Cation-Selective Transporters Enhance the Antitumor Efficacy of Metformin in Xenograft Mouse Models of Breast Cancer

Hao Cai1, Muhammad Wahajuddin2,3, Ruth S. Everett1, and Dhiren R. Thakker1

1Division of Pharmaceutical and Experimental Therapeutics, UNC Eshelman School of Pharmacy, The University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599

Current Affiliation: 2Central Drug Research Institute, Lucknow, India

INTRODUCTION

The leading drug for type II diabetes, metformin, exerts antitumor effects against several cancers, including breast cancer12. In diabetic patients, metformin is believed to suppress cancer cell growth through inhibiting insulin secretion and activation of the insulin pathway21 and to induce apoptosis via activation of its intracellular target, adenosine monophosphate-activated protein kinase (AMPK) (Figure 1). It has also been proposed that metformin selectively kills cancer stem cells (CSCs), which are believed to be responsible for chemoresistance and cancer metastasis.

Because metformin is hydrophilic and positively charged at physiological pH values, it requires cation-selective transporters for cellular entry. Previous studies in the Thakker laboratory showed varying expression of cation-selective transporters in human breast tumor tissues (Figure 2).

Figure 2: Expression of Cation-selective Transporters in Human Non-Malignant Breast Tissue and Human Breast Tumor Tissue

OCT3 and PMAT are the predominant transporters in human breast tumor tissue (n=15); OCT3 is the major transporter in human non-malignant breast tissue (n=5), at lower levels than that observed in breast tumor tissue.

Hypothesis: Transporter-mediated tumor accumulation of metformin is required for activation of its intracellular target AMPK and its antitumor efficacy in breast cancer.

Study Design: Tumors were produced in nude mice with MCF-7 human breast cancer cell line (low cation-selective transporter expression) and OCT3-expressing MCF-7 (OCT3-MCF7 cell line) that was generated by knocking in OCT3 as a representative cation-selective transporter. Anticancer activity of metformin [plus doxorubicin (DOX)] was evaluated in these two groups of animals.

MATERIALS AND METHODS

Materials: MCF-7 cells (American Type Culture Collection [ATCC], Manassas, VA), Amara Neuroblastoma® II (Lonza, NJ), mRNA purification kits (Qiagen, CA), antibodies (Cell Signaling Technology [MA]), quinidine (Waltman Pharmaceuticals [NJ]), estrogen pellets (Innovative Research of America [FL]), DOX (Sigma Aldrich [MO]), and Metformin® (RO Bioscience [SC]) were purchased from the sources indicated in the parentheses. Dose solution for metformin uptake studies comprised of a mixture of unlabeled metformin (Sigma Aldrich, MO) and [3H]metformin (Moravek Biochemicals, CA). Methods: OCT3-MCF7 cells were generated by electroporation. OCT3 expression was confirmed by assessing metformin uptake in the presence and absence of the pan transporter inhibitor quinidine. Xenograft mouse models bearing tumors developed from OCT3-MCF7 and MCF-7 cells were generated by subcutaneous injections of 2x10⁶ cells in 50% Matrigel® into the right flank of athymic nude mice. Estrogen pellets were implanted subcutaneously to stimulate tumor growth. Metformin and DOX treatment commenced when tumor volumes reached 100 mm³. Tumors were isolated from euthanized mice at 24 h after last metformin administration, and tumors were lyzed in water with a tissue homogenizer. Tumor lysates were analyzed by Western blot analysis to assess metformin-induced activation of AMPK pathway in tumors. Paraffin-embedded tumor tissue slides were prepared by the University of North Carolina Animal Histology Facility. Tumor micronuclei and CD44 in tumor tissues were assessed by immunohistochemical (IHC) staining.

RESULTS

Figure 3: Metformin Uptake in OCT3-MCF7 Cells vs. MCF-7 Cells

Metformin uptake in OCT3-MCF7 cells was 7-fold higher than in MCF-7 cells (65.07 ± 14.57 pmol/mg protein/min). Uptake was attenuated by 85% in the presence of 500µM of the pan transporter inhibitor quinidine. Data represent mean ± SD, n=3. *p<0.001. NS not significant.

Figure 4: Expression of Cation-selective Transporters in OCT3-MCF7 Cells vs. MCF-7 Cells

Relative mRNA expression was normalized to 18S rRNA. Expression of OCT3 was significantly increased in OCT3-MCF7 cells compared to MCF-7 cells (3.46 x 10⁶ vs. 1.61 x 10⁶). Data represent mean ± SD, n=3. *p<0.05. **p<0.001. NS not significant.

Figure 5: Antitumor Efficacy of Metformin in Xenograft Mice Bearing Tumors Generated with OCT3-MCF7 Cells vs. MCF-7 Cells

Tumor weights in each treatment group were compared between OCT3-MCF7 and MCF-7 tumors. Metformin showed greater inhibition of OCT3-MCF7 tumor growth compared to MCF-7 tumors (p<0.05 vs. 0.1027 mg). Data represent mean ± SD, n=8. *p<0.001. NS not significant.

Figure 6: Metformin-mediated Activation of AMPK Pathway in OCT3-MCF7 Cells vs. MCF-7 Cells

Western blot analyses showed that metformin (DOX) treatment caused greater increase in phosphorylation of AMPK and greater decrease in phosphorylation of P70S6K in OCT3-MCF7 tumors compared to MCF-7 tumors (using GAPDH as a loading control).

RESULTS

• OCT3-MCF7 cells showed higher intracellular uptake of metformin compared to MCF-7 cells.

• In vivo studies showed that metformin (DOX) caused significantly greater inhibition of growth in tumors generated with OCT3-MCF7 cells compared to tumors from MCF-7 cells.

• Metformin (DOX) caused greater activation of AMPK and its downstream signaling event (P70S6K) in OCT3-MCF7 tumors compared to MCF-7 tumors.

• Metformin (DOX) caused greater antiproliferative effects on CSCs in OCT3-MCF7 tumors compared to MCF-7 tumors.

• Collectively, results from this study provide clear evidence that the expression of cation-selective transporters plays an important role in the antitumor efficacy of metformin in breast cancer.

CONCLUSIONS

REFERENCES


ACKNOWLEDGMENT

Financial Support by NC TraCS Institute is acknowledged. Dr. Wahajuddin was supported by the Department of Biotechnology - Cutting-edge Research Enhancement and Scientific Training Award from the Ministry of Science and Technology, Department of Biotechnology, Government of India