ROLE OF MECONIUM IN THE REACTION OF AIRWAYS SMOOTH MUSCULATURE IN THE NEWBORN WITH MECONIUM ASPIRATION SYNDROME (MAS)

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Abstract

The role of meconium in the respiratory system was studied in newborns, who died from various causes (250 up to 3000 g of weight). We monitored tracheal rings response to dopamine, serotonin and ethanol in different concentrations (dopamine: 0,05 mg/ml, 0,5 mg/ml, 5 mg/ml; serotonin (5-HT): 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} mol/dm^3; ethanol: 0,02 ml, 0,5 ml, 1,0 ml; 96%). Tracheal smooth musculature tonus (TSM) was examined in 48 tracheal preparations taken after the newborn exitus due to different reasons. Based on functional researche of isolated preparations of tracheas, it may be concluded that: aspiration of meconium has not changed the response of TSM to dopamine, serotonin and ethanol ($p>0,1$) in comparison with the control group, which have died due to different lung inflammatory processes (e.g. pneumonia, bronchopneumonia, atelectasis, cerebral hemorrhage). The results suggest that meconium does not potentiate the constricting action of dopamine, serotonin and ethanol in tracheobronchial system.

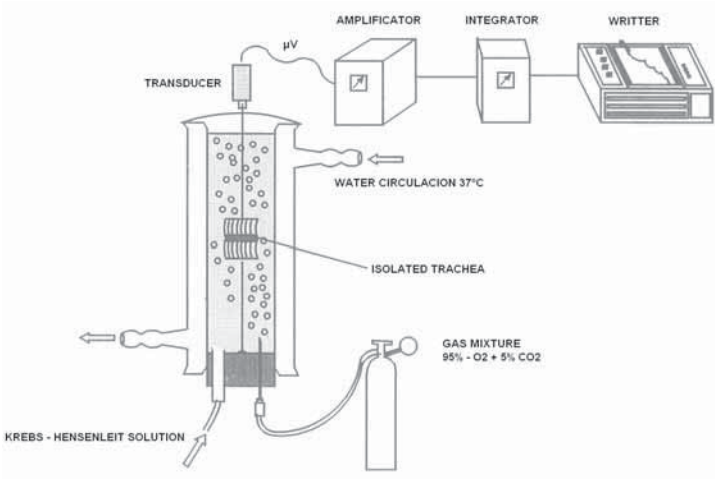
Meconium causes mild relaxation of the TSM through a mechanism that is not intermediated by the products of cyclooxygenases (prostaglandins, prostacyclins) from the tracheal epithelium or proteins. Also, as it seems, the direct activity of many tested acids in the smooth musculature has no significant impact on increase of the airways tonus in MAS syndrome.

KEY WORDS: Trachea, meconium, dopamine, serotonine, ethanol.

Introduction

Syndrome of the aspiration of the meconium liquid (MAS) is an important cause of respiratory mortality and morbidity in newborn. Mechanic obstruction of the airways, dysfunction of pulmonary surfactant, pulmonary inflammation and vasoconstriction are pathomechanisms interconnected with MAS. Damage of airways reactivity can also be interconnected with MAS (1,2). Obstruction of the airways can influence reflexive alterations of the airways tonus in connection with bronchoactive substances. Inter-reactions between individual pathogenic factors are not yet fully known. Meconium is present since 12th week of foetus gestation. Amniotic fluid consists slate cells, secretion of vernix caseosa, and it also contains gastrointestinal system cells (3). Meconium composition includes 4 different bile acids (e.g. choline, Chenodeoxycholic and lithocholic acid) and minerals such copper, zinc, manganese, calcium, iron, and phosphorus that are the frequent (4,5). It also contains plasmatic proteins (alpha-1 antitrypsin) (6,7). Meconium contains many other different substances such interleukins IL-1ß, IL-6 and IL8, necrotizing tumoral factor (TNF-alpha) (8) and phospholipases A2 (PLA2) (9) that may induce either direct or indirect pulmonary inflammation by increasing the production of cytokines and by activating leukocytes or epithelial and endothelial cells in the lung. *In vitro* exposure to meconium increases the release of IL-8, TNF-alpha (10), endothelium-1, trombocytes activating factor (PAF), leukotrienes, thromboxane A2, induces synthetase NO (11), NO (12), PLA2 and other substances that influence reactivity of the airways and inflammation. Development of adrenergic nervous system is closely related to the development of cholinergic nervous system. Both systems developed in parallel during intrauterine life (13). Whereas 5-HT is a product of mast cells and human trombocytes that causes bronchoconstriction in many species (14), but not in healthy people and asthmatics (15). 5-HT causes bronchoconstriction in mice, which can be blocked with atropine. This shows interdependence with cholinergic nervous fibres that innervate airways smooth musculature (16).

Ethanol causes constriction of smooth musculature in all species of animals. This constriction partially depends on the entry of calcium ions (Ca^{2+}) from extracellular space into intracellular space of the smooth tracheal musculature. However, constriction stimulating effect of ethanol depends on the release of (Ca^{2+}) from sarcoplasmatic reticulum that induces intracellular production of IP3 (inositol 1,4,5 triphosphate) and DAG (diacylglycerol), which potentiate the constriction of the airways smooth musculature (17). The aim of this work is to demonstrate the role of meconium in the newborns with MAS syndrome in modulating the activity of dopamine, serotonin and ethanol in the smooth musculature of tracheobronchial system in live and dead newborns.



SHEMA 1. Experimental model of the work: Measurement of the active tension of the tracheal rings smooth musculature.

Materials and Methods

Elaborate was performed in cooperation with the Gynaecology Obstetrics Clinic, Pathology Institute and Experimental Unit at Medical Faculty in Prishtina. Research was conducted in 33 experimental studies *in vitro* in the isolated tracheas of the passed away kids in different gestation weeks (with weight 250 up to 3000 g) taken immediately after the autopsy. Over the trachea bifurcation, 6 tracheal rings were taken by being placed in Krebs solution (pH = 7,4). During the development of the experiment, bathroom temperature was held in 37°C, and solution in the bathroom was aerosolized continuously with gas mixture (95% O2 and 5% O2), with continuous flow in the bathroom solution. Rings were prepared and serially connected in between themselves. Serial, composed of 6 rings, was placed in bathroom for isolated organs (50 ml volume), in order that lower part of the rings is connected for retainer, whilst upper part of the ring is connected to transducer with a thread („Force transducer", Statham UC2). Response of TSM was registered in a multi-channel registration (Watanabe HSE 6600). (See Scheme 1. of the experimental model in vitro). 30 minutes later, first tonus of tracheal rings was registered; afterwards preparation was exposed to different molar concentrations (dopamine: 0.05 mg/ml, 0.5 mg/ml, 5 mg/ml; serotonin: 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} mol/dm^3; ethanol: 0,02 ml, 0,5 ml, 1,0 ml; 96%). Doses have changed every 15 minutes, whilst effects of bronchi-constrictor agents, after the application, were monitored for 3 minutes. Afterwards, preparation got rinsed couple of times with Krebs solution, prior application of the other substance. Results were processed with statistical computer software GraphPad InStat III with T-test for comparison of two working groups.

Results

Classification was conducted based on hystopathologic analyses of samples: first study group (recently dead children from the aspiration of the amniotic fluid syndrome) histopathologically characterized by these changes: presence of amniotic fluid in airways, with granular proteinic eosynophyilic material and epithelial squama. Second controlling group in recently dead children by pneumonia, bronchopneumonia, atelectases and cerebral hemorrhage; histopathologically characterized by these changes: in air spaces up to the level of the alveoli, many inflammatory infiltrates of granulocyte, monocyte and erythrocytary extravasate types were observed. In bronchiole and in peribronchial part, proteinic eosynophyilic material, cellular detritus and many inflammatory infiltrates of the granulocyte and monocyte types were observed. Some parts of the lungs (alveoli) are not open. Results of the research in isolated tracheal preparations in dead newborn shows that dopamine, serotonin and ethanol were applied in different molar concentrations (dopamine: 0,05 mg/ml, 0,5 mg/ml, 5 mg/ml; serotonin: 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} mol/dm3; ethanol: 0,02 cm^3, 0,5 cm^3, 1,0 cm^3; 96%), which act on a different manner depending from the applied dose. In Table 1. and Figure 1., response of smooth musculature to different molar concentrations of dopamine in newborn of different ages with the syndrome of aspiration of amniotic fluid and the check up group is presented ($p<0,05$).

Groups	Dopamine 0,05 mg/cm^3	Dopamine 0,5 mg/cm^3	Dopamine 5,0 mg/cm^3
MAS	0,000±0,000	2,313±1,243	5,563±1,736
Control	0,2500±0,2500	2,750±0,7008	4,125±1,231

TABLE 1. TSM response to dopamine at newborn with MAS (X+SEM) n=16

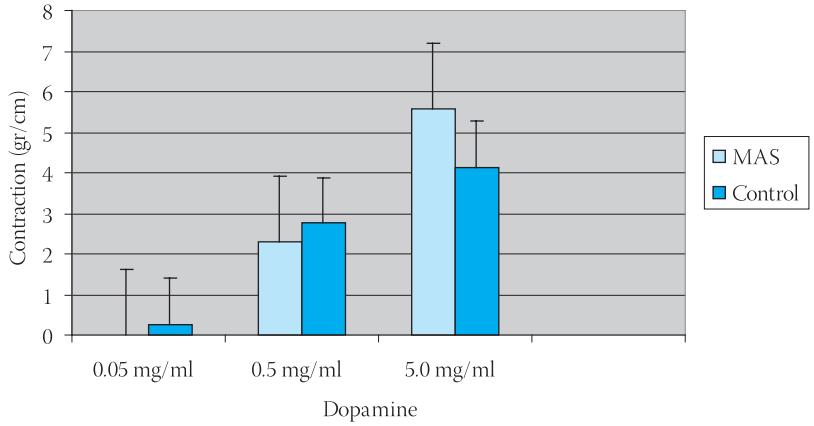


FIGURE 1. Cumulative action of dopamine in TSM at newborn with different maturity ages at MAS (X± SEM).

Serotonin	Log-4	Log-3	Log-2	Log-1
MAS	0,2500±0,2500	0,5000±0,5000	0,7500±0,7500	1,625±1,068
Control	0,3700±0,3750	0,2500±0,2500	-0,6250±1,603	3,375±1,614

TABLE 2. TSM response to serotonin at newborn with MAS (X+SEM) n=16

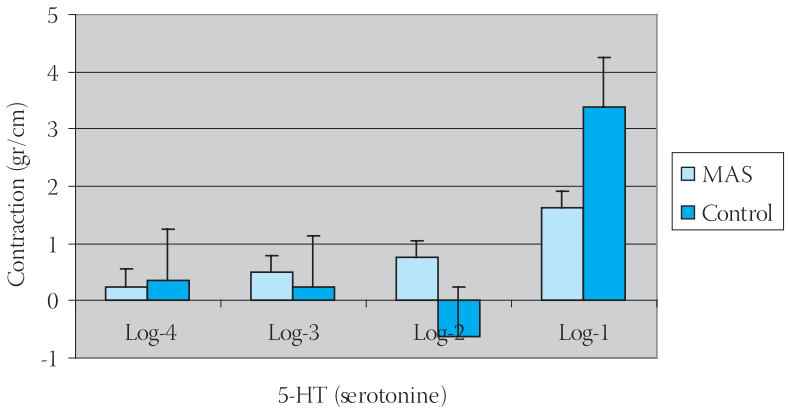


FIGURE 2. Cumulative action of serotonin in TSM at newborn with different maturity ages at MAS (X± SEM).

In Table 2. and Figure 2., serotonin's cumulative response in smooth musculature of newborn with different ages of maturity at the syndrome of aspiration of amniotic fluid and the check up group is presented ($p<0,05$). In Table 3. and Figure 3., ethanol's cumulative response in smooth musculature of newborn with different ages of maturity at the syndrome of aspiration of amniotic fluid and the check up group is presented ($p>0,1$).

In Figure 4, comparative difference of dopamine, serotonin and ethanol action in smooth musculature of newborn with different ages of maturity at the syndrome of aspiration of amniotic fluid and the check up group is presented ($p>0,1$).

DISCUSSION

Further studies of kids that had MAS syndrome in the neonatal period has shown abnormalities of lung's functional tests, airways reduced obstruction, episodes of bronchospasm, and also need for administration of bronchodilatators. Progressive pulmonary inflammation can also damage airways reactibility and administration of bronchodilatators along with anti-inflam-

Ethanol/cm³	0,02 cm³ (96%)	0,5 cm³ (96%)	1,0 cm³ (96%)
MAS	-3,625±3,156	-3,625±4,330	-9,125±5,580
Control	0,3750±1,322	3,500±3,157	11,125±5,878

TABLE 3. TSM response to ethanol at newborn with MAS (X+SEM) n=16

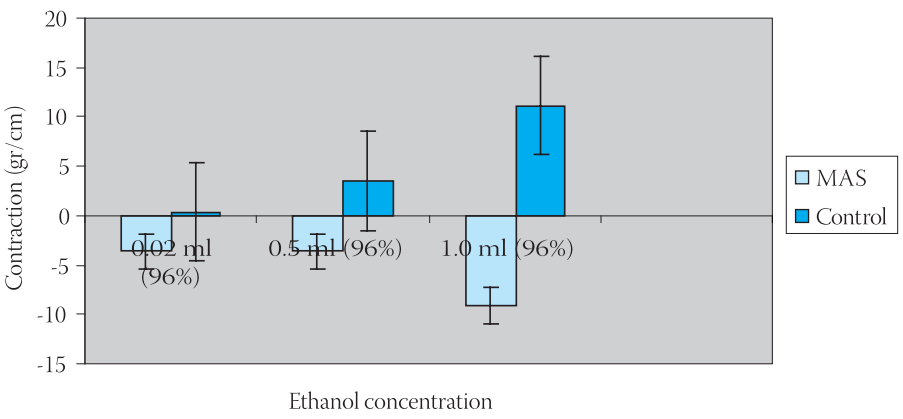


FIGURE 3. Cumulative action of ethanol in TSM at newborn with different maturity ages at MAS (X±SEM)

Groups	MAS	Control
Dopamine	2,650±1,613	2,375±1,134
Serotonin	0,781±0,299	0,843±0,872
Ethanol	-5,458±1,833	5,0±3,193

TABLE 4. TSM response to dopamine, serotonin and ethanol at newborn with MAS (X+SEM) n=16

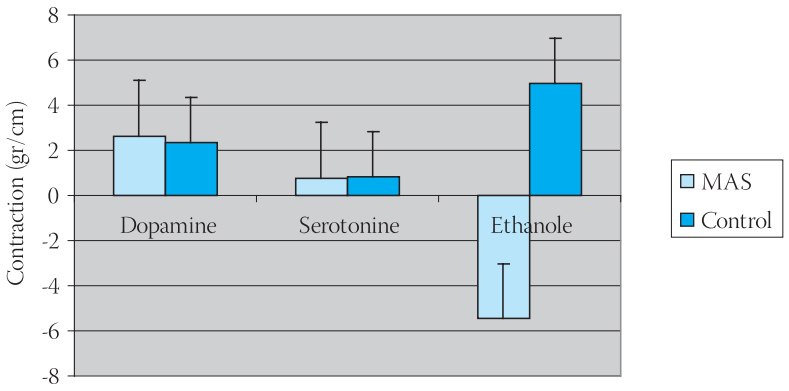


FIGURE 4. Comparative difference of dopamine, serotonin and ethanol action in smooth musculature of newborn with different ages of maturity at the syndrome of aspiration of meconium fluid (X± SEM)

matory medicines can be useful at MAS syndrome. Mechanisms that do contribute in increase of the airways reactibility at MAS syndrome are quite unclear. Meconium is a composition with biologically active substances with powerful contractile effect on smooth vascular and air musculature; such are leukotriene, PAF, ET-1 etc. Meconium contains high concentrations of fatty acids (18) and biliar acids (19), which can induce the contraction of airways smooth musculature. Assumption is that constriction of the airways smooth musculature depends from the concentration of aspired meconium (20). Constriction of the airways smooth musculature can increase in ratio to concentration of the meconium and exposure time towards meconium. In current studies, contractile responses of tracheal tissue rings increase gradually along with the increase of cumulative doses of dopamine, serotonin and ethanol. Relaxation *in vitro* of STM at mice previously was demonstrated by Collins et al. (21), but relaxing response increase along with the increase of the meconium's concentration. Reduced presence of the meconium's concentration in the amniotic fluid can represent a sign of physiologic maturity at newborn, assuming that this do not represent an inflammatory response in tissues. On the other side, high meconium's concentration can cause harmful changes that result with inflammation, and interconnect with the constriction of the airways and vascularity smooth musculature. Aforementioned results suggests that constriction of the airways smooth musculature is well bound with associating mechanisms of MAS such are hypoxia and production of cytokines as well as reactive products during the inflammation. Same response of the effect *in vitro* of human neutrofiles meconium at mice was also observed by (22). In current studies, response of tracheal smooth musculature against dopamine, serotonin and ethanol has shown a partial increase tendency depending from the increase of the doses of these mediators. Different responses may be related by the extension of the exposure of the meconium. Short time exposure *in vitro* may represent vaso and bronchodilatator effects, whilst long time exposure may have mainly constrictor effects in the smooth musculature that depends from the incubating medium time. Tracheal reactibility of the airways against cumulative doses of dopamine, serotonin and ethanol had increasing tendency. Reactivity mechanisms at damage of the airways in MAS syndrome are unclear and further experiments evaluates contractile response of airways in smooth musculature against meconium. Dopamine's action on bronchial musculature can be intermediated with indirect ways, through adrenergic-dopaminergic receptors, cholinergic transmission, and intracellular inhibition of the AMP creation (23). Stimulation of alpha-adrenergic receptors and dopaminergic receptors, as well as their role in the modification of the bronchomotor tonus remains to be clarified in the future. Dopamine, serotonin and ethanol do not cause significant response ($p>0,05$) of the tracheal smooth musculature. This happens because respective receptor system at newborn is not developed to the proper level in order to react in a signifi-

cant manner against abovementioned substances. Hypothesis that 5-HT causes release of the acetylcholine in vivo and *in vitro* depends directly from terminal postganglionar nerves. Activation of the receptors to 5-HT cause depolarization terminal cholinergic nerves of airways through the 5-HT3 receptor (one ionic channel related with ligande) as reported in human's airways and at guinea pig, stands as a possibility (24). By using a combination of pharmacologic and immunohistochemical methods, it was concluded that 5-HT causes release of Ach from the epithelium and from the nerves. Our results suggest that acetylcholine derived from the epithelium might be a final mediator of anaphylactic bronchoconstriction in mice and might play a role in the changed response of the airways in experimental models of the airways diseases study.

Contractile cholinergic response of 5-HT in the mice isolated trachea depends on non neural release of the acetylcholine that looks more like respiratory epithelium. 5-HT does not act directly on the localized receptors in SM, but it induces the release of Ach that causes the constriction. There is a chance that 5-HT can depolarize nerve endings through the action on ionic post-fastener channels of receptors to 5-HT3, which are found in other species. Ethanol's contractile effect on TSM can be caused regardless the age. Ethanol causes constriction of the TSM in newborn animals, developing animals and in those already fully developed. Constriction of TSM in different animals has the same mechanism; during the constriction of TSM, quantity of intra and extracellular calcium is important but it does not depend upon the age maturity (25). By the analyses of our results, we have concluded a developed receptor system for ethanol in TSM, which manifests with constrictor or relaxing effect, TSM response does not cause significant constriction in different weeks of gestation ($p>0,05$). Ethanol, in the airways structures causes the activation of the receptors family in a direct or indirect way and it cause increase of the IP3, DAG, Ca^{2+} concentrations and it can increase the range of generating of the tonus and prolonging of the concentration. Abnormalities in signalling of the airways are manifested with proliferation, desquamation, and inflammation of the airways at Respiratory Distress Syndrome at newborn that have low activity of the receptors for 5-HT and dopamine (26). In order to understand better abovementioned mechanisms, incubation for a certain time *in vitro* of tracheal rings and pulmonary blood vessels in different concentrations of the meconium would have been as necessary for further researches regarding clarification of exact role of the meconium in MAS.

Conclusion

- Dopamine and 5-HT have caused response of tracheal smooth musculature but that response is not significant one ($p>0,05$), which means that receptors for these mediators are not yet fully developed.
- Meconium in MAS syndrome does not potentiate the constrictor action of the dopamine in TSM in a significant manner ($p>0,1$).
- Meconium in MAS syndrome does not potentiate the constrictor action of the 5-HT in a significant manner ($p>0,1$).
- At newborn, in different weeks of gestation, in TSM receptor system for ethanol is not fully developed.
- In matured cases, low concentrations of ethanol cause constrictor action, whereas high concentrations cause relaxing effect.
- At immature newborn, ethanol causes relaxing effect regardless concentration.
- Meconium at MAS syndrome does not potentiate the constrictor action of the ethanol in a significant manner ($p>0,1$).

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