EXPERIMENTAL STUDY

Direct *in vitro* effects of meconium on airway reactivity in adult rabbits

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Abstract

Introduction: **Meconium aspiration syndrome (MAS) is a serious neonatal disease with multifactor pathogenesis and long-term sequelae on the developing respiratory system. Since the altered airway reactivity may play a role in MAS, this study investigated whether 1 hour-lasting in vitro incubation of rabbit tracheal and lung tissue strips with meconium would also increase the airway reactivity.**
Methods: **Trachea and lungs of adult healthy rabbits were excised. Smooth muscle reactivity was estimated by an in vitro method using organ chambers. After the adaptation of tissue strips, 1 ml of suspended meconium (Meconium) was added into two chambers with one tracheal and one lung strip for another 1 hour. The two other chambers were incubated only with Krebs-Henseleit's buffer (Control). Cumulative doses of histamine (10^{-8}–10^{-3} mol/l) were added after finishing the incubation period and a continuous recording of contractions was made.**
Results: **The contractile response of the lung tissue smooth muscle to histamine was recorded to decrease non-significantly in Meconium group when compared to Control group. The tracheal tissue reactivity to histamine at concentrations of 10^{-8}–10^{-4} mol/l was lower in Meconium group than in Control group. Significant differences appeared only at concentrations of 10^{-8}, 10^{-7}, and 10^{-6} mol/l ($p=0.041$, $p=0.033$, and $p=0.019$, respectively).**
Conclusions: **One-hour-lasting in vitro incubation of tracheal and lung tissue strips with meconium significantly decreased the tracheal reactivity to histamine and slightly, but non-significantly decreased the lung tissue reactivity to histamine *(Fig. 1, Ref. 6)*.**
Key words: meconium, airway reactivity, in vitro, rabbit, meconium aspiration syndrome.

Meconium aspiration syndrome (MAS) is a serious neonatal disease with multifactor pathogenesis and long-term sequelae on the developing respiratory system. Airway obstruction, surfactant inactivation, pulmonary inflammation and vasoconstriction are the most significant mechanisms participating in MAS. However, pro-inflammatory substances in meconium (1) are generated during inflammation (2), which may influence the airway reactivity. As recently shown, airway responsiveness to methacholine (3) and histamine (4) significantly increased in experimental animals after meconium instillation. To investigate the mechanisms of airway hyper-responsiveness in MAS, rabbit tracheal and lung tissue strips were incubated with meconium *in vitro*.

Methods

Meconium was collected from 20 healthy term neonates, lyophilized and stored at 20 °C. Before use, meconium was suspended at a concentration of 25 mg/ml in Krebs-Henseleit's buffer of the following composition: NaCl 110.00 mmol/l, KCl 4.80 mmol/l, $CaCl_2$ 2.35 mmol/l, $MgSO_4$ 1.20 mmol/l, $KHPO_4$ 1.20 mmol/l, $NaHCO_3$ 25.00 mmol/l and glucose 10.00 mmol/l in glass-distilled water.

The study design was approved by the Local Ethics Committee of Jessenius Faculty of Medicine. Seven adult healthy rab-

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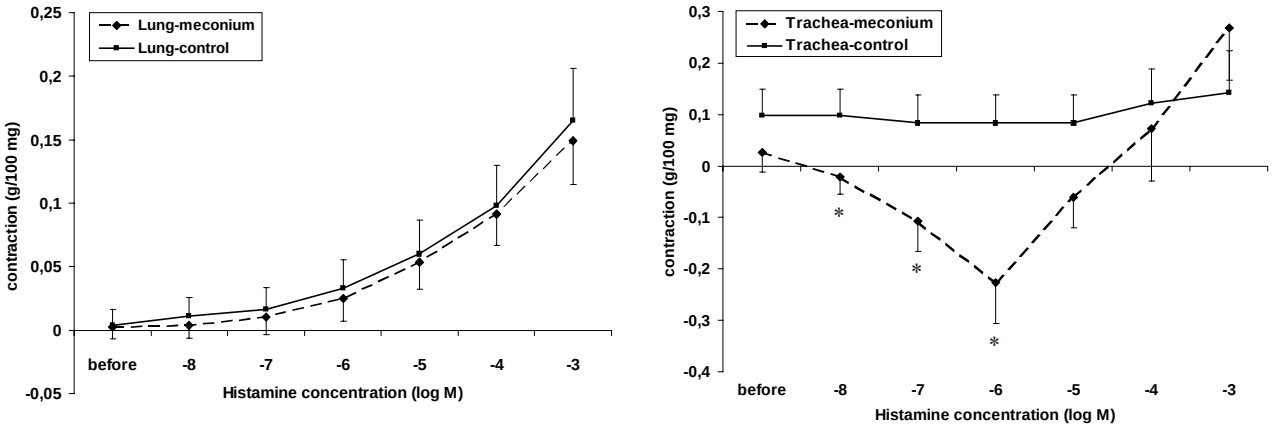

Fig. 1. Contractile responses of lung and tracheal smooth muscle after cumulative doses of histamine. Between-group comparisons: * p<0.05.

bits (Chinchilla) with mean body weight (b.w.) of 2.5±0.5 kg were killed by an overdose of ketamine (Narkamon, Spofa, Czech Republic) and trachea and lungs were excised.

Tracheal and lung smooth muscle reactivity was estimated by *in vitro* method (5). Tracheal smooth muscle strips (two tracheal rings cut on the opposite side of the smooth muscle) and lung tissue strips (2x2x15 mm) from the right lungs were mounted between two hooks and placed into 30-ml organ chambers containing Krebs-Henseleit's buffer. The chambers were maintained at 36.5±0.5 °C and aerated continuously with a mixture of 95 % O_2 and 5 % CO_2 to maintain pH 7.5±0.1. One of the hooks was connected to a force transducer (TSR 10G, Vyvoj, Slovakia) and an amplifier (M1101 SUPR, Mikrotechna, Czech Republic) and tension recordings were made on a Line Recorder TZ 4620 (Laboratorni pristroje, Czech Republic). The tissue strips were initially set to 4.0 gm of tension for 30 minutes (loading phase). Then, the tension in each strip was readjusted to the baseline of 2.0 gm for another 30 minutes (adaptation phase). During both periods, the tissue strips were washed at 10-minute intervals. Thereafter, 1 ml of suspended meconium (Meconium) was added into two of the chambers with one tracheal and one lung strip for 1 hour. The two other chambers were incubated only with Krebs-Henseleit's buffer (Control). During this period, one replacement of fresh Krebs-Henseleit's buffer was made with consecutive adding of meconium to the same strips. The cumulative doses (10^{-8} to 10^{-3} mol/l) of histamine (substance Sigma-Aldrich, Germany) were added after finishing the incubation period and a continuous recording of contractions was made.

Statistics: A Student's t-test was used for the statistical analysis. Data are presented in gram units recalculated to 100 mg of tissue (g/100 mg) as mean±SEM. $p<0.05$ was considered statistically significant.

Results

The cumulative doses of histamine led to a progressive increase in contractile responses of lung tissue strips in both groups of animals. Slight trend to lower contractile responses was recorded in Meconium group when compared to Control group, however, the between-group differences were not significant (Fig. 1). Tracheal tissue reactivity to histamine at concentrations of 10^{-8}–10^{-4} mol/l in Meconium group was lower than in Control group, with significant differences only at concentrations of 10^{-8}, 10^{-7}, and 10^{-6} mol/l ($p=0.041$, $p=0.033$, and $p=0.019$, respectively). At a histamine concentration of 10^{-3} mol/l, tracheal smooth muscle reactivity was non-significantly higher in Meconium group when compared to Control group ($p=0.207$) (Fig. 1).

Discussion

Complicated pathogenesis of MAS is still not completely understood. Since there is some evidence that airway reactivity is altered in MAS (3, 4), this study investigated the reactivity of rabbit lung and tracheal smooth muscles, when incubated with meconium for 1 hour *in vitro*. Despite our supposition, it was observed that when compared to the control group the tracheal reactivity in meconium group significantly decreased at low histamine concentrations and the lung tissue reactivity decreased slightly, but non-significantly at all histamine concentrations. Similarly, Collins et al previously demonstrated predominant *in vitro* relaxation of the rat tracheal smooth muscle being dependent on meconium concentration (6). Since the response to acetylcholine was not affected by pretreating the tracheal segments with indomethacine, removing the epithelium, using KCl, or by heating meconium above 60 °C for 1 hour, the authors supposed that the relaxation was caused by a mechanism that was not mediated by cyclooxygenase products, epithelium, or protein (6).

As opposed to the above-mentioned results, in our recent study both tracheal and lung smooth muscles *in vitro* reactivity to histamine in rabbits significantly increased within 5.5 hours of artificial ventilation after the meconium aspiration, when compared to saline controls (4). In the study by Khan et al, it was found that the *in vivo* airway responsiveness to methacholine in meconium-instilled mice increased on the 7th day (3). The discrepancy may result from the different methods and conditions influencing the tissue strips. Since in our recent study (4) and in

the study by Khan et al (3) the animals were exposed to the complex influence of all factors participating in MAS for a time period necessary for the development of inflammatory and biochemical changes, in these *in vitro* experiments excised trachea and lungs were exposed to meconium without the influence of other pathogenetic factors.

We conclude that the *in vitro* incubation of tracheal and lung tissue strips with meconium decreased the airway reactivity. Hence, the recently demonstrated increased airway reactivity in meconium-instilled animals could particularly result from the complex influence of the factors contributing to MAS, including the action of meconium, but not from the meconium effects alone. However, due to interspecies differences, the application of these results in neonatal MAS is limited and further experiments are needed to elucidate the role of altered airway reactivity in MAS.

References

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