

Lovastatin improves stool form in *Methanobrevibacter smithii* colonized rats with constipation.

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Evidence suggests that methane and colonization with *Methanobrevibacter smithii* (*M. smithii*) may be important in the pathogenesis of constipation and constipation-predominant irritable bowel syndrome. Direct *in vivo* infusion of methane into dogs slows the gut by 60%. Lovastatin, like other HMG-CoA reductase inhibitors, collectively known as statins, are produced by fungal species of the genus *Aspergillus*. Statins act on the rate-limiting enzyme in the mevalonate pathway important in cholesterol production in humans. In addition to this effect, lovastatin has specifically been shown to reduce the production of methane by inhibition of methanogenesis in *M. smithii*. In this study, we examine the effects of lovastatin in a rat model of diet-induced constipation and *M. smithii* proliferation.

Methods

30 adult, male Sprague-Dawley rats were placed on a high-fat diet (60.3% kcal from fat, Teklad high-fat diet TD.06414, Harlan Laboratories Inc, Madison, WI) for 7 weeks. The rats were assessed for increased *M. smithii* by qPCR before and after the diet, and then divided into 3 groups. Group 1 was given lovastatin in its lactone form, group 2 was

given lovastatin hydroxy acid (each 1.5 mg/rat), and the Group 3 was gavaged with a placebo. Each group was gavaged daily for 10 days. Three day stool collections were performed to assess average stool wet-weight and daily variability prior to commencing the high-fat diet, after 7 weeks of high-fat diet, and the final days of the lovastatin gavage (still on high-fat diet). On day 10 of the gavage, rats were then euthanized and DNA was extracted from contents of ligated bowel segments (ileum, jejunum, duodenum, cecum and left colon). qPCR was performed using primers for total luminal bacteria and *M. smithii*.

Results

Confirming previous studies, high-fat diet augmented stool *M. smithii* colonization in Sprague-Dawley rats ($7.58 \times 10^4 \pm 6.62 \times 10^4$ cfu/mL at baseline to $2.60 \times 10^5 \pm 1.95 \times 10^5$ after 7 weeks of high-fat) ($P < 0.01$). This was coupled with a reduction in the stool wet-weights (62.4% at baseline to 48.6% after 7 weeks) ($P < 0.01$). At this point rats were divided into 3 groups. With respect to the total bacteria by qPCR, levels were not different between placebo and either lovastatin group. For *M. smithii*, the ratio of *M. smithii* to total bacteria was significantly reduced in rats given the lovastatin lactone in the ileum. *M. smithii* levels in the colon were unaffected. Most importantly, there was an increase in stool wet-weight noted in rats receiving lovastatin lactone gavage.

Conclusions

Lovastatin lactone improved stool water content in constipated rats with higher *M. smithii*. Although rats do not produce large enough quantities of methane to show the effect on methane, lovastatin lactone produced a reduction (but not elimination) of *M. smithii* in the ileum by the lovastatin lactone.