Treatment of Overweight and Obesity by Suppressing Intestinal Epithelial Renewal in a Mouse Model of Obesity

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Treatment of Overweight and Obesity by Suppressing Intestinal Epithelial Renewal in a Mouse Model of Obesity

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Abstract

Obesity and overweight pose serious risk for many diseases and clinical conditions. However currently available weight loss strategies and pharmacotherapies of obesity are not satisfactory. This study shows that treatments with pH modifiers such as acids are not only well tolerated by obese mice but also very effective in reducing bodyweight and fat mass by depleting and suppressing the proliferation of proliferating epithelial cells of the intestinal villi. Therefore other cell proliferation inhibitors that suppress intestinal epithelial renewal can also be used for pharmacotherapy of obesity and overweight.

Introduction

According to the World Health Organization (WHO), 39% and 13% of the world's adults are overweight or obese, respectively, and over 340 million children and adolescents are overweight or obese based on the data of 2016. (1). Obesity and overweight increase the risk of many serious clinical conditions or diseases such as hypertension, type 2 diabetes, coronary heart disease, stroke, sleep apnea, cancers, clinical depression and pains. (2-4). Obesity and overweight also pose heavy economical burden on society. In the U.S. the medical cost of obesity in adults is estimated at $342.2 billion in 2013 dollars. (5).

The prevalence of overweight and obesity has been consistently trending higher. While the increase of the prevalence in developing countries may reflect the adoption of western style high calorie diets, the increase in developed countries such as the U.S. is an indication of the failure of various weight management strategies. (6). With regard to pharmacotherapy of obesity, current drugs are generally not well received by patients because their effects are rather modest and patients often regain body weight after the termination of the medication (7). Given the serious health risk and heavy economical burden of obesity and overweight, novel strategy of developing effective therapy of obesity and overweight is of great significance.

One way to reduce bodyweight is by limiting nutrient absorption. The absorption of nutrients is mainly mediated by the intestinal villi. The intestinal villi are maintained by constant renewal of the epithelial cells including all cell types of the intestinal epithelium through cell proliferation and differentiation. The current study explores a novel strategy of weight control by inhibiting intestinal epithelial renewal thereby limiting nutrient absorption.

Treatments with pH modifiers such as organic or inorganic acid preferentially deplete proliferating cells and suppress their proliferation, and the proliferative statuses of the cells are positively correlated with their susceptibility to the depletion and suppression. (Manuscript in preparation). It was therefore further hypothesized that pH modifiers could be used to inhibit the proliferation hence the renewal of intestinal epithelial cells.

Results

Reduction of bodyweight and fat mass by in vivo treatments with pH modifiers

Obesity was induced in C57BL/6 mice by feeding young female mice with high fat diet for 4 weeks. The mice were then switched to normal diet and randomly divided to groups to receive i.p. injection of saline or saline containing HCl, HOAc or NaOH. After the treatments, the saline-treated mice lost about 3% of body weight due to the change of diet; the HCl- and HOAc-treated mice lost about 25% and 15% of body weight, respectively, whereas the NaOH-treated mice lost about 17% of body weight. (Figure 1a).

The fat pads of the mice were analyzed to further determine whether the bodyweight reductions were attributable to the reduction of fat masses. The sizes of fat pads of HCl- and HOAc-treated mice were grossly smaller than those of the saline-treated mice; those of the NaOH-treated mice were also somewhat smaller. (Figure 1b). Fat (weight of all fat pads)/bodyweight ratios of the mice were calculated to normalize the effects of variations of body weight on fat mass. The saline-treated mice had an average fat/bodyweight ratio of 0.135 (13.5%). The average fat/bodyweight ratios of the HCl- and HOAc-treated mice were reduced to just 0.027 (2.7%) and 0.045 (4.5%), representing 5- and 3-fold reduction, respectively. On the other hand, despite 17% of bodyweight reduction, the average fat/bodyweight ratio of the NaOH-

treated mice was 0.106 (10.6%), only modestly lower than that of the saline-treated mice. (Figure 1c).

Shortening of intestinal length by treatments with pH modifiers

Gross examination of the entire intestines (from the end of stomach to anus) showed that the HCl- and HOAc-treated mice had drastically shortened intestines as compared with the saline-treated mice, whereas the intestines of the NaOH-treated mice were only slightly shortened. (Figure 2a). Quantitative measurements found that the HCl- and HOAc-treated mice had on average about 31% and 12% reduction of intestinal lengths, respectively, whereas the NaOH-treated mice had only about 2% reduction. (Figure 2b and c).

No significant reduction of masses of other major abdominal organs

Despite the significant reduction of body weights and shortening of the intestines, the masses of the other two major abdominal organs the livers and kidneys were similar among the different treatment groups. (Figure 3a and b).

Acid treatments reduced proliferating cells in the intestinal villi

To determine whether the fat mass reduction and shortening of the intestines were related to depletion of proliferating intestinal epithelial cells, tissue sections of intestines of representative mice of the different treatment groups were stained for Ki-67 with hematoxylin counter staining. Ki-67 is a reliable marker for proliferating cells, its level of expression positively correlates with rRNA and DNA synthesis. (8). As shown in Figure 4, there were more $Ki\text{-}67^{+}$ cells in the intestinal villi of saline- or NaOH-treated mice than in the HCl- or HOAc-treated mice. In addition, the Ki-67 staining was the strongest in the NaOH-treated mice, followed by that of the saline-treated mice, which was in turn stronger than those of the HCl- or

HOAc-treated mice. Thus, acid treatments depleted proliferating cells and inhibited cell proliferation, whereas alkaline treatment enhanced cell proliferation in the intestinal villi.

Discussion

This study shows that pH modifiers can potentially be used for pharmacotherapy of obesity and overweight. While all three pH modifiers tested in this study, HCl, HOAc and NaOH caused significant weight loss, acid treatments were particularly effective in reducing fat/bodyweight ratios. HCl treatments showed the strongest effects on bodyweight and fact mass reduction, and caused dramatic shortening of the intestines. On the other hand, while HOAc treatments also led to substantial reduction of bodyweight and fat mass, they caused only modest shortening of the intestines. The effects of the acid treatments appeared to be related to the depletion of proliferating cells in the epithelium of the intestinal villi and suppression of the proliferation. In contrast, NaOH treatments enhanced or at least maintained the proliferation of the intestinal epithelial cells. Such dichotomy of effects on cell proliferation and death is consistent with the notion that cell death and proliferation are controlled by a pH balance such that low pH induces cell death by apoptosis whereas high pH promotes cell proliferation. (Manuscripts in preparation).

While the current study tested only three pH modifiers of simple acids and base, it can be reasonably extrapolated that other organic or inorganic acids or bases, as well as pH buffering agents, may have similar effects. Further, pH modifiers may also include ionophores and stimulators or inhibitors of the expression or activities of enzymes, membrane transporters or ion channels that are responsible for the maintenance or alteration of pH in or around an intestinal

epithelial cell. For examples, some of such enzymes and membrane transporters may include carbonic anhydrases, monocarboxylate transporters, proton or bicarbonate transporters. (9-18).

Based on the current study, it can also be expect that any cell proliferation inhibitors that reduce intestinal epithelial renewal could potentially be repurposed for pharmacotherapy of obesity and overweight. Such cell proliferation inhibitors can be general cell proliferation inhibitors or inhibitors of the signaling pathways operating in the crypt of the intestinal villus. The former could be inhibitors and antagonists of DNA synthesis, cyclin-dependent kinases (CDKs) and Myc. (19-24). The latter could be inhibitors and antagonists of the Wnt-β-catenin signaling pathway, the EGFR signaling pathway and the Notch signaling pathway. (25-31).

Materials and Methods

Mice. Female C57BL/6 mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA), and housed in the animal facility of Charles River Accelerator and Development Lab (CRADL) (Cambridge, MA, USA). Animal studies were performed according to the protocols approved by the CRADL Institutional Animal Care and Use Committee.

Obesity model. The obesity model was similar to those previously described. (32, 33). Female C57BL/6 mice of 4 weeks age were first acclimated at CRADL for 1 week. After the acclimation, mice were switched to Gamma-irradiated high fat diet (Research Diets, New Brunswick, NJ, USA) (60% calories from fat) for 4 weeks.

Treatments with pH modifiers. After 4 weeks of high fat diet, the obese mice were switched back to regular diet, and randomly divided into 4 groups to receive i.p. injection of 200μl saline or saline plus 87.5mM HCl, HOAc or NaOH. The bodyweight of each mouse was recorded before each treatment. The mice continued to receive the treatments every other day

for 3 times. Four days after the final treatments, mice were sacrificed, their total body weights were measured, fat pads, and internal organs were collected, weighed, and analyzed.

Ki-67 immunohistochemistry. One day after 3 treatments with the pH modifiers, mice were sacrificed and intestines were fixed in 10% neutral formalin. Segments of the fixed jejunums were paraffin embedded and sectioned. Ki-67 staining of the tissue sections was carried out as described. (34). Briefly, after de-paraffinized and rehydrated, the tissue sections were heated in 10mM Sodium Citrate Buffer, pH 6.0, to boiling, followed by washing with PBS and incubation in 10% H_2O_2 at room temperature for 15 minutes. The tissue sections were then blocked with 5% BSA, and incubated with rabbit anti-mouse Ki-67 antibody (Servicebio, Wuhan, China) in PBS at 4^{o}C overnight. After washing with PBS plus 0.1% Tween 20, the tissue sections were incubated with Horseradish Peroxidase (HRP)-conjugated goat anti-rabbit antibodies (Invitrogen, Carlsbad, CA, USA) at room temperature for 1 hr. After washing the slides, color deposits were developed using a DAB Substrate Kit (ThermoFisher, Waltham, MA, USA) according to the manufacturer's instructions. After color was developed, the slides were washed with ddH_2O, and counter-stained with hematoxylin. The slides were scanned, and images were analyzed with QuPath software (https://qupath.github.io).

Declaration of conflict of interest

Wei-ping Zeng is the owner of Therazwimm Corporation, and inventor of patent application based in part on work described in this manuscript.

Author contribution

Wei-ping Zeng designed, carried out and funded the study, and wrote the manuscript.

Figure Legends

Figure 1. Reduction of body weights and fat masses in obese mice by pH modifiers. (a) Graph showing the average percentages of bodyweight reduction after treatments with different pH modifiers as indicated. (b) Photographs of fat pads comparing the sizes of the fat pads of representative mice of the different treatment groups. (c) Graph showing the average fat/bodyweight ratios of the mice in the treatment groups. Statistical significance of the differences of bodyweight reduction and fat/bodyweight ratios between saline group and the other treatment groups was determined by Student t test. **, $p < 0.01$. Data were pooled from 3 experiments.

Figure 2. Reduction of intestinal length by pH modifiers. (a) Photographs of the entire intestines of representative mice of the different treatment groups. (b) Graph showing the average lengths of the intestines of mice of the different treatment groups. Statistical significance of differences between saline group and other treatment groups was determined by Student t test. **, $p < 0.01$. (c) Graph showing the average percentages of reduction of the intestinal length of the acid- or alkaline-treated mice as compared with the saline-treated mice.

Figure 3. Effects of pH modifiers on the masses of livers and kidneys. (a and b) Graphs showing average weights of livers (a) and kidneys (b) of mice in the different treatment groups.

Figure 4. Modulation of cell proliferation in the epithelium of the intestinal villi by pH modifiers. Microscopic views of intestinal tissue sections stained for Ki-67 (brown) with haematoxylin counter staining (blue) of representative mice of the different treatment groups.

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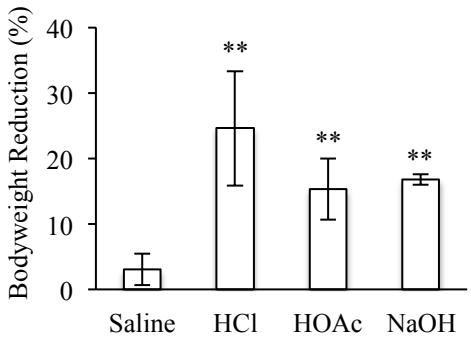
a)


Bodyweight Reduction (%)
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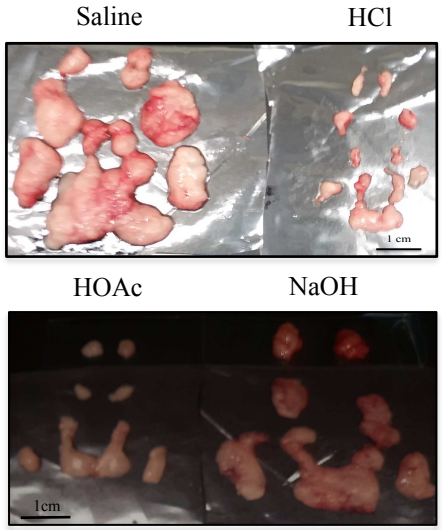
30
20
10
0
**
**
**
Saline
HCl
HOAc
NaOH

b)


Saline
HCl
1 cm
HOAc
NaOH
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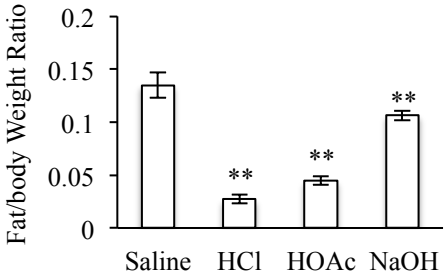
c)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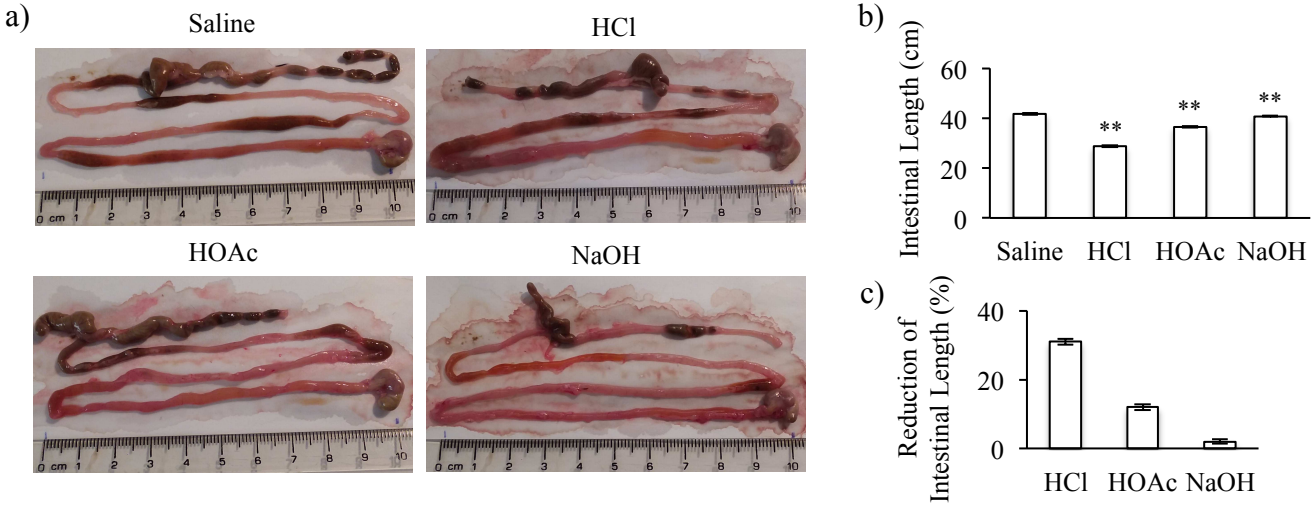
0.15
0.1
0.05
0
**
**
**
Saline
HCl
HOAc
NaOH


a)
Saline
HCl
HOAc
NaOH
b)
Intestinal Length (cm)
60
40
20
0
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Saline
HCl
HOAc
NaOH
c)
Reduction of Intestinal Length (%)
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20
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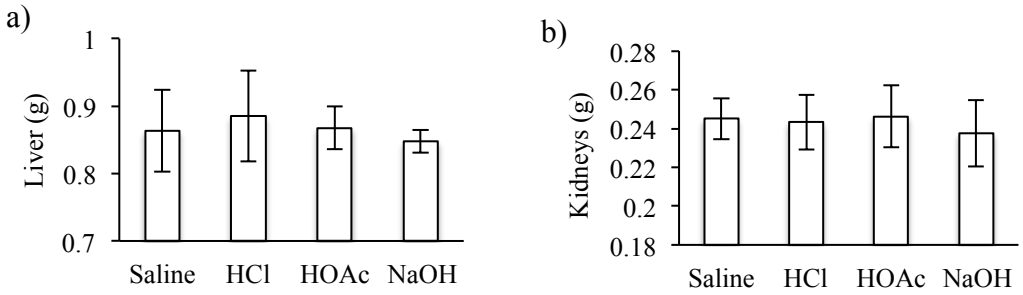
HCl
HOAc
NaOH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Saline
HCl
HOAc
NaOH
b)
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0.22
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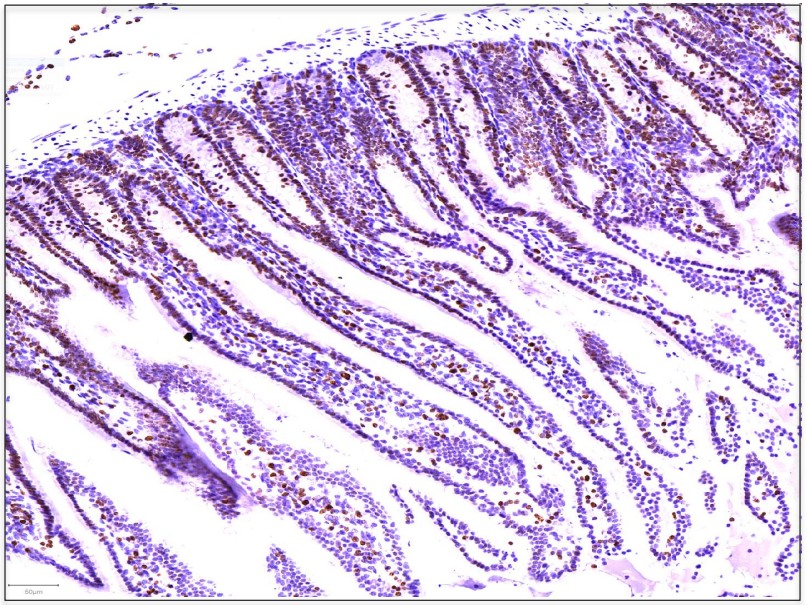
0.18
Saline
HCl
HOAc
NaOH

Saline



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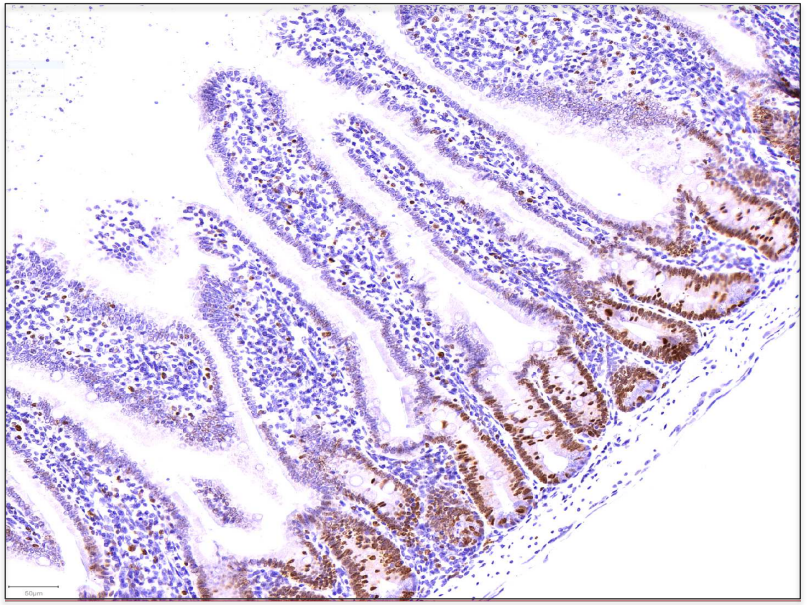

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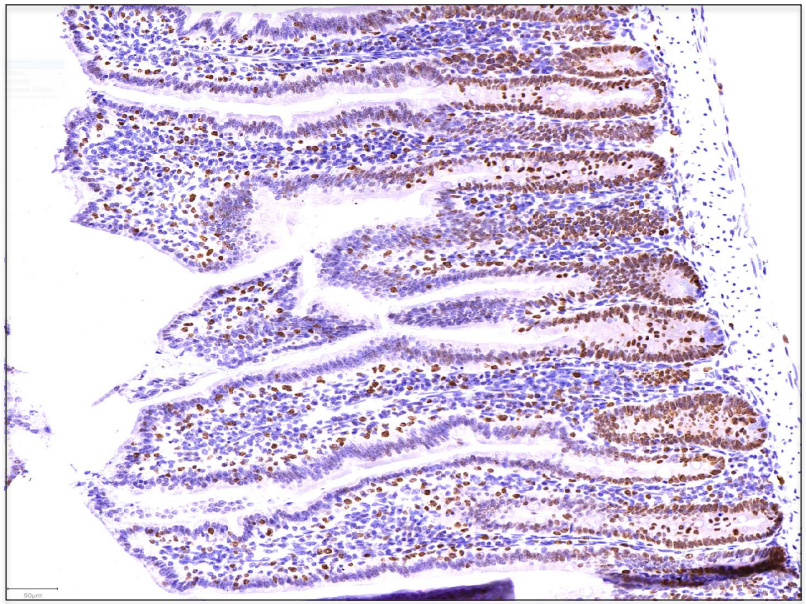

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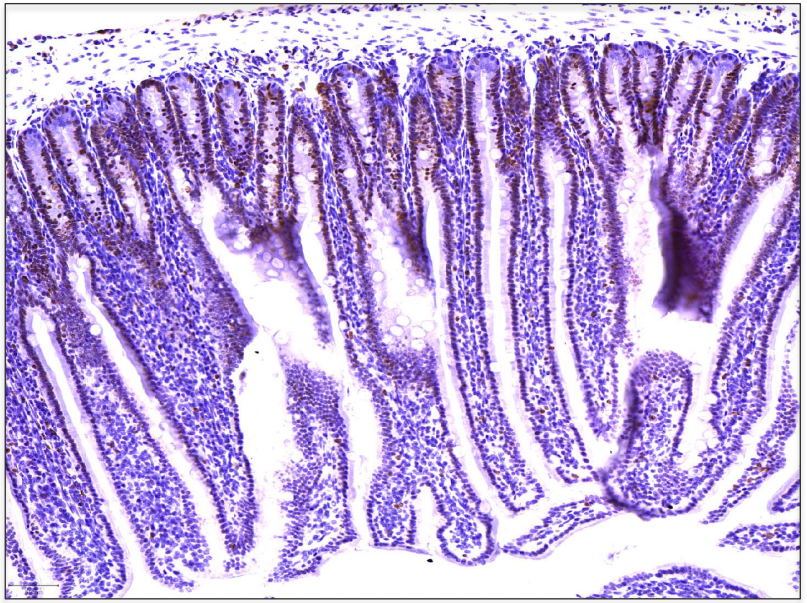

50μm