Preventive effects of oral probiotic on infantile colic: a prospective, randomised, blinded, controlled trial using Lactobacillus reuteri DSM 17938

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RESEARCH ARTICLE

Abstract

Infants were recruited in four centres in North-West Italy. 138 infants were assessed for eligibility, 113 ones underwent randomisation and 105 completed the study. Newborns aged less than 10 days of life, with gestational age between 37 and 42 weeks, birth weight from 2,500 to 4,300 g and normal physical examination were recruitable. Premature infants and infants affected by outcomes of perinatal hypoxia or necrotising enterocolitis have been excluded. Patients were randomly assigned to receive five drops containing Lactobacillus reuteri DSM 17938 (10⁸ cfu) with 400 UI of vitamin D3 or only 400 UI of vitamin D3 daily. The primary endpoints concern the administration of pain relieving agents (cimetropium bromide at least three times per week or simethicone at least five times per week) from baseline to 12 weeks. Additional analyses were done on the percentage of infants that switched from an exclusive breastfeeding to a partial or exclusive formula feeding from baseline to 12 weeks. Data concerning the number of calls to the paediatricians and the number of visits at paediatricians’ ambulatories due to infantile colic have been collected by paediatrician and analysed. Comparing the two groups, the relative risk was 0.04 (95% confidence interval (CI)=0.01-0.31) for cimetropium bromide, 0.24 (95% CI=0.14-0.41) for simethicone and 0.37 (95% CI=0.17-0.80) for the administration of infant formula, showing a protective action of L. reuteri. The treatment group showed a lower number of paediatric consultations related to episodes of infant colic than the control group (P<0.0001). L. reuteri DSM 17938 supplementation at the tested dosage could reduce parental discomfort due to infantile colic. The consumption of this probiotic is associated with a reduction of paediatric consultations for infantile colic, as well as use of pain relieving agents and of infant formula.

Keywords: infantile colic, probiotic, pain relieving agents, parental discomfort

1. Introduction

Infantile colic is a really common condition worldwide: about 20% of infants develop colic in the first three months of life (Drug and Therapeutics Bulletin, 2013; Savino et al., 2010). Although infantile colic is considered a self-limiting and benign affection, it is also a stressful problem for parents and a frequent and wrongly undervalued cause for paediatric consultation (Iacono et al., 2005). The lack of consensus concerning treatment increases parental discomfort (Kvitvaer et al., 2011). Infantile colic is defined as episodes of inconsolable crying in an otherwise healthy infant younger than three months of age, that last at least three hours a day and occur at least three days per week over the course of at least three weeks in a month, a definition first proposed by Wessel et al. (1954).

Recent studies have shown that colicky infants’ gut microbiota is characterised by a low amount of lactobacilli and an increased amount of coliform bacteria and this issue has been reported as a possible cause of gut dysmotility and increasing of flatulence production (De Weerth et al., 2013; Lehtonen et al., 1994; Pärtty et al., 2012; Savino et al., 2005, 2009). In the last years many scientists have
suggested the usefulness of vitamin D supplementation in newborns, which is routinely administered in early infancy. In animal models of experimental colitis, it has been shown that vitamin D deficit leads to great susceptibility to injury in the gut (Ananthakrishnan, 2013; Chen et al., 2014; Ooi et al., 2013).

Some clinical trials have reported that probiotics (Lactobacillus reuteri) may be useful in reducing crying time in breastfed colicky infants (Savino et al., 2007; 2010; Szajewska et al., 2013). A recent systematic review and meta-analysis by Sung et al. (2013) has found that although probiotics show promise in the treatment of infantile colic, there is still insufficient evidence to support the general use of probiotics for prevention in all infants (Praveen et al., 2014). Indrio et al. (2014) performed a double-blind randomised controlled trial that randomised 589 infants to receive L. reuteri versus placebo showing the usefulness of probiotic in reducing crying due to infantile colic. However, recently Sung et al. (2014) reported no effects of probiotics on infantile colic in a heterogeneous population of selected infants.

The objective of this study was to evaluate the efficacy of orally administered L. reuteri DSM 17938 with vitamin D3 from the age of ten days in reducing parental discomfort due to infantile colic in a population of otherwise healthy infants.

3. Materials and methods

This was a single blinded, prospective randomised-controlled, parallel-group study conducted in North-West Italy. Parent and clinicians, but not the Trial Outcome Assessor (TOA) knew medication type. The local ethics committee (Comitato Interaziendale AA.SS.OO. O.I.R.M./S. Anna-Ordine Mauriziano di Torino) approved the study before the start, and written informed consent was obtained from parents by paediatrician before inclusion of the infants in the study.

Parents received information about the study during the first paediatric consultation (within 10 days of life). In case of interest, parents were provided detailed information concerning infantile colic, how to recognise it and the available treatment by the paediatrician. However, paediatricians did not explain the potential effect of the probiotic in order not to influence parental observations. In compliance with the CONSORT statement, the number of subjects assessed for eligibility has been reported on a site-specific subject screening list. During the first visit, the investigator clarified whether the infant fit to the inclusion and exclusion criteria.

Objectives

In the current study we tested the hypothesis that an early administration of L. reuteri to newborns could reduce:
1. the use of pain relieving drugs (cimetropium bromide and simethicone) to treat infantile colic during the first 3 months of life;
2. the supply of infant formulas (formulas containing probiotics were not allowed);
3. the number of calls to the paediatrician due to infantile colic;
4. the number of visits at paediatrician’s ambulatory due to infantile colic.

Eligibility criteria for participants

Newborns aged less than 10 days of life, with gestational age between 37 and 42 weeks, birth weight from 2,500 to 4,300 g, and normal physical examination were eligible to the study. Premature infants (gestational age <35 weeks) and infants affected by outcomes of perinatal hypoxia or necrotising enterocolitis have been excluded.

Study settings

Infants were recruited from three general paediatricians in Genova (Italy) and outpatients at the Department of Paediatrics, University of Turin (Italy) (Regina Margherita Children Hospital) between January 2012 and November 2013.

Intervention

Patients were randomly assigned to receive five drops containing L. reuteri DSM 17938 (10<sup>8</sup> cfu) with 400 UI of vitamin D3 or only 400 UI of vitamin D3 daily (vitamin D is routinely administered to all newborns). The treatment group received a suspension of freeze-dried L. reuteri DSM 17938 in a mixture of sunflower oil and medium-chain triglyceride oil supplied with added vitamin D3, in a 5 ml dark bottle fitted with a dropper cap. The control product was a suspension of olive oil with colecaciferol (vitamin D) 2.5 mg (100,000 UI/ml), in a 10 ml dark bottle with a dropper cap. The two liquids looked and smelled similar.

Outcomes

The primary endpoints concern the administration of pain relieving agent (cimetropium bromide at least three times per week or simethicone at least five times per week) from baseline to 12 weeks. Cimetropium bromide is an antimuscarinic compound, anticholinergic drug (Scarpignato et al., 1985), which is well tolerated in infants when administered at the tested dosage and efficacious for crying episode (Savino et al., 2002, 2012). It is commonly used in Italy in the treatment of severe infantile colic (Savino et al., 2014).
Simethicone is a defoaming agent able to reduce surface tension of bubbles in the intestinal tract and promoting a more easy expelletion of abdominal gas; it has only been shown to be able to reduce meteorism, and so it is commonly considered as a placebo (Metcalfe et al., 1994; Perry et al., 2011; Sethi and Sethi, 1988). Nevertheless, in Italy it is usually employed as a first approach to infantile colic (Savino et al., 2014). These pain-relieving agents have been administrated after medical prescription.

Additional analyses were done on the percentage of infants that switched from an exclusive breastfeeding to a partial or exclusive formula feeding from baseline to 12 weeks. Data concerning the number of calls to the paediatricians and the number of visits at paediatricians’ ambulatories due to infantile colic have been collected by paediatrician and analysed in order to evaluate if there were significant differences between the two groups as an indirect indicator of parental discomfort.

In order to decrease anxiety of parents and to avoid bias during the study period, the paediatricians discussed infant’s colic with the caregiver: they underlined that infantile colic is not a serious disease and that it is a self-limiting and benign condition. In addition, physicians explained how to recognise infantile colic episodes according to Wessel’s criteria in order to avoid over-reporting and over-treatment.

**Study outcome measures**

The use of pain relieving agents (simethicone, cimetropium bromide) were reported each day by parents during the intervention period into the ‘diary’ and were assessed and reported in the table data by a study team member. The paediatrician noted the number of phone calls and visits due to infantile colic.

**Sample size**

We estimated that with 36 infants per group, we would be able to detect a prevalence of 20% in the treatment group and 40% in the control one (Savino et al., 1998) with 80% power (α=0.05) for the use of the cimetropium bromide. We have enrolled 113 infants (55 in the treatment group and 58 in the control group) in order to allow for a 20% drop out rate.

**Randomisation**

For allocation of the participants, a computer-generated list of random numbers was used. Randomisation sequence was created using Stata 9.0 (StataCorp, College Station, TX, USA) statistical software and stratified by center with a 1:1 allocation using random block sizes of 2, 4, and 6. Participants were randomly assigned following simple randomisation procedures (computerised random numbers) to control or treatments groups by an external collaborator. Patients and physicians allocated to the intervention group were aware of the allocated arm.

**Statistical methods**

Registered data were analysed on an intention-to-treat basis. The primary endpoint was the reduction of the use of cimetropium bromide or simethicone during infants’ first three months of life. Secondary efficacy endpoint included the use of infant formula after three months. Both outcomes were analysed using relative risks (RR) (associated to 95% confidence intervals (CI)) and Corrected Chi-square (P<0.05, β=0.20). We have used Corrected Chi Square because the sample size is lower than 200 patients. Other secondary outcomes (number of calls to paediatrician and number of paediatric visits) were analysed using t test (P<0.05).

3. Results

There were 138 infants admitted to our observation during the study period. Among these infants, 25 infants were excluded because they did not fit the eligibility criteria (n=10) and lack of parental consent (n=15). A total of 113 infants were enrolled in the study; we did not lose any infant to follow-up. Four infants in the control group and four infants in the treatment group were excluded (reasons reported in Figure 1). A total of 105 infants completed the study protocol. There were 51 infants in the study group and 54 infants in the control group (Figure 1).

Age-eligible participants were recruited from January 2012 to November 2013. Recruited infants attended clinic visits at the time of randomisation (baseline – Table 1) in the first ten days of life and at 3 months of age. The primary analysis was per-protocol and involved all patients who were randomly assigned. Parents of the treatment group infants showed less discomfort (monitored as the number of paediatric consultations) related to episodes of infant colic than control group infants’ ones: the number of calls to the paediatrician (5.04±2.64 for each infant in the treated group versus 8.40±3.58 for each infant in the control group) and the number of visits in the paediatric ambulatory (2.66±1.77 for each infant in the treated group versus 4.98±1.89 for each infant in the control group) are significantly lower (P<0.0001) in the treatment group than in the control one.

The outcomes (Table 2) show that the use of pain relieving agents is significantly lower in the treatment group than in the control one: cimetropium bromide was administrated to 1 infant in the treatment group and to 24 infants in the control group and simethicone was administrated to 11 infants in the treatment group and to 48 infants in the control group. Concerning the use of cimetropium bromide, we have calculated a RR of 0.04 (95% CI=0.01-0.31) and

**Additional analyses**

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Corrected Chi² of 23.806 (P<0.0001) from the obtained data, while for the use of simethicone we calculated a RR of 0.24 (95% CI=0.14-0.41) and a Corrected Chi² of 45.592 (P<0.0001). In the treatment group a significant lower use of infant formula was observed in the first three months: infant formula was given to 7 infants in the treatment group and 138 infants were assessed for eligibility

- 10 infants did not fit the eligibility criteria
- 15 families did not provide consent

113 infants underwent randomization

58 infants were assigned to the control group (A) (vitamin D3 only)

- 0 infants were lost to follow-up after 3 months
- 1 infant excluded because of urinary infection
- 3 infants excluded because of gastroesophageal reflux

54 infants were included in the intention-to-treat analysis

55 infants were assigned to the treatment group (B) (Lactobacillus reuteri + vitamin D3)

- 0 infants were lost to follow-up after 3 months
- 1 infant excluded because of urinary tract infection
- 1 infant excluded because of upper airways infection
- 3 infants excluded because of gastroesophageal reflux

51 infants were included in the intention-to-treat analysis

Figure 1. Flow chart of the study.

Table 1. Baseline data.

<table>
<thead>
<tr>
<th></th>
<th>Lactobacillus reuteri + vitamin D3 group (n=51)</th>
<th>Vitamin D3 group (n=54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nationality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italian</td>
<td>44 (86%)</td>
<td>45 (83%)</td>
</tr>
<tr>
<td>Not Italian</td>
<td>7 (14%)</td>
<td>9 (17%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25 (49%)</td>
<td>26 (48%)</td>
</tr>
<tr>
<td>Female</td>
<td>26 (51%)</td>
<td>28 (52%)</td>
</tr>
<tr>
<td>Feeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exclusively breastfed at recruitment</td>
<td>46 (90%)</td>
<td>48 (89%)</td>
</tr>
</tbody>
</table>

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to 20 infants in the control group, RR=0.37 (95% CI=0.17-0.80) and Corrected Chi$^2$ was 6.291 ($P=0.0121$).

4. Discussion

This is the first randomised controlled study that investigates if orally administered *L. reuteri* DSM 17938 with vitamin D3 in the first three months of life could be useful in order to reduce parental discomfort by reducing the intensity and frequency of infantile colic episodes as perceived by parents. Up to now, the majority of studies conducted with probiotics in breastfed colicky infants were carried out with strains of *Lactobacillus* and have demonstrated that the administration of these probiotics could reduce daily crying time (Praveen et al., 2014; Savino and Tarasco, 2010; Savino et al., 2004, 2007, 2009, 2010; Sung et al., 2013; Szajewska et al., 2013). Suggested mechanisms of action include immunomodulatory, nutritional and metabolic effects, but also action on gut motility has been proposed (Chassard et al., 2014; Wu et al., 2013).

Our study showed that *L. reuteri* DSM 17938 with vitamin D3 daily administered significantly reduced the use of pain relieving agents, such as simethicone and cimetropium bromide (registered by parents through a diary) and the number of phone calls and visits for colic registered by physician, compared to vitamin D3 only. Observed data concerning the use of infant formulas are really interesting: it is well known that infantile colic is one of the main reasons to switch formula (Forsyth et al., 1999; Iacovou et al., 2012; Polack et al., 1999) and we have registered how the administration of *L. reuteri* with vitamin D3 may reduce the use of artificial formulas in exclusively breastfed infants. Therefore, the probability to become a partially breastfed infant is significantly lower in the treated group of infants.

In this study, we have used indirect markers of parental discomfort in order to collect data concerning the perceived intensity of infantile colic in a population of otherwise healthy infants. Colicky infants usually have a great impact on parental stress. Many studies (Akman et al., 2006; Canivet et al., 2004, 2005; Landgren and Hallström, 2010; Miller et al., 1993; Rautava et al., 1993; Sondergaard et al., 2003; Van den Berg et al., 2009; Zuckerman et al., 1990) have reported how colicky infant’s parents experience great discomfort, mainly caused by the psychological sense of impotence in improving their child’s condition. In addition, they usually suffer a physical lack of rest due to sleep disorders related to excessive nocturnal infant crying (Brand et al., 2014), which has a strong effect on work efficiency and on the quality of everyday life. Taking such issues into account, we have observed that parents are prone to treat episodes of infantile colic with pain relieving agents, hoping that this approach could quickly improve their condition.

Our study has some limitations. It is not based on double-blinded collected data and only in a small population. Prospective studies on the therapeutic and preventive effects of probiotics on infantile excessive crying are growing, but the evidence concerning preventive effects is still limited (Indrio et al., 2014; Sung et al., 2013). We have evaluated the intensity and frequency of infantile colic using indirect and subjective data concerning the parental discomfort. Therefore, the associations we found provide evidence of a lower use of medication, provided by parents, in infants treated with *L. reuteri*. Furthermore,
our study does not clarify a possible role of vitamin D in preventing infantile colic. We should speculate that the observed effects were due to *L. reuteri* only, however we need further studies comparing the effects of *L. reuteri* alone against *L. reuteri* added to vitamin D3 in order to understand if the combination is more effective than this probiotic on its own.

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**Conflict of interest**

Francesco Savino has received a travel grant from companies, including Biogaia, Italchimici and Noos, to attend conferences to present the results of previous studies. These companies have had no role in the study neither in the preparation of the manuscript. Simone Ceratto has received a travel grant from Noos to attend conferences. This company has had no role in the study neither in the preparation of the manuscript. There are no other interests to declare.

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