GENERAL COMMENTS ON EFFICACY DATA

Second efficacy human study (Del Piano et al. Journal of Clinical Gastroenterology, 2010)

This was a double-blind, placebo-controlled, randomized study. Eighty subjects were enrolled in the placebo group and one hundred and ten subjects were in the active group receiving a formulation containing a total of 5 billion viable cells of mixed *Bifidobacterium breve* BR 03 and *Lactobacillus plantarum* LP 01.

The detailed statistical analysis of data is reported in the article. In any case, it is worth of being mentioned that 5 out of 6 parameters selected as outcome measures recorded markedly significant improvements already after 15 days of probiotic intake (A versus B column in Table 2). The most significant data came from the number of weekly evacuations ($p<0.001$) and abdominal bloating ($p<0.001$). The direct statistical comparison between placebo and active group at T_0, T_{15}, and T_{30} is able to subtract any possible placebo effect, that is to say an even slight, not statistically significant improvement which could be recorded in the placebo group at T_{15} compared with T_0 or at T_{30} compared with T_0. No statistically significant differences were recorded before the beginning of the supplementation (T_0) in the active group compared to placebo (A versus B column in Table 2). This confirms that the enrolled subjects were homogeneously distributed into the different groups, which is central to assess the effectiveness of probiotics in the restoration of a physiological intestinal motility. No statistically significant improvement was recorded in the placebo, neither at T_{15} nor at T_{30} compared with T_0 in any of the parameters.

The supplementation with mixed *Bifidobacterium breve* BR 03 and *Lactobacillus plantarum* LP 01 for 15 additional days was able to slightly further improve 5 out of the 6 parameters taken into account. At T_{30} five p values are <0.001 and one is $=0.001$ (consistency of feces). It's really interesting to point out how the number of weekly evacuations has remained around 7 at T_{15} (7.29) and at T_{30} (7.26), but it was significantly increased at T_{15} (7.29) compared with T_0 (5.30). This strongly suggests the sustainability of the beneficial effects over time. After restoration of an optimal number of weekly evacuations, together with the improvement of other parameters associated with bowel movements, the intake of the same amount of mixed *B. breve* BR 03 and *L. plantarum* LP 01 for 15 additional days was able to maintain the benefit over time. A very similar argument applies to abdominal bloating, significantly reduced after the first 15 days of probiotic supplementation and then only slightly further improved in the following 2 weeks (2.59 compared with 2.48).

The effective dose is 2.5 to 5 billion viable cells of each strain per day, to be taken for at least 14 days. In detail, the effective dose in subjects reporting abdominal pain and IBS symptoms is 5 billion viable cells of each strain/day with a supplementation period of at least 14 days in order to gain efficacy. On the other side, the effective dose in subjects reporting altered bowel habits and abdominal bloating is 2.5 billion viable cells of each strain/day with a supplementation period of at least 15 days. The beneficial effects mediated by these probiotics are very likely to be sustained over time, especially if their intake is prolonged for some additional weeks or months. In this sense, after relieve of abdominal pain, IBS symptoms and restoration of an optimal intestinal transit, a combination of *B. breve* BR 03 and *L. plantarum* LP 01 would be able to maintain this physiological condition over time.