Clinical Study Report

A 12 week, randomized, double blind placebo controlled, fixed-dose , parallel group pilot feeding study to establish the safety and efficacy of a probiotic in patients with mild to moderate Psoriasis

Protocol No: AH-PSR-01

Development Phase: Phase 11

Name of Investigational Product: *Bifidobacterium Infantis 35624*

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Alimentary Health

Alimentary Health

The study was designed and managed in compliance with the principles of Good Clinical Practice.

Alimentary Health Ltd: Signature

Protocol No: AH-PSR-01

Study Title: A 12 week, randomized, double blind placebo controlled, fixed-dose parallel group, pilot feeding study to establish the safety and efficacy of a probiotic in patients with mild-to-moderate Psoriasis.

Dr. Barry Kiely; Barry Kiely **Date;** 30/Jan/2012.
{CEO}

SIGNATURE PAGE

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Investigator's Statement of Agreement: "I read this report and confirm that, to the best of my knowledge, it accurately describes the conduct and results of the study".

Investigator Name: Dr. Johnny Bourke
Department of Dermatology,
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Signature: .....

Date: 30/11/11

Alimentary Health Ltd: Signatures:

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Dr. Jenny Roper: _____ **Date;** 29/11/2011
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Dr; Liam O Mahony; _____ **Date;** 14.11.2011
{Study Director}

Executive Summary

Objective: A 12 week randomised, double blind placebo controlled, fixed-dose parallel pilot study to establish the safety and efficacy of a probiotic in patients with mild to moderate psoriasis.

Rationale

Bifidobacterium infantis 35624 is a commensal microbe originally isolated from the human gastrointestinal mucosa and has been extensively studied for its ability to regulate inflammatory responses, both in mice and humans. This microbe has been shown to attenuate inflammation in murine models of colitis and arthritis mainly through the suppression of pro-inflammatory cytokine production, for example, TNF-α. (McCarthy et al., 2003; O'Mahony et al., 2001; Sheil et al., 2004). This anti-inflammatory effect is mediated, in part, via the induction of T regulatory cell activity and consequent inhibition of the pro-inflammatory signal transduction molecule NF-κB (O'Mahony et al., 2008). In addition, human clinical trials in patients with Irritable Bowel Syndrome (IBS) have confirmed that feeding with this bacterial strain is associated with clinical benefit (O'Mahony et al., 2005; Whorwell et al., 2006). This prompted us to assess the effect of *Bifidobacterium infantis* 35624 in psoriasis. It is the first trial of probiotic bacteria in psoriasis as far as we are aware and was designed as a pilot study to detect any effect on disease severity, to assess its safety, and to examine any effect on proinflammatory biomarkers in psoriasis.

Study Design/Intervention:

The study was conducted in winter and early spring to minimize the confounding effect of ambient Ultra Violet light. It was a randomized, double-blind, fixed-dose placebo-controlled parallel-group pilot study to obtain data on size and variance of treatment effects. The target sample size of 60 was selected as a practical figure for recruitment purposes in one centre over 3 months. After a 2 week washout during which only emollients were applied to their psoriasis, patients attended for baseline assessment of psoriasis severity, and blood tests. Subjects attended every 4 weeks for 12 weeks for clinical assessment and blood tests.
PASI, PDI, PGAI and PGA were assessed at each visit, as were full blood count, serum electrolytes, renal and hepatic function. Nine mls of whole blood were collected in EDTA tubes for cytokines analysis at week 0, week 4, week 8 and week 12. Samples were centrifuged immediately and plasma frozen at -80°C. Measurements of plasma C - reactive protein (CRP), IL-1β, IL-6, IL-8, IL-10, IL-12p70, IL-17, TGF-β, and TNF-α and IFN-γ were performed using an electrochemiluminescence multiplex system Sector 2400 imager from Meso Scale Discovery (Gaithersburg, MD, USA) where antibodies labelled with Sulfo-tag reagents emitted light upon electrochemical stimulation. This is an ultra-sensitive method which has a detection limit for CRP of 0.7ng/ml, IL-1β of 0.3pg/ml, IL-6 of 0.3pg/ml, IL-8 of 1.0pg/ml, IL-10 of 1.9pg/ml, IL- 12p70 of 1.1pg/ml, IL-17 of 2.5 pg/ml , TGF-β of 3.2 pg/ml of TNF-α of 0.3pg/ml, and IFN-γ of 0.8pg/ml

Treatments

Each patient received either 10{10} CFU viable *Bifidobacterium infantis* 35264 or 5g Maltodextran placebo product in identical packing. Treatment was allocated according to a pre-prepared randomization list and both patients and

investigators were blinded with regard to treatment. Subjects were given detailed instructions on the storage and administration of product. Product was to be stored in a refrigerator at a temperature between 4°Celius and 6°Celius. A single sachet was to be taken before or after a meal, at the same time, each day. Compliance was monitored by return of all test product containers (used and unused).

Primary Endpoints:

Changes in the psoriasis area and severity index (PASI) between baseline and end of feeding.

Secondary Endpoints:

To compare the effects of *Bifidobacterium infantis* 35624 and placebo on:

Psoriasis Disability Index {PDI}
Patients Global Assessment {PGA}
Physician's Global Assessment Index {PGAI}
Digital Image Data

Main findings:

Psoriasis area and severity index (PASI)

The mixed models analysis revealed a statistically significant interaction between treatment and visit (p=0.04), implying that the pattern of response over time differed between *Bifidobacterium infantis* 35624 and placebo. This was due to a transient improvement of PASI in the treatment group at 4 weeks which, however, was not statistically significantly different from placebo (p=0.12). The relationship between the patterns of cytokine response and efficacy was investigated by defining as responders those patients who had increased IL-10 and decreased IL-17 and TGF-β. The clinical response of *Bifidobacterium infantis* 35624 responders (i.e. cytokine response) was significantly different to the clinical response of *Bifidobacterium infantis* 35624 cytokine non-responders. The responders (n=7) exhibited a significant improvement in PASI scores when compared to the non-responders (n=5) at weeks 4 and 8 on 2 way ANOVA analysis (p=0.05).

Psoriasis disability index (PDI)

There was a gradual reduction from baseline in PDI score of between 3 and 4 points up to the end of study in the active group which was not seen in the placebo group: this difference in response pattern fell short of statistical significance (p=0.09).

Global assessment indices

There was no evidence of any difference between treatments for the physician's global assessment index (PGAI, odds ratio for improvement=0.6, 95% CI 0.18 to 1.88). However, there was an interaction between treatment and visit on patient's global assessment (PGA) implying that the treatment effect is different for each visit (p=0.003). An initial apparent advantage of active treatment was reversed at the end of the study with odds ratios of improvement on *Bifidobacterium infantis* 35624 relative to placebo ranging from 4.25 at week 4 to 0.27 at week 12. Generally, the patients were more likely to consider their psoriasis to have improved than were the investigators.

The study showed evidence of a statistically significant difference between *Bifidobacterium infantis* 35624 and placebo in terms of the pattern of response over time for PASI and patient's assessment of treatment effect and also, but to a lesser extent, PDI. However, at no individual time point was the treatment comparison statistically significant. For both PASI and patient's global assessment, the results indicated some improvement with active treatment compared to placebo after the first four weeks of treatment. Any difference was no longer apparent later in the study. Conversely, for PDI the effect was more marked later in the study, although it was complicated by the fact that there was a difference between the treatment effect for males and females, with males being more likely to respond better to active treatment than placebo.

Although the number of withdrawals was relatively small, the clinical response shown by patients who were withdrawn in the two groups may have introduced some bias at the end of the study. Five patients were withdrawn from the *Bifidobacterium Infantis* 35624 group for adverse events, three of whom had shown some improvement (as rated by the patients) before withdrawal. The two patients withdrawn before their final assessment from the placebo group both rated their condition as unchanged.

There was no difference in the incidence of side effects between the two groups. There were no significant changes in serum biochemical or haematological parameters during the study. In the active group, one patient developed arthritis and one vasculitis while in the placebo group one patient developed paresthesiae and one had a flare of Crohn's disease. All of these settled within a few weeks and were felt to be unrelated to the trial medication. The vasculitis was felt to be due to a dental infection. The patient was treated with antibiotics, systemic steroids and dapsone. His vasculitis resolved after 6 weeks. There were no serious adverse events.

Plasma pro-inflammatory biomarker levels

Blood for immunological assessment were obtained at baseline, Week 4, Visit 4, Week 8 and Week 12, {end of feeding}. The TH1 cytokines IFN-γ, TNF-α and IL-12p70 were significantly elevated in the plasma of psoriasis patients at study entry as were the TH17 cytokines IL-17, IL-6 and TGF-β In addition, plasma IL-1β levels were significantly increased in the patient group while there were no significant differences in plasma IL-5, IL-8 or IL-10 levels. In patients with psoriasis, a strong positive correlation was found, by linear regression analysis ($P<0.001$, $r2=0.70543$), between baseline PASI scores and IL-17 levels. Furthermore, baseline plasma CRP levels were significantly elevated in the psoriasis patients.

Bifidobacterium infantis 35624-feeding was associated with a time-dependent modulation of patient plasma cytokine levels. TNF-α ($p=0.0380$), IL-17 ($p = 0.0410$) and TGF-β1 ($p=0.0490$) were significantly lower at week 8 while IL-10 levels significantly increased ($p=0.023$) at weeks 4 and 8 in probiotic-fed patients compared to placebo-fed patients. However, these cytokine differences were no longer evident at week 12, end of feeding. Plasma CRP levels were significantly decreased in *Bifidobacterium infantis* 35624-fed patients at week 4, week 8 and week 12, with the overall reduction in CRP level being nearly 50% greater with *Bifidobacterium infantis* 35624 than with placebo ($p=0.04$).

A post-hoc investigation of cytokine patterns over the course of the study in the psoriasis patients suggested the presence of a distinctive responder pattern characterized by an "anti-inflammatory" phenotype which was only observed in the

Bifidobacterium infantis 35624 treatment group. At week 8, seven of twelve psoriasis patients who were fed *Bifidobacterium infantis* 35624 displayed increased IL-10 levels associated with decreased IL-17 and TGF-β levels. None of the placebo-treated patients displayed this biomarker pattern. Using canonical variate analysis, this biomarker pattern was significantly associated with *Bifidobacterium infantis* 35624 feeding ($p=0.035$), compared to placebo.

Discussion

Although there was no strong evidence in this pilot study that *Bifidobacterium infantis* 35624 is more effective than placebo, there were some indicators of a treatment effect, particularly in regard to systemic pro-inflammatory biomarkers of disease. Both clinical variables and cytokine levels showed a parallel improvement at weeks 4 and 8. We found, in accordance with the existing literature (Arican et al., 2005; Takahashi et al., 2010), that our cohort of psoriatic patients had raised CRP, TH1 and TH17 cytokines at baseline and that IL-17 correlated well with PASI scores. Treatment with *Bifidobacterium infantis* 35624 resulted in a transient reduction in TH1 and TH17 cytokines and a rise in IL-10 which was paralleled by a transient reduction in PASI. Both clinical and inflammatory markers, with the exception of CRP, returned to levels similar to baseline at the end of the treatment period indicating a possible loss of therapeutic effect. What was particularly interesting was the identification of a sub-group of responders who showed dramatic changes in cytokine levels in parallel with improvements in clinical scores. Though acknowledging the limitations of post hoc analyses, this suggests that *Bifidobacterium infantis* 35624 may have an immunomodulatory role, possibly mediated via IL-10, in the normalization of exaggerated immune responses in psoriasis patients.

IL-10 is a regulatory cytokine which suppresses both TH1 and TH17 cytokine production (Asadullah et al., 1999; McInnes et al., 2001). There are some reports of lower levels of IL-10 in patients with psoriasis (Asadullah et al., 2000; Kang et al., 1998; Kormeili et al., 2004; Olaniran et al., 1996; Toichi et al., 2006) although Deeva et al (2010) found higher levels in mild to moderate disease. Systemic administration of IL-10 has also been used to treat psoriasis with variable effects, possibly due to its short half-life (Asadullah et al., 2001; Friedrich et al., 2002; Kimball et al., 2002). In the present study, there was no significant difference observed for baseline plasma IL-10 levels in patients with psoriasis. After *Bifidobacterum infantis* 35624 treatments, levels of IL-10 increased significantly in plasma from psoriasis patients after 4 and 8 weeks; however, surprisingly this increase was not maintained at week 12. Interestingly, Asadullah et al., 2000 reported that during established antipsoriatic therapy, patients showed higher IL-10 mRNA expression of peripheral blood mononuclear cells than before therapy. This finding is in agreement with our results where the increase in plasma IL-10 in *Bifidobacterium infantis* 35624-treated subjects resulted in significant improvement in the PASI score for up to 8 weeks, especially when subjects had a higher baseline PASI score (>5). All responders had a higher PASI. The subjects with a baseline PASI score lower 5 showed no change with treatment as there was no room for improvement. Therefore, our study supports the importance of IL-10 in psoriasis and shows that commensal microbes which are known to induce IL-10 *in vivo* could represent a new therapeutic approach for inflammatory skin diseases.

TNF-α is believed to contribute to the pathogenesis of psoriasis through its ability to both promote immune cell trafficking to the skin and induce keratinocyte proliferation (Gottlieb et al., 2003) and anti-TNF-α therapy is highly effective in psoriasis. Elevated TNF-α mRNA expression has been demonstrated in psoriatic plaques and in serum (Ameglio et al., 1994; Schopf et al., 2002; Toichi et al., 2006). In the present study, we found that elevated baseline plasma TNF-α levels fell after 8 weeks of treatment in the *Bifidobacterium infantis* 35624 group but not in the placebo group.

TH17 cytokines are produced by TH17 cells, a novel subset of CD4-positive T cells, (Lowes et al., 2007) which are thought to have an important role in the pathophysiology of psoriasis (Zheng et al., 2007). Our data indicate that TH17 cytokines (IL-1β, IL-6, IL-17, and TGF-β) were significantly elevated in the plasma of psoriatic patients supporting the hypothesis that TH17 cells may play a role in the pathogenesis of the disease. We also found a direct correlation between IL-17 levels in plasma and PASI which is consistent with some reports in the literature (Arican et al., 2005; Takahashi et al., 2010). Interestingly the elevated IL-17 levels at pre-treatment were significantly decreased after 8 weeks of treatment in the *Bifidobacterium infantis* 35624 group, but not in the placebo group. TGF-β, which is important for induction of TH17 cytokines, may be important in the pathogenesis of psoriasis. Flisiak et al., 2003 reported a correlation between TGF-β and PASI, although levels did not differ significantly from control patients in their study. In contrast, we found significantly higher levels of TGF-β1 in our psoriasis patients compared to controls but no correlation between plasma levels and PASI possibly because of the small numbers in our study. Elevated TGF-β1 levels at baseline were significantly decreased after 8 weeks of treatment with *Bifidobacterium infantis* 35624 but not with placebo.

CRP is a plasma protein synthesized primarily by hepatocytes as an acute phase reactant and is commonly elevated in many different inflammatory processes, primarily due to its induction by pro-inflammatory cytokines such as IL-6, IL-1β and TNF-α. Our study confirms previous findings that CRP levels are elevated in psoriasis patients. *Bifidobacterium infantis* 35624 significantly reduced plasma CRP levels at 4, 8 and 12 weeks, while levels in the placebo group were unchanged.

Conclusion

We have shown in this small pilot study in psoriasis patients, that *Bifidobacterium infantis* 35624-feeding is associated with a significant reduction in plasma CRP levels, a time-dependent modulation of the cytokine milieu and a transient effect on clinical measures. The presence of a subgroup of apparent responders within the active group where cytokine responses were more clearly delineated offers further evidence of an immunomodulatory effect of *Bifidobacterium infantis* 35624 associated with changes in clinical response. The apparent loss of efficacy of *Bifidobacterium infantis* 35624 after week 8 is difficult to explain. Perhaps dose is an issue and a more detailed study examining multiple doses of this probiotic should be performed. The small study size, the mild nature of the disease and the presence of outliers may also affect results. Nevertheless, it is clear that additional studies are required to determine whether probiotics are useful tools in the treatment of psoriasis.