Proton pump inhibitors induce changes in colonocyte gene expression that may affect *Clostridium difficile* infection

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Background. *Proton pump inhibitors seem to promote* Clostridium difficile *infection (CDI). Although the current literature suggests that this association is mediated through gastric acid suppression, there has been little investigation into whether a direct effect on expression of colonocyte genes may also have a role. The aim of this study was to investigate the effect of omeprazole on genome-wide gene expression in a human colonic cell line.*
Methods. *T84 cell monolayers were treated with acid-activated omeprazole at 0, 1, 10, or 100 μmol/L for 48 hours. Cells were lysed and total RNA samples were reverse transcribed and used to generate biotinylated cRNA. Whole-genome transcript expression levels were then quantified using an Illumina HT-12 BeadChip microarray targeting 25,440 genes. Transcripts with a stringent minimum absolute fold change of 1.5 and an adjusted nominal* P *value <.05 (false discovery) were identified as being differentially expressed.*
Results. *Significant changes in expression were observed for 322 colonocyte transcripts, including genes with potential implications for susceptibility to CDI. These genes include roles in cell junctions, toxin susceptibility, and bile acid metabolism and transport.*
Conclusion. *Omeprazole treatment decreases the expression of genes that have important functions in colonocyte integrity. Such impairment in colonocyte function may promote CDI. (Surgery 2014;156:972-8.)*

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PROTON PUMP INHIBITORS (PPIs) are benzimidazole drugs that decrease gastric acid secretion, and are widely used in conditions such as dyspepsia, gastroesophageal reflux, Zollinger–Ellison syndrome, and peptic ulcer disease. An association between PPIs and an increased risk for development of *Clostridium difficile* infection (CDI) has been well noted in the literature and is acknowledged by the US Food and Drug Administration.[1,2] This association has implications for the rather common use of PPIs among hospitalized patients, where the frequent liberal use of PPI therapy may pose a substantial risk for a potentially serious nosocomial infection.

The mechanisms through which PPIs promote CDI are unknown, although most assume principle mediation via gastric acid suppression. Our group provided the first seminal evidence that PPIs can exert an effect on *C. difficile* toxin gene expression.[3] Whether PPIs might also induce changes in the gut as the environment of the bacteria which lead to CDI has not been evaluated previously. To investigate this possibility, the present study utilized an in vitro model of human colonic T84 monolayers to identify PPI (omeprazole)-induced changes in gene expression that may have a role in increasing susceptibility to CDI.

Presented at on Friday March 7, 2014, Indianapolis, Indiana.

Accepted for publication June 27, 2014.

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0039-6060/$ - see front matter

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http://dx.doi.org/10.1016/j.surg.2014.06.074

MATERIALS AND METHODS

This study was performed at the authors' institution using *C. difficile* isolates from an institutional review board–approved tissue biobank.

Cell culture. The human colonic epithelial cell line T84 (CCL-248; American Type Culture Collection, Manassas, VA) was cultivated in DMEM/F-12

medium supplemented with 15 mm HEPES (pH 7.4), 0.5% sodium pyruvate, 6% fetal bovine serum, 100 U/mL penicillin, and 100 U/mL streptomycin in a humidified atmosphere of 5% CO_2 at 37°C. To model the polarized phenotype of native colonic epithelia, cells were seeded at 5×10^5 cells onto 12-mm, 0.4-μm pore Transwell polycarbonate membranes (Corning Inc, Corning, NY) and incubated for 14–21 days until formation of confluent monolayers with a trans-epithelial electrical resistance between 500 and 600 Ω*cm^2 was measured. A concentrated stock of omeprazole (Sigma Aldrich, St. Louis, MO) was prepared by initially solubilizing powder into a minimal volume of DMSO and then acid activating this preparation in culture medium at pH 5.5 for 30 minutes to convert the omeprazole into its active sulfenamide form.[4] Activated omeprazole was added as required to DMEM/F12 (pH 7.4) medium to immediately bilaterally treat T84 monolayers at doses of 0 (empty DMSO-adjusted media) 1, 10, or 100 μmol/L for 48 hours in matched biologic triplicates. This dose range included physiologically relevant concentrations, as human C_{max} plasma omeprazole concentrations range from 2.6 to 5.7 μmol/L with daily oral doses of 20 and 40 mg, respectively.[5]

Extraction of high-quality total RNA. Confluent colonic cell monolayers were washed with ice-cold Dulbecco's phosphate-buffered saline and were then directly lysed by rapid collection into 0.4 mL TRIzol reagent (Invitrogen, Carlsbad, CA). Lysates were extracted once with chloroform and were diluted with equal volumes of ethanol, followed by absorption onto RNeasy mini columns (Qiagen, Valencia, CA) according to the manufacturer's protocol with the addition of a 20 minute on-column DNase digestion. Total RNAs were eluted from washed columns with RNase-free water. RNA integrity (values of >9.5) was accessed on RNA 6000 Nanochips using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Total RNA concentrations were determined with a Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA), adjusted to 300 ng/μL, and stored at −70°C.

BeadChip microarray expression profiling. Whole-genome expression analysis was performed using an Illumina Human HT12 v3 Expression BeadChip microarray (Illumina, San Diego, CA). Total RNAs collected from omeprazole-treated and untreated control cultures were reverse transcribed and were biotinylated-UTP-labeled using the Total Prep RNA Amplification Kit (Ambion, Austin, TX). In brief, 300 ng of RNA was reverse-transcribed into single-stranded, full-length cDNAs with an oligo-dT primer bearing a T7 RNA polymerase promoter. Second strand synthesis was followed by a magnetic-based cleanup to produce a template for overnight transcription using T7 RNA polymerase to generate single-stranded, biotinylated, antisense cRNAs. Microarray hybridization of biologic triplicates was performed using 0.75 μg of biotin-labeled cRNA at 58°C for 16 hours to the HT-12 BeadChip. Hybridization was detected with 1 μg/mL Cy3-Streptavidin (GE Healthcare, Piscataway, NJ) and image data from the arrays extracted using GenomeStudio software (Illumina) at the Genomics Core of the Cleveland Clinic Lerner Research Institute. Data were log2 transformed and quantile normalized using the open-source Bioconductor package *lumi*. The complete BeadChip array data will be deposited in the open access NCBI Gene Expression Omnibus (GEO) repository.

Identification of colonic genes affected by treatment with omeprazole. Beadchip data quality was initially screened using scatter plots and principal component analysis to exclude possible outliers. Differentially expressed genes (DEGs) were defined as significant by calculating the fold-difference between experimental groups. Significance (P values) from Bayes-moderated t tests were adjusted by Benjamini and Hochberg's method to correct for false discovery from multiple testing. Transcripts with a minimum absolute-fold change of 1.5 and an adjusted nominal $P < .05$ (false discovery) were assigned as differentially expressed. Lists of DEGs with normalized-fold change differences in expression were accessed by Gene Ontology enrichment analysis using WebGestalt software[6] and the Panther 8.1 classification system,[7] and the top affected canonical pathways were identified using IPA software (Ingenuity Systems, Redwood City, CA).

Validation of BeadChip microarray data by reverse transcriptase polymerase chain reaction. Validation of observed microarray expression changes for 3 genes of interest (*JAM1/F11R*, *RHOA*, and *TFF3*) was carried out by reverse transcriptase polymerase chain reaction (RT-PCR) amplification of cDNAs from controls and from all 3 omeprazole concentrations using specific exon-spanning primers designed with PrimerQuest software (Integrated DNA Technologies, Coralville, IA). First-strand syntheses of cDNAs were performed using 800 ng RNA primed with oligo (dT) 12-18 primers and SuperScript III transcriptase (Life Technologies, Gaithersburg, MD). Synthesis products were digested with RNase-H

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