cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Interleukin 10 Restores Gastric Emptying, Electrical Activity, and Interstitial Cells of Cajal Networks in Diabetic Mice

Kyoung Moo Choi,[1,*] Simon J. Gibbons,[1,*] Lei Sha,[1] Arthur Beyder,[1] Pieter-Jan Verhulst,[1] Gianluca Cipriani,[1] Jessica E. Phillips,[1] Anthony J. Bauer,[2] Tamas Ordog,[1] Jon J. Camp,[1] Xin Ge,[1] Adil E. Bharucha,[1] David R. Linden,[1] Joseph H. Szurszewski,[1] Purna C. Kashyap,[1] and Gianrico Farrugia[1]

[1]*Enteric NeuroScience Program, Mayo Clinic School of Medicine, Rochester, Minnesota;* [2]*Department of Integrative Physiology and Pharmacology, Liberty University College of Osteopathic Medicine, Lynchburg, Virginia*

SUMMARY

Interleukin 10 reversed delayed gastric emptying in diabetic mice. It increased heme oxygenase 1 expression and normalized electrical activity and networks of interstitial cells of Cajal in the stomach. Thus, interleukin 10 is a potential therapy for diabetic gastroparesis.

BACKGROUND & AIMS: Gastroparesis is a complication of diabetes characterized by delayed emptying of stomach contents and accompanied by early satiety, nausea, vomiting, and pain. No safe and reliable treatments are available. Interleukin 10 (IL10) activates the M2 cytoprotective phenotype of macrophages and induces expression of heme oxygenase 1 (HO1) protein. We investigated whether IL10 administration could improve gastric emptying and reverse the associated cellular and electrical abnormalities in diabetic mice.

METHODS: Nonobese diabetic mice with delayed gastric emptying were given either IL10 (0.1–1 μg, twice/day) or vehicle (controls). Stomach tissues were isolated, and sharp microelectrode recordings were made of the electrical activity in the gastric muscle layers. Changes to interstitial cells of Cajal (ICC), reduced nicotinamide adenine dinucleotide phosphate diaphorase, and levels and distribution of HO1 protein were determined by histochemical and imaging analyses of the same tissues.

RESULTS: Gastric emptying remained delayed in vehicle-treated diabetic mice but returned to normal in mice given IL10 (n = 10 mice; $P < .05$). In mice given IL10, normalization of gastric emptying was associated with a membrane potential difference between the proximal and distal stomach, and lower irregularity and higher frequency of slow-wave activity, particularly in the distal stomach. Levels of HO1 protein were higher in stomach tissues from mice given IL10, and ICC networks were more organized, better connected, and more evenly distributed compared with controls.

CONCLUSIONS: IL10 increases gastric emptying in diabetic mice and has therapeutic potential for patients with diabetic gastroparesis. This response is associated with up-regulation of HO1 and repair of connectivity of ICC networks. *(Cell Mol Gastroenterol Hepatol 2016;2:454–467; http://dx.doi.org/10.1016/j.jcmgh.2016.04.006)*

Keywords: Alternatively Activated Macrophages; Heme Oxygenase 1; Electrical Slow Wave.

Gastroparesis is a complication of diabetes defined by delayed gastric emptying without obstruction that is accompanied by early satiety, nausea, vomiting, and pain.[1–3] No safe and reliable treatments are available.[4] Identification of the cellular changes associated with gastroparesis in human beings[5,6] and in mouse models of gastroparesis[7,8] have identified possible therapeutic targets. The nonobese diabetic (NOD) mouse in particular has proved to be a useful model of human diabetic gastroparesis.[7,9–11]

In previous studies using a noninvasive gastric emptying test, a proportion of diabetic NOD mice developed delayed emptying of solids while the remainder remained resistant to this complication.[11] Although most animals show loss of neuronal nitric oxide synthase upon the development of diabetes, the development of delayed gastric emptying was concurrent with reduced expression of the receptor tyrosine kinase, Kit, a marker for interstitial cells of Cajal (ICC).[7] This is consistent with studies showing impairment to ICC networks and loss of neuronal nitric oxide synthase in mice with long-standing diabetes.[9,10] Subsequent studies determined that animals resistant to the development of delayed emptying maintained high expression of heme oxygenase-1 (HO1) in alternatively activated M2-macrophages in stomach muscle layers.[7,8] In studies of the human gastric body, a correlation between the numbers of ICC and CD206-positive M2-macrophages was found.[12]

These studies have suggested that targeting HO1 may ameliorate diabetic gastroparesis. Indeed, treatment of

*Authors share co-first authorship.

Abbreviations used in this paper: **CO, carbon monoxide; HO1, heme oxygenase 1; ICC, interstitial cells of Cajal; IL10, interleukin 10; MDA, malondialdehyde; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NOS, nitric oxide synthase; NOD, nonobese diabetic; PBS, phosphate-buffered saline.**

Most current article

2352-345X
http://dx.doi.org/10.1016/j.jcmgh.2016.04.006

diabetic mice with delayed gastric emptying by inducing HO1 normalized gastric emptying and Kit expression levels.[7,8] These therapies were accompanied by increased numbers of HO1-positive, M2-macrophages in gastric muscularis propria.[8] Hemin (as Panhematin, Ovation Pharmaceuticals, Deerfield, IL) also up-regulates HO1 expression in human beings, although administration of hemin must be performed under close supervision.[13] These data suggest that HO1 is a promising therapeutic target for diabetic gastroparesis,[14] but treatment with hemin has significant disadvantages including the delivery method and side effects.

Consequently, a need exists to determine other modalities to target HO1. In the present study we tested interleukin 10 (IL10) as a treatment for delayed gastric emptying in NOD mice. The rationale was that IL10 expression is up-regulated during treatment by carbon monoxide (CO) of postoperative ileus in mice[15] and that IL10 had an obligatory role in postoperative intestinal recovery.[16] IL10 up-regulates HO1 expression[17] and is expressed at high levels in M2-macrophages. IL10 increases the numbers of M2-macrophages[18] directly and suppresses expression of M1-macrophages.[19] In addition, in diabetic mice with delayed emptying, IL10 messenger RNA levels were decreased.[8]

In previous studies on diabetic mice and rats, ICC networks and the electrical slow-wave activity that originates from ICC were absent or abnormal, especially in the distal antrum.[9,20] In human beings, electrical dysrhythmias also were reported in diabetic gastroparesis.[21–23] However, electrical properties and immunohistochemical differences were not comparatively studied in rodents with delayed gastric emptying or after normalization of gastric emptying. Thus, for the current study we made electrical recordings from multiple locations in the stomach of treated mice and subsequently immunolabeled for HO1 and Kit. Therefore, we were able to examine associations between gastric emptying of the mice and physiological and anatomic changes to the stomach, and the response of those changes to treatment with IL10.

Materials and Methods

Animals and Experimental Design

Animal procedures were performed using protocols approved by the Mayo Clinic's Institutional Animal Care and Use Committee. Female NOD/ShiLtJ mice were used as previously described.[7,8,11] Blood glucose levels were measured every week until the onset of diabetes, after which point they were measured daily. Single drops of blood were collected from the vascular bundle located at the rear of the jaw bone of the mice. The amount of blood collected was monitored carefully to avoid anemia. Mice were considered diabetic when the glucose levels were higher than 250 mg/dL. The incidence of diabetes was 57% in this study. Subtherapeutic insulin (Lantus insulin glargine; Sanofi-Aventis US LLC, Bridgewater, NJ) was injected once daily intraperitoneally when the glucose levels were higher than 500 mg/dL to keep the diabetic mice alive, yet also keep the blood glucose levels between 400 and 600 mg/dL to allow complications of diabetes to develop. To determine the levels of oxidative stress in the diabetic mice, the concentration of malondialdehyde (MDA) was measured in blood plasma. The concentration of thiobarbituric acid–reactive substances was calculated as malondialdehyde equivalents using a commercial kit (Oxi-Tek; Zeptometrix Corp, Buffalo, NY). Five microliters of plasma sample was mixed with an equal volume of sodium dodecyl sulfate solution and 125 μL of 5% thiobarbituric acid/acetic acid reagent. Samples were incubated for 60 minutes at 95°C. After centrifugation at 1600*g*, the absorbances of supernatants from samples were read at 532 nm using a spectrophotometer (NanoDrop Technology, Wilmington, DE).

Gastric emptying of solids was measured using a ^{13}[C]-octanoic acid breath test.[11] Three baseline values of times to half maximal emptying ($T_{1/2}$) for gastric emptying were obtained before the development of diabetes. After the onset of diabetes, gastric emptying was measured weekly. After the development of delayed gastric emptying, mice were assigned to treatment with vehicle or IL10 (1 or 0.1 μg intraperitoneally, twice a day; GenScript, Piscataway, NJ; or Insight Genomics, Falls Church, VA). We alternated the assignment to treatment based on the development of delayed gastric emptying only and without regard to any metabolic parameters such as blood glucose or MDA levels. Treatment began after 2 consecutive measurements of delayed gastric emptying and continued as 2 doses every day until 2 consecutive normal values for $T_{1/2}$ were obtained or a maximum of 10 weeks after the start of treatment. The duration and frequency of vehicle treatment was matched to the length of IL10 treatment. All mice with delayed gastric emptying also had MDA levels that were significantly greater than the upper limit of the normal range for MDA in diabetic NOD mice (>73 nmol/mL).[7] At the completion of the study, mice were killed by carbon dioxide exposure followed by cervical dislocation. Whole stomachs were cut along the lesser curvature and the mucosa was removed. Electrical recordings and immunohistochemical studies then were performed on the tissues. The experimental protocol is outlined in Figure 1*A*.

Electrophysiological Recordings From the Gastric Smooth Muscle

Smooth muscle membrane potential and electrical slow waves were recorded from the circular muscle of every tissue from the treated mice at 12 defined regions distributed from the proximal body to distal antrum (Figure 1*B*). Sharp glass microelectrodes filled with 3 mol/L KCl (input resistances, 40–70 MΩ) were used to record membrane potential and electrical slow wave. The locations of the recording sites were documented on a digital image, after which the tissue was fixed in the recording dish (Figure 1*B*). Electrical slow-wave events were analyzed using Clampfit (Molecular Devices, LLC, Sunnyvale, CA). An analysis was performed on recordings longer than 2 minutes in duration in which at least 12 slow-wave events were observed as follows. Slow-wave events from stable recordings were identified using the "template search" event discriminator function in Clampfit. For each trace, a template was derived