic maturity in newborn babes and do not present inflammatory response of tissues. On the other side, a high concentration of meconium may cause harmful changes resulting in inflammations and related to constriction of vascular and respiratory airways of the smooth musculature. While a lower concentration of meconium increases secretion of surfactant in isolated alveolar tip II cell [20] and inhibit oxidative blast of neutrophils and

phagocytosis [21]. This antioxidative capability of meconium could be partially responsible for the lower incidence of MAS syndrome in case of amniotic fluid aspiration of newborn babes.

In vitro condition, the aspiration of 20% of meconium in rats significantly increases the response of the respiratory airways to methacholine after 7 days and is associated with the lymphocytic and eosinophilic inflammation, metaplasia of goblet cells and increased concentrations of IL-5 and IL-3 in bronchoalveolar wash [1]. Is noticed progression of the polymorphonuclear inflammation in ventilated rats for 5.5 hours after inhalation of meconium, who also was associated with increased tracheal reactivity to histamine [2]. The above results suggest that the contraction of smooth musculature of the respiratory airways is associated with concomitant mechanisms at MAS syndrome, such hypoxia, cytokine production and the reactive products during inflammations.

There is no full correlation between contractile response and meconium concentration. Tracheal reactivity of the respiratory airways to the cumulative dosage of histamine and acetylcholine increases with increased concentration of meconium, but the reactivity of lung tissue tends to decreases. Mechanisms of reactivity in affected respiratory airways in MAS syndrome are unclear, and further experiments show for constrictor response of smooth musculature of the respiratory airways to meconium.

In the present studies, during increased concentrations of meconium, reactivity response of smooth tracheal musculature to the acetylcholine and histamine is shown with a tendency to partial decreases depending from increases dosage of these mediators. The different responses may be related to the duration of exposure to meconium. Exposure for a short time in vitro may present vasodilator and bronchodilator effects, while exposure for a long time may have mainly constrictor effects in smooth musculature that depends on the time of inhibiting medium. For a better understanding of above mechanisms, incubation for a short period in vitro of pulmonary rings and blood vessels in different meconium concentrations would be necessary.

Based on the results obtained experimentally can be concluded that:

Tracheal smooth musculature response in MAS syndrome to acetylcholine in cases that have exited without aspiration of amniotic fluid is significant ($P < 0.01$).

Meconium in MAS syndrome does not potentiate the constrictor action of acetylcholine in smooth tracheal musculature insignificant way ($P > 0.1$).

Tracheal smooth musculature response in MAS syndrome to histamine in cases that have not exited from meconium aspiration (but for other reasons) is significant ($P < 0.01$).

Meconium in MAS syndrome does not potentiate the constrictor action of histamine compared to group control ($P > 0.1$).

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