Testosterone and 11-ketotestosterone are metabolized at different rates in prostate cancer cells leading to temporal differences in their androgenic response

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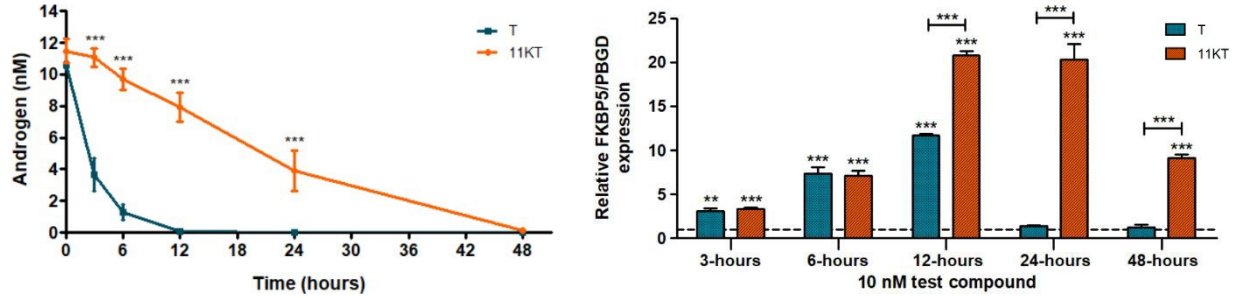

11-ketotestosterone is metabolized at a lower rate than testosterone (left) in LNCaP cells leading to prolonged androgenic activity as shown here for the androgen dependent gene FKBP5 (right).

Introduction: Recent studies have shown that 11β-hydroxyandrostenedione (11OHA4), a steroid produced abundantly by the human adrenal gland, is a precursor to the potent androgen 11-ketotestosterone (11KT).Following the discovery of 11KT in humans, it is now recognized as a full androgen receptor (AR) agonist, displaying a potency and efficacy similar to that of testosterone (T)[1]. However, we have observed that treatment of androgen dependent prostate cancer (PCa) cells with T and 11KT do not yield the same androgenic response. Given that PCa cells are known to inactivate androgens by metabolism, we set out to investigate if differential metabolism of T and 11KT in PCa cells may be responsible for the differences in androgenic response.

Methodology: The model PCa cell line LNCaP was treated with either 10 nM T, 11KT or a vehicle control and incubated over a 48-hour period. Samples were collected at several time points for the quantification of steroid levels by UHPLC-MS/MS and the analysis of gene expression by qPCR. LNCaP cells were also transiently transfected to express a yellow fluorescent protein tagged human AR to monitor the cellular localization of the AR in response to treatment with T or 11KT.

Results: 11KT was metabolized at a significantly lower rate than T in LNCaP cells. T was depleted after 12 hours, while 11KT was detectable up until 48 hours after treatment. Both T and 11KT resulted in the upregulation of the androgen-dependent genes, TMPRSS2, FKBP5 and KLK3. For T, the increases in geneexpression peaked at 12 hours and returned to baseline by 24 hours. Conversely, for 11KT, expression levels of all three genes remained significantly elevated at 24 hours, with only KLK3 returning to baseline by 48 hours. Though both T and 11KT induced translocation of the AR from the cytoplasm into the nucleus, the AR gradually translocated back into the cytoplasm after 24-hour treatment with T, while the AR remained predominantly nuclear following treatment with 11KT for up to 48-hours.

Discussion and Conclusion: Our results clearly show that while both T and 11KT are potent androgens which induce the nuclear translocation of the AR and the induction of AR-regulated gene expression, there are temporal differences in these responses due to the differences in the rate at which these androgens are inactivated by metabolism. T is metabolized at a substantially higher rate than 11KT, which likely resultsin its androgenic effects being short-lived compared to those of 11KT. Moreover, our results suggest that 11KT may accumulate at higher levels than T in prostate cancer tissue and may be an important driver of this androgen-dependent disease.

References: [1]Pretorius et al. (2016) PLoS ONE 11(7): e0159867.

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