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Alteration of GI symptoms in a cow with Johne disease by the dietary organosulfur, 2-mercaptoethanol

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Sub-phenotypes of inflammatory bowel disease (IBD)—Crohn disease, ulcerative colitis and some cases of irritable bowel syndrome—are generally considered a consequence of gastrointestinal inflammation of unknown etiology. Conventional therapy and more recently biologic agents, all with varying degrees of drawbacks, have resulted in improved control of these diseases. However, as the incidence and prevalence continue to rise, needs for prevention, permanent remission and cures remain unmet, plus there still remain needs for improved control of symptoms, such as pain and diarrhea. The case report herein describes a serendipitous, novel means for curtailing these symptoms associated with a bovine gastrointestinal disease that may have applicability for patients with diseases characterized by abdominal-visceral pain and diarrhea.

The case report herein describes a serendipitous, novel means of curtailing pain and diarrhea associated with a bovine gastrointestinal disease (Johne disease) that may potentially be beneficial for relieving abdominal-visceral pain and diarrhea in patients with gastrointestinal diseases.

Johne disease—a world-wide disease primarily in ruminants, most prevalent in cattle, sheep and goats, but even in wildlife—has many manifestations in common with the human IBD, Crohn disease (CD),[1-4] including pain and debilitating diarrhea (in cattle). As with CD, there are no preventive/curative therapies for Johne disease outside a probiotic Dietzia therapy.[5-7] The etiologic agent of Johne disease is *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Natural exposure of cattle to MAP, which usually results in clinical disease at > 2 y of age, occurs primarily in utero and/or by oral ingestion of MAP by neonates.[8] For disease to be manifested, infection with MAP is followed by intestinal inflammation.

Interestingly, humans are also systemically infected with MAP. However, the incidence of infection in the "normal" population is lower than in those with Crohn disease,[9-13] ulcerative colitis[13] and irritable bowel syndrome,[13] three diseases characterized in whole or part by gastrointestinal pain and diarrhea. Unfortunately, the consequences—cause, perpetuate, bystander—of such associations are highly controversial and remain to be unequivocally clarified.

The initial investigations on alteration of cellular processes by 2-Me originated in this laboratory when various murine immunological reactivities in vitro were found to be dramatically enhanced by, or dependent upon, sulfhydryl compounds—of those studied, the most potent was 2-Me.[14-17] Subsequently, a number of in situ processes/diseases were found to also be altered by 2-Me. For example, 2-Me (1) reversed the loss of immune functions associated with aging,[18,19] (2) increased longevity and quality of life,[19-22] (3) prevented high-fat-diet obesity,[21] (4) altered systemic lupus erythematosus,[22] (5) completely prevented development of mammary tumors of unknown etiology[23] and (6) slowed progression of spontaneous or cigarette-smoke-induced liver cancers,[19,20] virally caused mammary tumors[23] and leukemia (Click RE, in preparation). Some of these diseases have also been found to be altered by organosulfur compounds/derivatives derived from Allium and Brassica foods; the most extensively investigated are processes associated with tumorigenesis—anticarcinogenic and apoptosis induction.[24-26]

Of all the processes altered by 2-Me, the finding that 2-Me altered leukemia progression in AKR/Cum mice—a strain that naturally harbors murine leukemia virus (MuLV) and at least four ecotropic, endogenous MuLV proviral DNA sequences[27]—was instrumental in why the protocol reported herein was tested in cattle. An adult, four-year-old multiparous dairy cow (#16) was found positive (sero-specific ELISA OD of 3.85 determined by the Univ Minn Vet Diagnostic Lab) for bovine leukemia virus (BLV). Cattle infected with this virus may develop lymphomas/leukemia;[28] however, the incidence of disease is quite low relative to the number of positive animals in any given herd, likely because dairy cows in general are not productive much beyond 5–6 y of age. Twenty-two months after #16 tested BLV positive, clinical symptoms indicative of lymphoma developed

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(lack of appetite, weight loss and enlarged fore-udder lymph nodes). In addition, although not having pipestream diarrhea, her feces were a bit loose, a symptom not typical of BLV infection. It should be pointed out that these symptoms are in general characteristic signs of early, clinical Johne disease. However, a diagnosis of Johne disease was considered unlikely because she had tested MAP negative[29] multiple times over the prior two years. Based on the presumptuous diagnosis of BLV-induced leukosis and since there was/are no effective treatments for BLV disease in cattle, she was considered an ideal, non-rodent candidate to assess the safety and efficacy of the murine, 2-Me-leukemia protocol. Therefore, she was started on a once daily, feed additive regimen of 1.38 mg/kg body weight 2-Me, a dose slightly lower than the lowest effective dose for mice.[21] After treatment was initiated, both fecal consistency and appetite returned to normal (reduced GI pain?) and both remained normal up until the day she succumbed. During this "beneficial" four-month interval, she unexpectedly continued to lose weight, which is not characteristic for BLV-caused leukosis, nor for the Dietzia probiotic reversal of Johne disease symptoms. This, plus the earlier pretreatment, atypical fecal consistency, indicated that retesting for MAP should once again be done. Surprisingly, this time she tested serologically positive by ELISA (an OD value of 2.4), but was serologically negative for AGID (a serological test that defines a more advanced disease than an ELISA[29]) and negative for fecal MAP shedding, all determined by Allied Monitor, Inc.[29] Three weeks after serum and feces were submitted for testing, she succumbed with Johne disease; anticipated because of the continued weight loss, but still not understood since treatment curtailed diarrhea and reduced GI pain sufficiently to return appetite to normal. This is striking because neither of these symptoms ever reversed in over 60 end-stage, non-treated or unsuccessfully treated animals that succumbed from Johne disease.[6,7] The results do suggest that 2-Me "normalized" the gastrointestinal tract in some manner, a result that may have value for IBD patients. Interestingly, a similar phenomenon—decreased macroscopic and microscopic colonic damage—was found for experimentally-induced colitis in rats by dietary methylsulfonylmethane,[30] an organosulfur molecule with a chemical structure such that it likely functions via a mechanism similar to that postulated for 2-Me; i.e., generation of sulphane sulfur.[31]

In conclusion, treatment of a cow with early signs of clinical Johne disease (plus two cows with BLV-induced leucosis that were negative for Johne disease; manuscript in preparation) with 2-Me was safe, just as found in rodents, and it had ameliorative effects for symptoms of a bovine intestinal bowel disease. The findings raise two intriguing questions: By what mechanism does intestinal "well-being" occur and would IBD patients, if treated with either food or xenobiotic organosulfurs, have a similar health-related quality of life benefit?

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