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The Aging Gut Motility Decay: May Symbiotics Be Acting as "Implantable" Biologic Pace-Makers?

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ABSTRACT

Motility recording of small and large intestine was performed in old Wistar rats divided into three groups: (a) standard diet, (b) standard diet plus a symbiotic preparation, and (c) standard diet plus a heat-inactivated symbiotic preparation. SCM-III. significantly increased the myoelectric activity of small intestine and colon ($p < 0.01$ versus [a] and [c]) paralleling "young" values of 4-month-old rats and increased the spike burst frequency in the proximal-distal colon ($p < 0.05$). SCM-III significantly increased the frequency and duration of spike bursts in the jejunum, transverse-distal colon, and defecation frequency, while decreasing the intervals of migrating motor complex in the colon ($p < 0.01$) to "young" values with an increased mRNA expression of VIP ($p < 0.05$). Gut flora manipulation aimed to modulate myoelectric activity can tentatively help reversing age-related motility decay.

INTRODUCTION

THE COLONIC MICROBIOTA mediates many cellular and molecular events in the host that are important to health. It has been shown in recent years how dysbiosis may affect the gut motor and sensory activity.[1,2] Further, physiologic changes in the gastrointestinal tract (including aging) and modifications in diet and host immunity may affect the composition and metabolism of gut microbiota. In clinical practice this is supported by several reports on the onset of irritable bowel syndrome–like symptoms when the gut ecosystem is altered by antibiotic treatments or following gastroenteritis.[3,4] Moreover, besides a number of associated diseases,[5] during aging a relentless derangement of the enteric nervous system occurs,[6] as well as a composition shift of gut flora and its metabolic activities.[7,8] The aim of this study was to probe a possible treatment of aging gut motility disorders from a biologic approach through probiotics.

MATERIALS AND METHODS

Four bipolar copper electrodes were implanted into the muscular wall of the small intestine of anesthetized rats at 5, 15, 25, and

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35 cm distal to the duodenojejunal junction. An additional two electrodes were implanted in the proximal and distal colon. The seventh wire was used as ground and implanted in a subcutaneous fold. The abdominal incision was then sutured and the electrodes were tunneled subcutaneously to exit at the interscapular region and the wires connected to a computerized system. After an average postoperative period of 5 days, control recording of basal myoelectric activity (four activity fronts of the MMC propagated over all recording sites during a period of 60 min) were obtained. Afterward, rats were randomly coded and divided into three groups. Group A was given a standard chow diet, group B diet was mixed with the symbiotic preparation, and group C was treated as group B but with a heat-inactivated symbiotic preparation. Four-month-old rats served as the "young" control group.

Motility study procedure

The experiments were performed in conscious rats and in a fasting state because it has been shown that the intestinal microflora modulates fasting rather than postprandial gut myoelectric activity.[9] The electromyographic recordings were monitored by a polygraph (Sky-Electric-Laboratory, Tokyo, Japan) and motility recordings for each channel were simultaneously analyzed for contractions and relaxations. Subsequently, six consecutive phase IIIs, migrating at least from J5 to J25, were recorded. Phase III was defined as clearly distinguishable periods of regular spiking activity at the maximum frequency and amplitude observed at each electrode, indicated by at least doubling of baseline myoelectric activity.[10] The sensitivity was set to 0.25 mV/cm. The interval between phase IIIs was regarded as the MMC period. The MMC migration ratio was calculated by the ratio between MMC period J5(J25) and MMC period J5 as previously defined.[11] When six consecutive phase IIIs migrating from J5 to J25 were recorded, this ratio was estimated by $5/(N_{J5} - 1)$, where N_{J5} is the total number of phase IIIs at J5 regardless of their aboral migration.

The defecation pattern (frequency, daily fecal weight, fecal mass per each defecation) also was recorded.

Vasoactive intestinal polypeptide analysis: plasma and tissue concentration and gene expression

Small intestine samples were used to measure the concentration of vasoactive intestinal polypeptide (VIP) by radioimmunoassay (after addition of ^{125}I-labeled VIP and separation by adding 0.4 mL of activated charcoal, coefficient of variation <8%) and their immunofluorescence was analyzed by a computerized system. Related gene expression was assessed on cryosections by *in situ* hybridization.

Statistical analysis

Significance was established by analysis of variance and the level of significance was assessed by a Duncan's multiple-range test. Data were expressed in the text as means plus or minus the standard deviation (SD) with a probability value of $p < 0.05$.

RESULTS

The pattern of spike bursts of the small intestine appeared as packets of cyclic MMCs occurring at regular intervals (14.3 ± 4.6 min) and were propagated from the duodenum to the jejunum at 2 to 3 cm/min^{-1} as already described.[12] The overall colonic myoelectrical activity showed a random spike bursts activity at a frequency varying from 0.4 ± 0.04/min to 0.6 ± 0.06/min. The analysis showed that old rats had a statistically significant decrease in myoelectric activity when compared with the "young" group ($p < 0.05$). However, no significant change was observed in the defecation pattern. SCM-III exerted an excitatory action on the myoelectric activity of the small intestine and colon as compared with age-matched rats fed a standard diet or the inactivated symbiotic ($p < 0.01$) with values comparable to those recorded in "young" rats. In 37% of treated rats the increased myoelectric activity was not uniform across the different leads and the analysis of intragroup variability showed that this was wider for recordings from leads J5 to J15. Overall, the most intense excitatory effects of SCM-III was recorded at distal jejunal sites (J25 to J35), where it scored up to a 59% increase over baseline. In particular, SCM-III not only

normalized this diminished activity to a "young" level, but also increased it compared with the "young" baseline ($p < 0.05$). The administration of inactivated symbiotics produced only a significant increase of frequency of spike burst in the transverse and distal colon ($p < 0.05$ versus A). SCM-III was the only treatment causing a significant increase of the frequency and duration of spike bursts in the jejunum, proximal, and distal colon while decreasing the intervals of migrating motor complex in the colon ($p < 0.01$). As compared with group A, baseline B, and "young" rats, SCM-III also increased the frequency of defecation during the daytime (time/hour: 0.65 versus 0.19, <0.05 and caused a trend decrease during nighttime (time/hour: 0.18 versus 0.29, $p = 0.062$).

Vasoactive intestinal polypeptide analysis

Plasma concentration of VIP was comparable among groups and did not change during symbiotic treatment. Old rats showed a decreased number of VIP-immunoreactive nerve fibers when calculated for a 100 μ^2 tissue area ($p < 0.01$ versus "young") and a decreased tissue concentration (VIP μg/g: 4.4 ± 1.1 versus 6.7 ± 1.3, $p < 0.05$). SCM-treated rats showed a not significant trend increase of VIP concentration but its quantitative analysis of mRNA showed a significant increase during symbiotic treatment ($p < 0.05$ versus baseline and versus group C).

DISCUSSION

Although the pattern and composition of the microflora in healthy people is thought to be fairly stable over the years, it represents a dynamic ecosystem that is subjected to a continuous although vital interplay with dietary live microbes and prebiotics substrates. Indeed, recent studies suggest that aging *per se* affects the intestinal microflora with a decrease in anaerobes and bifidobacteria population and an increase in enterobacteria and in species diversity of the dominant fecal microflora overall.[13] Although it remains to be clarified whether the type of overgrowth flora is an epiphenomenon of a disease or the overgrowth flora itself may contribute to disease pathophysiology, these changes and the reduced age-related intestinal immunity can be partly reconciled with the increased susceptibility to degenerative or infectious diseases observed in the elderly. Moreover, it is has been demonstrated that antibiotics, by altering the gut ecosystem, may bring about a derangement of intestinal motility; this effect may persist even for months afterward. Thus, it is likely that a healthy gut bacterial balance with a net prevalence of the fermenting anaerobic microbiota promoting a regular spike burst activity in small intestine is one of the crucial protecting phenomena mediated by the intestinal microflora.[14] Although the authors did not perform any microbiologic analysis in this study, previous data with same symbiotic had shown its significantly beneficial effect on gut flora composition.[15,16] Finally, although the modulation of gut motility involves multiple pathways with different messengers, hormones, luminal bacterial metabolites, and several other neurotransmitters, the present symbiotic intervention seemed to significantly enhance VIP mRNA expression despite histologic signs of age-related paucity and degeneration of nerve plexus, as described elsewhere.[17–19] Taken altogether, such preliminary data suggest that new effector sites of probiotics in the gut are amenable to more comprehensive age management.[20]

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