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Prospective evaluation of the molecular effects of metformin on the endometrium in women with newly diagnosed endometrial cancer: A window of opportunity study☆

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HIGHLIGHTS

- Evaluating molecular changes on the endometrium in a window of opportunity study was feasible.
- Metformin resulted in changes in serum markers of insulin sensitivity and glucose metabolism.
- Metformin resulted in tissue level changes consistent with mTOR inhibition.

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ABSTRACT

Objective. Metformin reduces cancer incidence and improves overall survival in diabetic patients. In preclinical studies, metformin decreases endometrial cancer (EC) cell growth by activation of AMPK/mTOR inhibition. We sought to determine the effects of metformin on serum/tumor biomarkers in women with EC.

Methods. In this prospective trial, newly diagnosed EC patients underwent pre-treatment blood draw/endometrial biopsy, were administered oral metformin 850 mg daily for ≥7 days, and underwent post-treatment blood draw/definitive surgery. Pre- and post- serum analyses were performed. Tumor samples were evaluated for changes in AMPK, PI3K/AKT pathway, proliferation, and apoptosis by immunohistochemistry.

Results. Twenty patients completed the trial. Median age and BMI were 57 years (range: 27–67) and 34.5 kg/m^2 (range: 21.9–50.0). Median duration of metformin was 9.5 days (range: 7–24). A majority of women had endometrioid adenocarcinomas (90%) and were early stage (85%). After metformin, there were significant decreases in serum IGF-1 ($p = 0.046$), omentin ($p = 0.007$), insulin ($p = 0.012$), C-peptide ($p = 0.018$), and leptin ($p = 0.0035$). Compared to baseline, post-treatment tissue showed decreased phospho-AKT in 18/20 patients (90%, $p = 0.0002$), decreased phospho-S6rp in 14/20 patients (70%, $p = 0.057$), and decreased phospho-p44/42MAPK in 15/18 patients (83.3%, $p = 0.0038$). There was no difference in Ki67, phospho-ACC, or caspase 3. Changes did not correlate with BMI, grade, or KRAS mutation.

Conclusion. In this prospective window of opportunity study, we demonstrated that relevant serum and molecular changes occur in patients with newly diagnosed EC after a short course of metformin. Ongoing clinical trials will help determine the appropriate role for metformin in the treatment of women with EC.

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1. Introduction

Metformin is one of the most widely prescribed oral hypoglycemic agents used to treat type 2 diabetes [1]. Over the last decade, a number of studies have suggested therapeutic potential for metformin in the prevention and treatment of cancer. This hypothesis was supported by the observation that metformin has the potential to reduce the incidence of cancer in diabetic patients taking metformin compared to other hypoglycemia agents [2]. In an observational cohort study of diabetics, incident cancer was diagnosed among 7.3% of 4085 metformin users compared with 11.6% of 4085 comparators ($p < 0.001$) [3]. Since then, multiple publications have suggested that taking metformin may benefit patients with pancreatic, breast, colorectal, ovarian, and endometrial cancer [4–7].

Metformin is thought to have both a direct and indirect effect on cell growth and metabolism (Fig. 1). In the direct model, metformin activates AMPK, which results in phosphorylation of tuberous sclerosis 2 protein. This inhibits mTOR signaling which ultimately inhibits cell growth. Metformin also acts indirectly by increasing insulin sensitivity, increasing uptake of glucose in the cell, and subsequently decreasing circulating levels of insulin. Both insulin and IGF-1 are known factors that promote cell growth, thus, decreasing insulin would have a negative effect on cell proliferation.

Both diabetes and insulin resistance are risk factors for endometrial cancer [8,9]. Preclinical data have shown that increasing doses of metformin were associated with a decrease in cell proliferation in several endometrial cancer cell lines [10,11]. Based on these data, the objective of our study was to determine the effects of oral metformin on the endometrial cancer cells in women with newly diagnosed endometrioid endometrial cancer. We hypothesized that treatment with oral metformin would decrease endometrial cancer cell growth by activation of AMPK and inhibition of MTOR.

2. Materials and methods

After approval from the Institutional Review Board at MD Anderson Cancer Center and Lyndon Baines Johnson Hospital (LBJ), patients with newly diagnosed endometrial cancer who were candidates for definitive surgery were approached. Patients were eligible if they were a surgical candidate and had [1] histologically confirmed endometrioid adenocarcinoma, any grade, or a mixed tumor with at least an endometrioid component, [2] documented non-fasting plasma glucose level of ≤125 mg/dL or a fasting plasma glucose level of ≤125 mg/dL, [3] a creatinine clearance >60 cm^3/min documented by the Cockcroft Gault formula, and [4] serum bilirubin <2.5 mg/dL. Exclusion criteria included [1] known history of diabetes or currently taking any hypoglycemic agents, [2] use of metformin or an mTOR inhibitor in the previous 2 years, [3] prior cytotoxic or biologic therapy for endometrial cancer, or [4] any contraindication to metformin.

Once informed consent was obtained, the pre-treatment evaluation included [1] anthropometric testing including height, weight, waist circumference, and blood pressure, [2] fasting blood and urine collection, and [3] an office endometrial biopsy. Part of the tissue was flash frozen and the remaining tissue was formalin-fixed and paraffin embedded. A hematoxylin and eosin stain was performed to confirm presence of tumor tissue.

Once the baseline testing was completed, patients were started on metformin 850 mg by mouth daily for a minimum of 7 days and maximum of 30 days prior to scheduled surgery. If a patient was scheduled for CT imaging, the metformin dose was held for 48 h after the

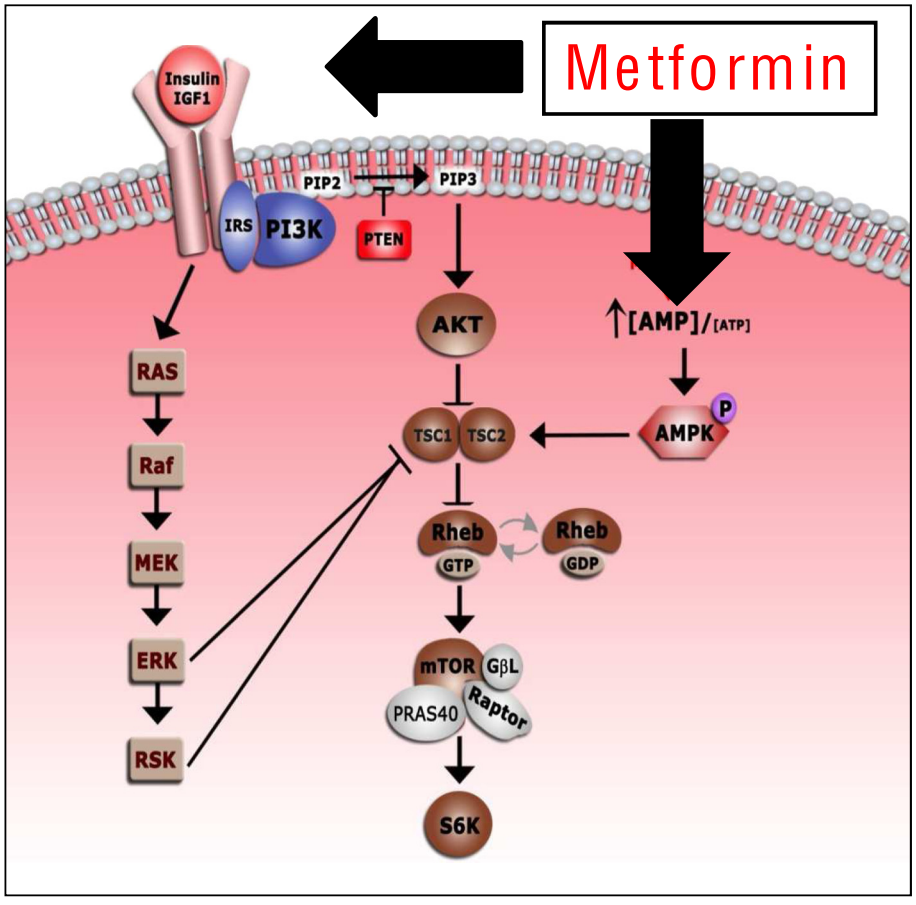

Fig. 1. The indirect and direct effects of metformin on cell proliferation.

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