Metformin Does Not Reduce Markers of Cell Proliferation in Esophageal Tissues of Patients With Barrett's Esophagus

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BACKGROUND & AIMS: **Obesity is associated with neoplasia, possibly via insulin-mediated cell pathways that affect cell proliferation. Metformin has been proposed to protect against obesity-associated cancers by decreasing serum insulin. We conducted a randomized, double-blind, placebo-controlled, phase 2 study of patients with Barrett's esophagus (BE) to assess the effect of metformin on phosphorylated S6 kinase (pS6K1), a biomarker of insulin pathway activation.**

METHODS: **Seventy-four subjects with BE (mean age, 58.7 years; 58 men [78%; 52 with BE >2 cm [70%]) were recruited through 8 participating organizations of the Cancer Prevention Network. Participants were randomly assigned to groups given metformin daily (increasing to 2000 mg/day by week 4, n = 38) or placebo (n = 36) for 12 weeks. Biopsy specimens were collected at baseline and at week 12 via esophagogastroduodenoscopy. We calculated and compared percent changes in median levels of pS6K1 between subjects given metformin vs placebo as the primary end point.**

RESULTS: **The percent change in median level of pS6K1 did not differ significantly between groups (1.4% among subjects given metformin vs −14.7% among subjects given placebo; 1-sided $P = .80$). Metformin was associated with an almost significant reduction in serum levels of insulin (median −4.7% among subjects given metformin vs 23.6% increase among those given placebo, $P = .08$) as well as in homeostatic model assessments of insulin resistance (median −7.2% among subjects given metformin vs 38% increase among those given placebo, $P = .06$). Metformin had no effects on cell proliferation (on the basis of assays for KI67) or apoptosis (on the basis of levels of caspase 3).**

CONCLUSIONS: **In a chemoprevention trial of patients with BE, daily administration of metformin for 12 weeks, compared with placebo, did not cause major reductions in esophageal levels of pS6K1. Although metformin reduced serum levels of insulin and insulin resistance, it did not discernibly alter epithelial proliferation or apoptosis in esophageal tissues. These findings do not support metformin as a chemopreventive agent for BE-associated carcinogenesis. ClinicalTrials.gov number, NCT01447927.**

Keywords: HOMA-IR; Diabetes Drug; Cancer Development; Tumorigenesis.

Abbreviations used in this paper: **AE, adverse event; AMP, adenosine monophosphate; AMPK, adenosine monophosphate–activated protein kinase; BE, Barrett's esophagus; BMI, body mass index; EAC, esophageal adenocarcinoma; EGD, esophagogastroduodenoscopy; HOMA-IR, homeostatic model of insulin resistance; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; IGF-1R, insulin-like growth factor-1 receptor; IR, insulin receptor; mTOR, mammalian target of rapamycin; NSAID, nonsteroidal anti-inflammatory drug; pS6K1, phosphorylated S6 kinase 1.**

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Obesity has been linked to a variety of malignancies.[1–4] Recent studies suggest that one explanation for the role of obesity in the development of cancer is activation of the insulin/insulin-like growth factor (IGF) pathway.[5–7] A diet high in energy, high in animal fat, and low in fiber in combination with physical inactivity contributes to insulin resistance and resulting hyperinsulinemia. Complex interactions of increased levels of insulin, IGF-1, and members of the serum IGF binding protein (IGFBP) family (IGFBP1 through IGFBP6) determine the levels of insulin and IGF that are available to mediate effects at the cellular level through the insulin receptor (IR) and the IGF-1 receptor (IGF-1R).[3,6–8] Activation of IR and IGF-1R stimulates cellular proliferation and inhibits apoptosis via molecular pathways that are mediated by PI3K, AKT, mammalian target of rapamycin (mTOR), S6K1, and other signaling molecules.

Central adiposity is a risk factor that is independently and consistently associated with Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC).[9] Activation of the insulin/IGF pathway is associated with Barrett's-mediated carcinogenesis.[10,11] Metformin is an insulin sensitizer commonly used to treat diabetes mellitus. It lowers serum insulin levels and directly inhibits cell growth. Besides inhibiting gluconeogenesis, this biguanide derivative activates adenosine monophosphate (AMP)–activated protein kinase (AMPK) in epithelial cells by an LKB-dependent mechanism. AMPK appears to be a key target for cancers associated with diabetes mellitus and obesity.[6,12] Activation of AMPK by metformin increases insulin-stimulated glucose uptake and inhibits mTOR via TSC2/1, resulting in decreased protein synthesis mediated by the down-regulation of ribomosomal protein S6 kinase 1 (S6K1). This decrease in phosphorylated S6K1 (pS6K1) inhibits cell proliferation. Metformin also has AMPK-independent, indirect antiproliferative effects related to lower systemic levels of insulin. Recent studies have shown its potential as a cancer prevention drug in other common obesity-associated cancers.[13–18]

The prognosis for EAC patients has remained poor, with the large majority dying of cancer-related causes within 5 years.[19] Novel interventions, such as chemoprevention in BE, are a high research priority. The goal of this study was to investigate the potential for metformin as a chemoprevention agent by determining its effect on phosphorylated ribosomal S6K in Barrett's epithelium.

Methods

All aspects of the study protocol were reviewed and approved by the appropriate Institutional Review Board for human research at each participating site. Mayo Clinic in Rochester, MN served as the coordinating research base. The Data and Safety Monitoring Board of the Mayo Clinic Cancer Center reviewed safety data every 6 months. All authors had access to the study data and reviewed and approved the final manuscript.

Recruiting Sites

Participants were recruited at 8 Cancer Prevention Network member organizations: University Hospitals Case Medical Center, Cleveland, OH; Kansas City VA Medical Center, Kansas City, MO; Massachusetts General Hospital, Boston, MA; Mayo Clinic, Rochester, MN; St Michael's Hospital, Toronto, ON, Canada; University of Pittsburgh, Pittsburgh, PA; University of Pennsylvania, Philadelphia, PA; and the University of Puerto Rico, San Juan, PR.

Study Participants

Seventy-five eligible participants were enrolled between February 2012 and January 2013. The target population included participants ($\geq$18 years) with histologically confirmed BE, defined as the presence of specialized columnar epithelium in the tubular esophagus with $\geq$2 cm of involvement and no evidence of high-grade dysplasia or cancer on the basis of both clinical surveillance and additional research biopsies. Participants were required to have documented intestinal metaplasia with goblet cells in $\geq$1 of 4 research biopsy samples ($\geq$50% intestinal metaplasia), use of a proton pump inhibitor before enrollment, and no history of diabetes mellitus. Women of childbearing potential were required to document a negative pregnancy test before enrollment. General exclusion criteria were history of confirmed esophageal high-grade dysplasia, esophageal carcinoma, or any cancer; vitamin B_{12} deficiency; history of lactic acidosis; medication for weight loss $\leq$2 months before enrollment; treatment with other oral hypoglycemia agents or biguanides; receipt of other investigational agents $\leq$3 months before enrollment; history of allergic reactions attributed to compounds of similar composition to the study agent; elective surgery during the study period; genetic disorders such as family history of hereditary gastrointestinal polyp disorder; or comorbidities that might limit adherence to the study protocol.

Baseline Evaluation

After informed consent, participants completed a focused interview, physical exam, peripheral blood draw, anthropometric measurements (height, weight, calculated body mass index [BMI], and waist-hip ratio), and esophagogastroduodenoscopy (EGD) with biopsies for eligibility testing.

Baseline Endoscopy

Endoscopic landmarks including the diaphragmatic hiatus, end of the tubular esophagus as marked by the proximal margin of gastric folds, and squamocolumnar junction were recorded. Extent of the circumferentially involved BE segment was determined by using the