Cation-selective Transporters are Critical in the Efficacy of Metformin against Breast and Endometrial Cancer

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Introduction

The antidiabetic drug metformin has significant anticancer effects as a combination therapy with other chemotherapeutic agents1. Retrospective studies showed that diabetic patients with breast cancer receiving metformin and neoadjuvant chemotherapy have a higher pathologic complete response rate than diabetics not receiving metformin2 (Figure 1). In vitro studies showed that metformin also exerts potent anti-proliferative effects on endometrial cancer cell lines3.

Study Goals

Preliminary studies in our laboratory to determine metformin transporter-competent and transporter-deficient breast cancer cell lines show that the BT-20 cell line expresses negligible mRNA levels of metformin transporters, and the MDA-MB-231 cell line has high gene expression of OCT1 and MATE1. These two transporter are also expressed in breast tumor tissue and non-malignant breast tissue. This study aims to:
- demonstrate the role of transporters in metformin uptake in human breast cancer cell lines;
- compare metformin transporter expression in endometrial tumor tissue and adjacent non-malignant tissue;
- determine transporter expression profiles in human endometrial cancer cell lines and tissues, and identify a relevant cell line for investigating the anticancer effects of metformin.

Methods

Cell Culture. Human breast cancer cell lines, MDA-MB-231 and BT-20, and human endometrial cancer cell lines, ECC-1, Ishikawa and SVEC-2 cells (ATCC) were cultured at 37°C in 5% CO2 and humidified air after 90% confluency using tyrosin-EDTA and plated in 25-cm2 T-flasks.

Human endometrial Tissue. 15 human endometrial tumor tissues and 5 adjacent non-malignant endometrial tissues (snap frozen) were procured from the UNC Tissue Procurement Facility with IRB exemption and stored at -80°C.

Real-Time RT-PCR. Total RNA from tissues was subjected to real-time reverse transcription PCR to determine the relative expression of OCT1-3, PMAT, and MATE-1, which were normalized to 18S (RNA).

Uptake Kinetics. Cells were preincubated in transport buffer for 30 minutes. Experiments were initiated by replacing transport buffer with 0.3 mL of transport buffer containing 10-4 M metformin in the absence or presence of transport-specific chemical inhibitors. At the end of the uptake period, cells were washed 3× with 4°C transport buffer and lysed. Protein content was determined by the BCA protein assay and 10-4 M metformin was measured using scintillation spectrometry.

Statistical Analysis. The statistical significance between transporter expression in tumor tissues (n=5) versus corresponding adjacent non-malignant tissues (n=5) was determined by Wilcoxon rank sum test. An independent t-test was performed to compare metformin uptake between control and inhibitor-treated cells. In all cases, P<0.05 was accepted for statistical significance.

Results

Metformin uptake into transporter-competent MDA-MB-231 cells was ~14-fold higher than in transporter-deficient BT-20 cells. OCT1-3 and MATE-1 inhibitor famotidine (50 μM) and the pan-cation inhibitor MPP+ (200 μM) decreased metformin uptake into MDA-MB-231 cells by ~50% and ~80%, respectively (p<0.05), confirming the role of cation transporters in metformin uptake.

Metformin transport across human breast cancer cell lines and endometrial cancer cell lines is mediated by OATP subfamily transporters, which suggests that metformin uptake in breast and endometrial cancer cells must be mediated by cation-selective transporters.

Figure 1. Effect of Metformin on Pathologic Complete Response (pCR) in Breast Cancer

Figure 2. Mechanisms of Cellular Entry and Antidiabetic/Anticancer Effects of Metformin

Figure 3. Metformin

Figure 4. Metformin Uptake in MDA-MB-231 and BT-20 Breast Cancer Cell Lines in the Presence or Absence of Cation-selective Transporter Inhibitors

Figure 5. Relative Transporter Expression in Human Endometrial Cancer Cell Lines

Figure 6. Relative Transporter Expression in Endometrial Tumor Tissues and Adjacent Non-malignant Tissues

Figure 7. Relative MATE1 Expression in Human Endometrial Tumor Tissues and Adjacent Non-malignant Tissues

Figure 8. Metformin Uptake as a Function of Time in the ECC1 Endometrial Cancer Cell Line

Conclusions

1. Metformin uptake studies in the two breast cancer cell lines suggest that OCT3 and MATE1 mediate metformin uptake into transporter-competent MDA-MB-231 cells, whereas uptake is negligible in the transporter-deficient BT20 cells. These data suggest that transporters are necessary for metformin cellular uptake accumulation in vivo in tumors, and demonstrate the need to screen cancer cell lines for transporter expression prior to selecting relevant cell lines for investigating the anticancer effects of metformin.

2. MATE1 is the predominant metformin transporter expressed in endometrial tumor tissues and adjacent non-malignant tissues. Based on our data, MATE1 is likely to be down-regulated in tumors, although more tissue specimens need to be analyzed to reach a conclusion.

3. ECC1 and Ishikawa cell lines are appropriate cellular models for elucidating the anticancer efficacy of metformin in endometrial cancer.

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References


