Acta Biomed. - Vol. 87 - N. 3 - December 2016 | ISSN 0392 - 4203

ACTA BIOMEDICA REPRINT

ATENEI PARMENSIS | FOUNDED 1887

Official Journal of the Society of Medicine and Natural Sciences of Parma

The Acta Biomedica is indexed by Index Medicus / Medline Excerpta Medica (EMBASE), the Elsevier BioBASE

POSITIVE CLINICAL OUTCOMES DERIVED FROM USING A PROPRIETARY MIXTURE OF SELECTED STRAINS DURING PREGNANCY

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Mattioli 1885

Pubblicazione trimestrale - Poste Italiane s.p.a. - Sped. in A.P. - D.L. 353/2003 (conv. in L. 27/02/2004 n. 46) art. 1, comma 1, DCB Parma - Finito di stampare october 2016

Acta Biomed 2016; Vol. 87, N. 3: 1-7

Original article

Positive clinical outcomes derived from using a proprietary mixture of selected strains during pregnancy

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Summary. *Background*: While the use of antibiotic prophylaxis is clearly advantageous to prevent streptococcal infection, it alters the composition of the gut microbiota in mothers and infants. *Enterococcus faecium* L3 is one of the best studied probiotic strains and shows strong antagonistic activity against *Streptococcus agalactiae* due to the production of bacteriocins able to inhibit common gut and vaginal pathogens. *Methods*: We tested an L3-based probiotic formula (iNatal®) on 127 pregnant women attending our gynaecological unit in 2015. We compared the study subjects with 279 pregnant women enrolled in the same year and with 892 other pregnant women who attended our gynaecological unit in 2013 and 2014. *Results*: The findings demonstrate: (a) the safety profile of the product; (b) its ability to reduce gut disorders; (c) a 6% decrease in the incidence of streptococcal colonization; (d) an approximately 30% decline in episodes of premature rupture of membranes; (e) fewer caesarean sections during labour; and (f) a reduction in pathological umbilical cord blood pH. *Conclusions*: Our results demonstrate that a probiotic treatment during pregnancy could have unexpected but favourable clinical results. Further randomized, double-blind, placebo controlled studies are now needed to confirm our preliminary findings. (www.actabiomedica.it)

Key words: *Enterococcus faecium* L3, PROM, *Streptococcus agalactiae*, Intrapartum Antibiotic Prophylaxis, iNatal

Introduction

Streptococcus agalactiae, a gram-positive commensal bacterium present in the gastrointestinal and genitourinary tracts of 10-30% of pregnant women, is the primary source of early-onset bacterial sepsis in newborns, a major cause of neonatal morbidity and mortality (1). The newborn can be exposed to streptococcus in utero, as the organism can reach the amniotic fluid through ruptured membranes and/or the birth canal during delivery. The introduction in many countries of universal screening for the presence of streptococcus in all pregnant women and subsequent intrapartum ampicillin prophylaxis in *S. agalactiae*-positive women has resulted in a significant reduction in early-onset infection, which has fallen from 1.7 to 0.25 cases per 1000 births in developed countries (2). While the use of antibiotic prophylaxis is clearly advantageous for the prevention of streptococcal infection, it alters the composition of the gut microbiota of infants and results in a higher relative abundance of ampicillin-resistant *Enterobacteriaceae* and in a dramatic reduction in bifidobacteria (3). Recently, it has been suggested that mother-only supplementation with probiotics during pregnancy could reduce vaginal carriage of *S. agalactiae* (4). One of the best studied probiotic strains with strong activity against *S. agalactiae* is *Enterococcus faecium* L3. Its antagonistic activity is due to the produc-

tion of two well-known bacteriocins called enterocin A and enterocin B (5). In addition to *S. agalactiae*, this bacteriocin-releasing strain has also shown activity against the *in vitro* growth of *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus pyogenes*, *Klebsiella pneumonia*, *Mycoplasma hominis*, *Candida albicans and Helicobacter pylori* (6). Strain L3 is also pharmacologically active *in vitro* against herpes simplex virus type 1 (HSV-1). In particular, light and immunofluorescence microscopy showed an anti-virus effect on HSV-1 grown on Vero cell cultures in the presence of strain L3 or in the presence of its two bacteriocins (7). Clinically, in mature newborns receiving antibiotic therapy (penicillins, aminoglycosides, cephalosporin), administration of *E. faecium* L3 reduced the incidence and persistence of *Clostridium difficile* infection as well as a number of opportunistic microorganisms in the intestinal microbiota while preserving the growth of bifidobacteria and lactobacilli (8). In premature infants receiving full or partial parenteral nutrition and antimicrobial therapy (carbapenems, fluoroquinolones, glycopeptides), the use of strain L3 resulted in increased body weight, decreased the frequency of infectious complications, promoted conservation of immunomodulatory function and restricted the growth of nosocomial flora (9,10). A recent paper has also shown that the use of *E. faecium* L3 in children in their first year of life reduced the incidence of acute respiratory infections in the winter-spring period by more than 50% and improved the infants' nutritional status resulting in a significantly higher body mass index (11). Finally, a pilot uncontrolled study performed on 112 pregnant women indicated that strain L3 had anti-candida and anti-streptococcal properties. Administered during the last trimester, the strain reduced gynaecological infections (12). In this study, we examined whether the antibiotic-like properties of *E. faecium* L3, clearly reported as *in vitro* results or seen clinically in mature and preterm newborns, would also be demonstrated if administered to women during pregnancy. We have therefore evaluated whether administration of a probiotic mixture containing strain L3 during the last 10 weeks of pregnancy could affect outcomes such as the occurrence of *S. agalactiae* or premature rupture of membranes (PROM).

Materials and Methods

Methods

The trial was conducted at the Gynecology and Obstetrics Department, Arco Hospital, Trento, Italy and in accordance with the Declaration of Helsinki, with the approval of the local ethics committee. Each participant was told of the study procedures and objectives and signed an informed consent form and a form covering personal data handling in accordance with privacy law. The objectives of the study were to examine: (a) the safety and tolerability of a probiotic treatment; (b) the number of rectal-vaginal swabs positive for *S. agalactiae*; (c) episodes of PROM; (d) the rate of caesarean sections during labour; and (e) umbilical cord arterial blood pH.

Inclusion and exclusion criteria

The study enrolled 406 healthy pregnant women attending the department from January to December 2015. Exclusion criteria included: age below 18; the presence of a neurological disorder, heart, lung or kidney disease, or a severe metabolic disorder; a past history of cancer; and refusal to sign the informed consent and/or privacy form.

Group assignment

We performed a non-randomized, controlled, open-label study. The probiotic product, iNatal®, was administered to 127 pregnant women, while 279 served as controls. Subjects were assigned to the treatment or control group as follows. Of the 406 women attending our department during 2015, 166 reported having episodes of constipation, colitis, diarrhoea or bladder infections in the trimester before enrolment or had evidence of vaginosis, vulvo-vaginitis or undiagnosed vulvar itching. These 166 women were offered treatment with the probiotic product. Of these 166 women, 127 accepted and were assigned to the treated group, while the remaining 39 and the other 240 women not reporting any gut or gynaecological disorder in the previous trimester served as controls (untreated).

Treatment

iNatal® was administered daily 2 hours after breakfast from the 30th to the 40th week of pregnancy. Acetaminophen and antibiotics could be taken if required. However, the probiotic treatment was suspended during antibiotic therapy but was then resumed and continued until the scheduled end day. iNatal® is a nutritional supplement notified at the Italian Ministry of Health by Omeopiacenza (Pontenure, Italy), according to the provisions of law No. 169 of 2004, on 4 September 2014 (notification number: 71640) and contains *Enterococcus faecium* L3 LMG P-27496 at the concentration of 5×10^9 colony forming units/dose (CFU/dose), *Bifidobacterium animalis* ssp. *lactis* BB12 DSM 15954 at 3×10^9 CFU/dose, *Lactobacillus casei* RO215 CNCM I-3429 at 3×10^9 CFU/dose and *Lactococcus lactis* ssp. *lactis* SP 38 DSM 26868 at 3×10^9 CFU/dose.

Swab test

A rectal-vaginal swab (Todd Hewitt CNA Regular Flocked Swab Kit; Copan Diagnostics, Murrieta, CA, USA) was taken between the 36th and 37th weeks of pregnancy to check for the presence of *S. agalactiae*.

Statistical analysis

Equivalence between the two groups (treated and control) regarding age, parity, ethnicity, education and type of employment was determined using Fisher's exact test and the two-tailed Wilcoxon-Mann-Whitney test. The difference between the two groups in terms of side effects and clinical outcomes was determined using the two-tailed Wilcoxon-Mann-Whitney test. JMP 10 statistical software for Mac OsX and statistical significance was set at 95%.

Results

A total of 406 pregnant women were enrolled in a non-randomized, controlled, open label clinical study during which 127 pregnant women were administered a proprietary mixture of selected probiotic strains, while 279 served as controls. Despite the large difference in numbers of subjects in the two groups, statistical analysis showed that the groups were comparable as regards age, parity, ethnicity, education and employment (Table 1). The study was carried out to examine whether the administration of iNatal® was safe and well tolerated, and could reduce the number of rectal-vaginal swabs positive for *S. agalactiae*, the occurrence of PROM, the rate of caesarean sections during labour and the presence of pathological umbilical cord arterial blood pH. As shown in Table 2, the product was found to be safe and well tolerated with no particular side effects. It is noteworthy that gut side effects, such as bowel pain, constipation, colitis, meteorism, flatulence and diarrhoea, were less frequent in the treated group than in control subjects. This finding is likely due to the probiotic effect of the tested formula. Rectal-vaginal swabs were positive for the presence of streptococcus in 27 of

Table 1. Characteristics of the 406 women enrolled in the study and attending our unit in 2015

	Treated (127)	Untreated (279)	p Value*
Age			
18-19	0	1	ns
20-24	12	31	ns
25-29	30	70	ns
30-34	37	83	ns
35-39	34	69	ns
40-44	14	25	ns
Children			
1	56	125	ns
2	37	99	ns
3	34	55	ns
Ethnicity			
European	82	186	ns
North African	28	62	ns
Far Eastern	11	20	ns
Other	6	11	ns
Education			
Primary school	1	2	ns
Middle school	34	59	ns
High school	65	139	ns
University	27	79	ns
Employment			
None/housewife	43	97	ns
Yes	84	182	ns

*Non-significant (ns) differences between groups

Table 2. Adverse events (number of global episodes) during 10-week treatment with the proprietary mixture of selected strains and in the control group

	Gastric[1]	Gut[2]	Skin[3]	Headache	Insomnia
Treated (127)	60	11*	1	15	68
Control (279)	136	75	3	39	152

[1]Pain, reflux, nausea, vomiting, spasm; [2]pain, constipation, colitis, meteorism, flatulence, diarrhoea; [3]rash, erythema, dermatitis, acne-like reaction.
*p<0.01 versus control.

the 127 women in the treated group (21.3%) and in 76 of the 279 (27.3%) women in the control group (Table 3). The significant reduction in swab positivity is confirmed when positive findings rates in the treated 2015 group are compared with the higher rates in untreated women from two previous years (2014 and 2013). No episodes of PROM (Table 4) were seen in the treated group compared to 87 episodes in the control group (31.2%), which findings were similar to the 156/290 (40%) and 180/502 (35.9%) PROM episodes in the previous two years. In 2015, seven (5.51%) and 29 (10.39%) caesarean sections were performed during labour in the treated and control groups, respectively, because of concerning cardiotocography (CTG) findings (Table 5). According to the Robson classification (13) the seven caesarean sections of the treated group were ranked as Group 1 as well as 25 out of 29 of the control one. The remaining four of the control group were ranked as Group 3 (data not shown). The number of caesarean sections in the untreated group is very similar to the 43/390 cases (11.02%) in 2014 and 57/502 cases (11.35%) in 2013. Finally, we considered pathological the umbilical cord arterial blood pH when lower than 7.2. As shown in Table 6, there were no cases of blood pH <7.2 in the treated group but there were 17 cases out of 279 (6.09%) in the control group. Values in the two previous years were comparable to those in the control group, with 20/390 (5.12%) in 2014 and 29/502 (5.79%) in 2013.

Table 3. Number of women attending our gynaecological department in 2013-2015 with rectal-vaginal swabs positive for *Streptococcus agalactiae*

	Treated 2015	Untreated 2015	All 2014	All 2013
Positive/total	27/127*	76/279	125/390	138/502
%	21.3	27.3	32.05	27.5

*p<0.05 versus Untreated 2015, All 2014 and All 2013.

Table 4. Episodes of PROM° in all women attending our gynaecological department, 2013-2015

	Treated 2015	Untreated 2015	All 2014	All 2013
PROM/total	0/127*	87/279	156/390	180/502
%	0.0	31.2	40	35.9

*p<0.001 versus Untreated 2015, All 2014 and All 2013.
°PROM: premature rupture of membranes.

Table 5. Caesarean sections during labour in all women attending our gynaecological department, 2013-2015

	Treated 2015	Untreated 2015	All 2014	All 2013
Caesarean/total	7/127*	29/279	43/390	59/502
%	5.51	10.39	11.02	11.75

*p<0.05 versus Untreated 2015, All 2014 and All 2013.

Table 6. Umbilical cord arterial blood pH <7.2 in all women attending our gynaecological department in 2013–2015

	Treated 2015	Untreated 2015	All 2014	All 2013
<7.2/total	0/127*	17/279	20/390	29/502
%	0.0	6.09	5.12	5.79*

p<0.01 versus Untreated 2015, All 2014 and All 2013.

Discussion

The use of intrapartum antibiotics likely affects the biodiversity of neonatal microbiota and is associated with mother and infant gut microbiota dysbiosis (14). Alteration of gut microbiota in infants caused by caesarean section can have later negative consequences such as chronic immune disease, allergic rhinitis, atopy, asthma, overweight and obesity (15-18). Since intrapartum antibiotics are often administered because of rectal and/or vaginal colonization by *S. agalactiae*, we have tested the role of *E. faecium* L3, a probiotic select-

ed strain known to counteract streptococcal *in vitro* growth, in reducing the clinical occurrence of *S. agalactiae* as evaluated by rectal-vaginal swab. No pharma- or nutritional supplement-grade products containing just *E. faecium* L3 are available in Italy as the strain is only offered in a probiotic product also containing *Bifidobacterium animalis* ssp. *lactis* BB12, *Lactobacillus casei* RO215 and *Lactococcus lactis* ssp. *lactis* SP 38. Strain BB12 has been intensively investigated. It has immune-modulating properties (19), is capable of potentiating a specific IgG response to vaccination (20) and can reduce the incidence of upper respiratory tract infections (21). *L. casei* is a probiotic described as having anti-inflammatory properties likely mediated by IL-10 (22) and useful for potentiating the role of mesalazine in diverticular disease (23). In particular, strain RO215 (data not shown) is a strong producer (29.8 mg/L) of L-tryptophan, which is converted to melatonin in the gastrointestinal tract during the day when pineal gland synthesis is inhibited (24). Clinically, gastrointestinal melatonin has potential for the prevention or treatment of ulcerative colitis, irritable bowel syndrome, childhood colic diarrhoea and necrotizing enterocolitis in premature infants (25,26). *L. lactis* ssp. *lactis*, previously known as *Streptococcus lactis* (27), has lactose-fermenting ability and proteinase activity likely mediated through two distinct plasmids (28). On the basis of these findings, the L3-based mixture of strains likely confers benefits on both pregnant women and their infants. Probiotic intervention during pregnancy is an opportunity to promote the health of both the mother and the child (29). Probiotic strains administered to pregnant woman can be found in the infant gut and temporary colonization of an infant may be possible by colonizing the pregnant mother before delivery. Colonization is stable for as long as 6 months, and in unexplained circumstances may persist for up to 24 months (30). Administration of specific probiotics to pregnant women positive for the skin prick test was found to be a safe and effective method for reducing the risk of eczema in infants evaluated at 6, 12 and 24 months (31). Our study has shown that administration of iNatal® is safe and well tolerated. As expected, the mixture reduces the gut discomfort typically affecting pregnant women, and therefore acts as a eubiotic. The mixture also decreases swab positivity for *S. agalactiae*, thereby reducing the use of intrapartum antibiotics. We expected a greater decrease, but some recent findings could explain why only a 6% reduction was observed (32,33). In fact, the very high colonization index (100% in healthy female volunteers; data not shown) of the enterococcus strain used in the product may be partially responsible for the minimal result being the enterococcus strain confused with *S. agalactiae*. However, following iNatal® treatment, only 21.3% of women in the treated group required intrapartum antibiotics compared to 27.3% in the control group. Episodes of PROM were also reduced in the treated group. PROM and preterm premature rupture of membranes (pPROM) are associated with an elevated risk of intra-amniotic infection which increases perinatal morbidity and mortality. Nevertheless, PROM is only very rarely life-threatening and it might be caused by gynaecological dysbiotic conditions (34). We believe that the reduction in the number of PROM episodes is mainly due to the antimicrobial effects of the L3 strain which is a strong producer of bacteriocins, although the immuno-modulating properties of BB12, or some other beneficial and still unknown actions of the other strains, cannot be excluded. Finally, we do not know why use of the probiotic product reduced the number of unplanned caesarean sections during labour or why the treatment reduced the incidence of umbilical cord blood pH <7.2. However, we suggest that the product improved the general condition of the mother and fetus. Our work has of course a number of limitations. First, the two groups are very different in terms of numbers and subjects are not randomized. This difference, and the lack of randomization, are anyway due to the established criteria for enrolling the women to be treated. As clearly shown in Table 1, in spite of this difference the two groups are then absolutely comparable according to their main features. Second, our study is not double-blind and placebo controlled. In spite of these two last important bias, our study shows an important number of controls. The outcomes measured have been in fact compared with 1171 untreated pregnant women attending our operative unit from 2013 to 2015. Nevertheless, our results prompt us to perform a new clinical trial with randomization, blinding and the presence of a probiotic treatment for the control group.

Conflict of interest

FDP is a member of the scientific council of a company selling iNatal®. The other authors have no conflicts of interest.

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Received: 19 July 2016
Accepted: 25 August 2016
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