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The SGLT2 Inhibitor Canagliflozin Reduces Androgen Receptor Expression and Nuclear Localization of Beta-Catenin in Human Prostate Cancer Cells

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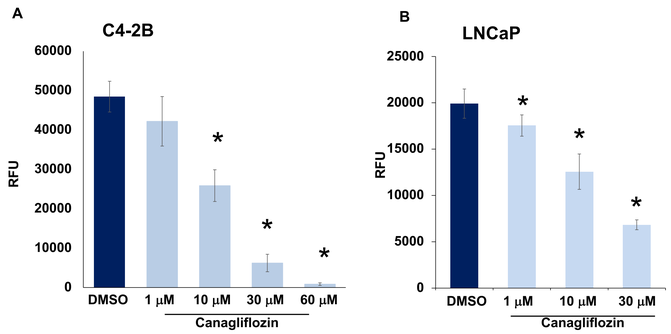
Canagliflozin-induced decreases in AR may be due in part to reductions in nuclear beta catenin levels.

ABSTRACT

Drugs that inhibit the protein sodium-glucose cotransporter-2 (SGLT2) are commonly used in the United States to treat type 2 diabetes. Recent studies suggest that SGLT2 inhibitors reduce growth of lung, liver, and breast cancer cells *in vitro*. Therefore, administration of SGLT2 inhibitors may also limit the growth of malignant tumors. Previous work from our laboratory has shown that one SGLT2 inhibitor, canagliflozin, reduces the proliferation of C4-2B cells, a castration-resistant human prostate cancer cell line. Concentrations of canagliflozin that reduce proliferation also lower levels of the androgen receptor (AR), a protein known to promote growth of prostate cancers. The goal of this study was to define the mechanism by which canagliflozin lowers AR protein levels. Since canagliflozin induces phosphorylation of adenosine monophosphate-activated protein kinase (AMPK), we initially investigated the effect of the AMPK pathway on this response. AMPK is a kinase that regulates cellular energy homeostasis. Western blot analyses demonstrated that the AMPK -within the C4-2B cells. This suggests the AMPK pathway does not significantly contribute to these reductions in AR. We next explored whether canagliflozin might alter localization of beta-catenin, a transcription factor known to increase AR expression. Western blotting showed that canagliflozin reduces the amount of nuclear beta-catenin and AR within C4-2B cells. These data suggest that reductions in beta-catenin signaling may be the mechanism by which canagliflozin lowers AR in prostate cancer cells.

RESULTS

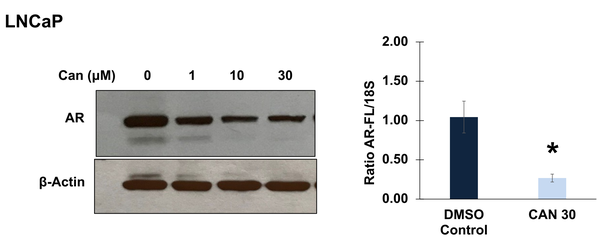
Figure 1. Canagliflozin reduced the proliferation of castration-sensitive and castration-resistant prostate cancer cells.



Each cell line was plated in 24 tissue culture plates at a density of 10,000 cells/well and allowed to attach overnight. The cells were treated for three days with DMSO vehicle or the indicated concentrations of canagliflozin. During the last two hours of treatment, cells were incubated with the Presto Blue Cell Viability Reagent. The level of cell proliferation in each well was then assessed according to the Presto Blue protocol. Each bar represents the mean ± SD for four wells. *, $P<0.05$ compared to DMSO vehicle.

Summary: Canagliflozin produced a significant decrease in the number of viable C4-2B cells and LNCaP cells. We saw the greatest decrease in cell viability at canagliflozin concentrations at ≥ 30μM.

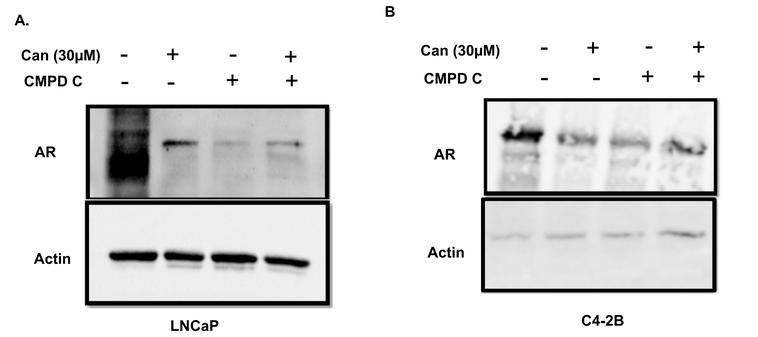
Figure 2. Canagliflozin reduces AR protein and mRNA levels.



Methods: LNCaP cells were plated in 10 cm dishes at a density of 750,000-1,000,000 cells per dish and allowed to attach for forty-eight hours. The cells then were exposed for 24 hours to DMSO vehicle or canagliflozin (30μm). A subset of cells were harvested by scraping into the media and lysed in RIPA buffer. Western blot analysis was then performed to measure AR and β- actin protein levels in each sample. In parallel plates, total RNA was extracted from treated cells. Quantitative RT-PCR was performed to measure the amount of full length AR mRNA and 18S RNA in each sample. Each bar in the bar graph represents the mean ± SD for three independent samples. *, $P<0.05$ compared to DMSO vehicle.

Summary: Canagliflozin reduces the amount of androgen receptor (AR) protein and mRNA in the LNCaP cell line. The greatest reduction was seen at Canagliflozin concentrations of 30μM.

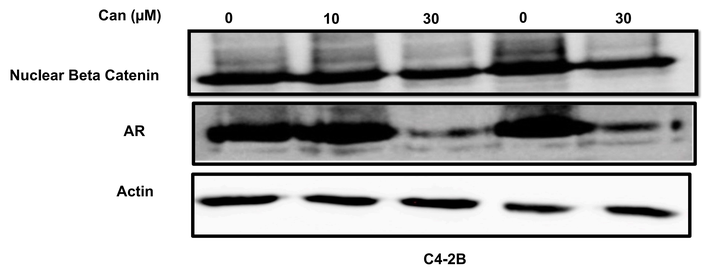
Figure 3. Compound C does not prevent canagliflozin-mediated reductions in AR protein levels within LNCaP and C4-2B cells.



Methods: LNCaP cells (A) and C4-2B cells (B) were plated in 10 cm dishes at a density of 750,000 cells per dish and allowed to attach for forty-eight hours. The cells were first pretreated with either DMSO or or compound C (5μm). One hour later, the cells were exposed for 24 hours to DMSO vehicle or canagliflozin (30μm). The cells were harvested by scraping into the media and lysed in RIPA buffer. Western blot analysis was then performed to measure AR and β- actin protein levels in each sample.

Summary: Both Compound C and Canagliflozin lowered levels of AR protein within the LNCaP and C4-2B cells. Furthermore, the combination of Compound C and Canagliflozin lowered AR protein levels as well. Therefore, the reductions in AR produced by Canagliflozin are not altered by addition of the AMPK inhibitor Compound C.

Figure 4. Canagliflozin reduces the amount of nuclear beta catenin in C4-2B cells.



Methods: C4-2B cells were plated in 10 cm dishes at a density of 750,000-1,000,000 cells per dish and allowed to attach for forty-eight hours. The cells were exposed for 24 hours to DMSO vehicle or canagliflozin (30μm). The cells were harvested by scraping into the media and lysed using the Sigma–Aldrich Nuclei EZ Prep Nuclei Isolation Kit. Western blot analysis was then performed on nuclear extracts to measure nuclear beta catenin, AR and actin protein levels in each sample.

Summary: Canagliflozin exposure not only lowered the amount of AR protein within the nucleus but also reduced the level of nuclear beta catenin in the cell. The greatest reduction of beta catenin was seen in cells treated with 30μM of canagliflozin.

SUMMARY & CONCLUSIONS

1. Canagliflozin reduces the proliferation of both the castration-sensitive LNCaP and castration-resistant C4-2B prostate cancer cells.
2. Concentrations of canagliflozin that inhibit prostate cancer proliferation also reduce androgen receptor (AR) levels.
3. Canagliflozin-stimulated reductions in AR may be due to the ability of canagliflozin to decrease the level of nuclear beta catenin in prostate cancer cells.

ACKNOWLEDGEMENTS

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