

Meconium Enhances the Growth of Perinatal Bacterial Pathogens

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Abstract

Objective: To demonstrate the effects of meconium on growth of bacterial pathogens, which are common causes of intra-amniotic infection and neonatal sepsis.

Methods: Meconium collected from 9 healthy neonates was suspended as a 20% solution using sterile saline. In experiment 1, separate test tubes of meconium solution and sterile saline (the control) were individually inoculated with 10^6 colony-forming units of a single species of the following test pathogens: *Escherichia coli*, *Enterococcus faecalis*, group B *Streptococcus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes*. After incubation at 37°C for 24 hours, 1 μ L each of the bacterial-meconium and bacterial-saline solutions was inoculated onto 5% sheep blood agar. After 24 hours of incubation, the number of developing colonies was counted. In experiment 2, equal volumes of meconium and saline solutions were inoculated with 10^5 colony-forming units of either *E. coli* or group B *Streptococcus*. At intervals of 6, 9, and 24 hours post-incubation, 1 μ L each of the bacterial-meconium and bacterial-saline solutions was inoculated onto 5% sheep blood agar plates, and colonies were counted after overnight incubation.

Results: In the first experiment, 24 hours of incubation resulted in bacterial amplification in the meconium solution from an initial inoculum of 10^6 colony-forming units/mL to 10^9 colony-forming units/mL. In contrast, the same inoculation of saline solution (control) showed no increase in colony counts over the same time interval. For *E. coli* and group B *Streptococcus* in experiment 2, growth enhancement in meconium was seen as early as 6 hours, as colony counts of a test species increased from 10^5 colony-forming units/mL to 10^9 – 10^{10} colony-forming units/mL.

Conclusion: Enhanced growth of perinatal pathogens in meconium was constantly observed, and can occur as early as 6 hours after bacterial interaction of meconium.

Key Words: Meconium, chorioamnionitis, intra-amniotic infection, bacterial pathogens, neonatal sepsis, amnioinfusion, *Escherichia coli*, group B *Streptococcus*.

Background

MECONIUM HAS BEEN ASSOCIATED with an increased incidence of intra-amniotic infection because it alters the bacteriostatic properties of amniotic fluid and inhibits host immune de-

fenses. Several investigators working with *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and group B *Streptococcus* (GBS) have shown that the addition of meconium to amniotic fluid enhances the growth of these bacterial species (1, 2). In this study, our goal was to utilize a broad range of common obstetric and neonatal pathogens associated with chorioamnionitis and neonatal sepsis to address the following questions: (a) Does meconium enhance bacterial growth under otherwise sterile conditions? And if so, (b) Over what time period does enhanced growth take place?

Methods

Experiments 1 and 2

Fresh meconium was collected within 18 hours of delivery from singleton, term, healthy

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infants of women whose pregnancies were uncomplicated. Meconium was taken from a diaper and placed into a sterile container. Immediately after collection, a solution of meconium (weight/volume) was prepared in sterile saline lacking bacteriostatic additives. The meconium was brought into solution by mechanical agitation. This solution visually corresponded to the clinically defined “thick meconium” or “pea soup” appearance. The concentration was such that when a bacterial solution was added to the meconium, the final concentration was 20%. Sterility of the meconium solution was assured by plating 1 mL of this freshly prepared meconium solution onto 5% sheep blood agar (SBA) prior to bacterial addition and observing for growth after 24-hour incubation. This was done concurrently with all trials in both experiments. The bacterial species tested in this study were isolated from clinical specimens (blood, urine) submitted to the microbiology laboratory at The Mount Sinai Hospital. Stock solutions for each bacterial species were prepared by emulsifying several colonies of a given bacterium obtained from a 24-hour culture grown on SBA plates. Bacterial solutions were prepared by addition of the following bacteria to sterile saline without bacteriostatic additives: *E. coli*, *Enterococcus faecalis*, GBS, *S. aureus*, *Pseudomonas aeruginosa*, and *L. monocytogenes*. Stock bacterial solutions were made to the concentration of 10^9 colony-forming units per milliliter (10^9 cfu/mL). This was accomplished by visually adjusting the optical density of each stock bacterial solution through use of a McFarland #3 density standard (3) to equal 10^9 cfu/mL. All incubations of SBA plates occurred for 24 hours post-inoculation at 37°C in ambient air; solutions were incubated under the same conditions but at variable times, depending on the specified protocol.

Experiment 1

One μ L of a test bacterial solution was individually transferred to 1.0 mL of sterile saline or to 1.0 mL of 20% meconium solution. After gently mixing the contents, 1 μ L of each solution was plated onto SBA at time zero (T_0); the test and control mixtures were then incubated for 24 hours, and colonies counted. The remaining meconium and sterile saline solutions were incubated for 24 hours at 37°C in ambient air, after which 1 μ L of each solution was plated to SBA at time 24 hours (T_{24}) and incubated for 24 hours; then colonies were counted. This proto-

col was repeated 5 times with meconium obtained from 5 different neonates. In each trial, stock bacterial solutions were freshly created so as to deliver approximately 10^6 cfu of the test pathogen to the meconium and sterile saline solution. Within each trial, solutions were plated in duplicate.

Experiment 2

This experiment was repeated 4 times in duplicate with meconium obtained from four other neonates. For *E. coli* and GBS, fresh stock solutions were prepared to a concentration of 10^6 cfu/mL. Then, 0.1 mL of a test pathogen was delivered to 0.9 mL sterile saline and similarly to 0.9 mL of 22.2% meconium solution. Immediately thereafter, 1 μ L of each solution was plated onto duplicate SBA (T_0), and the plates were incubated. The remaining meconium and saline solutions were incubated. At 6, 9, and 24 hours post-inoculation, 1 μ L of each solution was plated onto SBA plates and incubated for 24 hours; the resultant colonies were counted.

Statistics

Due to small sample sizes and not assuming a normal distribution, nonparametric testing was used to analyze the data (Mann-Whitney U test). For experiment 2, logarithmic transformation of the mean values was performed, thus allowing analysis by t test.

Results

Statistical analysis was performed to verify that at T_0 , there was no difference in the amount of microorganisms delivered to both saline and meconium solutions in both experiments ($p > 0.1$, data not shown). In experiment 1 (Table 1), for each test microorganism, the colony counts at 24 hours post-inoculation in saline solution fell to 10^2 cfu/mL (with the exception of *Pseudomonas*, which remained at 10^6 cfu/mL). Significant enhancement of growth, however, was seen in the meconium solution group at 24-hours post-inoculation ($p < 0.05$), which resulted in confluent growth of colonies (i.e., the agar surface was covered by a lawn of colonies). Because individual colonies could not be counted, in order to assess the actual cfu number that resulted in confluent growth, 1 μ L from the bacterial-meconium solution was transferred to 1.0 mL of sterile saline, and then 10-fold serial dilutions were performed, plating 0.1 mL onto

TABLE 1

The Growth of Various Pathogens in Saline and Meconium Solution 24 Hours after an Initial Inoculum of $\approx 10^6$ Colony-Forming Units/mL

Pathogen	Saline (Control)	Meconium (Study)
<i>Escherichia coli</i>	10^2	10^9
<i>Enterococcus faecalis</i>	10^2	10^9
Group B <i>Streptococcus</i>	10^2	10^9
<i>Staphylococcus aureus</i>	10^2	10^9
<i>Listeria monocytogenes</i>	10^2	10^9
<i>Pseudomonas aeruginosa</i>	10^6	10^9

The numbers show the amount of bacterial pathogens in colony-forming units/mL.

Each experiment was replicated 5 times. The difference between meconium and saline is statistically significant.

SBA at each dilution. Through this method it was determined that confluent growth of bacteria occurred when the count was 10^9 – 10^{10} cfu/mL. This was performed with only one of the trials, and for statistical analysis, confluence was assumed to be 10^9 cfu/mL in all five trials.

To further quantitate the role of meconium enhancement of bacterial growth, colony counts of *E. coli* and GBS were assessed by sampling the bacterial-saline and bacterial-meconium mixtures at different time intervals (experiment 2, Tables 2 and 3). Subculture of saline inoculated with *E. coli* demonstrated no growth after 6 hours (3 of 4 trials, Table 2). In the one contrary instance, 10^5 cfu/mL of *E. coli* were present at 6, but at 9 and 24 hours *E. coli* were absent. This may have been due to a sampling error. In contrast, co-cultivation of *E. coli* in meconium resulted in growth increase from 10^5 cfu/mL to 10^9 cfu/mL at 6, 9, and 24 hours ($p=0.01$). Similar results were recorded for GBS (Table 3). GBS persisted in the saline control in trial 1 until 9 hours post-inoculation; for trials 2–4, GBS was absent by 6 hours post-inoculation. Meconium inoculated with GBS, however, demonstrated significant growth as early as 6 hours ($p=0.009$).

Conclusions

Despite the antibacterial properties of amniotic fluid, when intra-amniotic infection occurs, it poses a significant risk both for the mother and the neonate. The inhibitory effect of amniotic fluid on bacterial growth may be explained by the presence of trace elements, lysozyme and immunoglobulins, and by the balance between the zinc and phosphate content of the am-

TABLE 2

The Growth of *Escherichia coli* in Saline and Meconium Solution at Varying Time Intervals after an Initial Inoculum of $\approx 10^5$ Colony-Forming Units/mL

Inoculated Solution	6 Hours	9 Hours	24 Hours
Saline 1	0	0	0
Meconium 1	10^9	10^9	2×10^{10}
Saline 2	0	0	0
Meconium 2	3.4×10^9	10^9	10^9
Saline 3	10^5 *	0	0
Meconium 3	10^9	10^9	10^9
Saline 4	0	0	0
Meconium 4	10^9	10^9	10^9

* see text

TABLE 3

The Growth of Group B *Streptococcus* in Saline and Meconium Solution at Varying Time Intervals after an Initial Inoculum of $\approx 10^5$ Colony-Forming Units/mL

Infected Solution	6 Hours*	9 Hours*	24 Hours*
Saline 1	10^5	10^7	0
Meconium 1	10^9	10^9	10^9
Saline 2	0	0	0
Meconium 2	3.4×10^9	10^9	10^9
Saline 3	0	0	0
Meconium 3	10^9	10^9	10^9
Saline 4	0	0	0
Meconium 4	10^9	10^9	10^9

The numbers show the amount of group B *Streptococcus* in colony-forming units/mL.

* = statistically significant numbers

niotic fluid (4–6). Changes in the ideal composition of the amniotic fluid can lead to its loss of antibacterial properties, and can even promote bacterial growth. Meconium-contaminated amniotic fluid is suspected to be an important factor which can lead to such alteration.

Meconium-related enhancement of bacterial infection was first demonstrated in a mouse model (1). Meconium injected with *E. coli* intraperitoneally, in comparison to bacterial-saline or bacterial-amniotic fluid injections, effected a reduction in LD50 (organisms/mouse) by a factor of 7.5–6800. The mechanism causing meconium enhancement of bacterial growth is unclear. In recent years, an increase in intra-amniotic infection in patients with meconium-stained amniotic fluid has been documented in clinical studies (7, 8).

In this *in vitro* study, we demonstrated growth-enhancing effect of meconium for dif-

ferent neonatal pathogens. A similar experimental design by Florman and Teubner (2) demonstrated that meconium at a concentration of 1% enhanced growth for *E. coli*, *S. aureus* and *L. monocytogenes* (2). Our study (a) confirmed their results for those three species but also expanded the spectrum of species tested, and (b) demonstrated that growth enhancement occurred at a higher meconium concentration (20% versus 1%). In experiment 1, all five trials demonstrated that meconium enhanced bacterial growth in comparison to saline-inoculated solution (controls). Experiment 2 clearly showed that bacterial growth enhancement in meconium occurred as early as 6 hours after cocultivation. In this setting, bacterial colony counts for the tested species increased from an initial 10^5 cfu/mL to 10^9 – 10^{10} cfu/mL at 6, 9, and 24 hours.

Several related issues were not addressed in this study. The study by Florman and Teubner (2) used amniotic fluid rather than saline as the “solvent” to suspend meconium. Amniotic fluid may vary from infant to infant; our goal was to test the effect of meconium alone on bacterial growth enhancement rather than meconium combined with amniotic fluid. However, the design in our study may more closely reflect the clinical setting where there is rupture of membranes and thick meconium present, or where a

transcervical amnioinfusion is administered to reduce the thickness of the meconium.

With respect to clinical practice, one should be aware of the enhancement of growth of various microorganisms in the presence of meconium. Further research is needed to determine whether antibiotics and/or amnioinfusion are useful strategies to decrease infectious morbidity associated with thick meconium.

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