Effects of AMD & DME on LNCaP C-81 cell proliferation in SR conditions

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Abstract

LNCaP C-81 is a model prostate cancer (PCa) cell line that represents the majority of advanced PCa population in clinic. There is increased interest in LNCaP C-81 cells due to their ability to express a functional androgen receptor (AR), secrete prostate-specific antigen (PSA) under androgen-deprived conditions and exhibit intracrine ability. In this study, we investigated ribonucleotide reductase (RR) inhibitors, AMD and DME, for their growth inhibitory activity on LNCaP C-81 cells in steroid-reduced conditions. Our results showed that both AMD and DME can inhibit the growth of LNCaP C-81 cells under regular and androgen-deprived conditions.

Introduction

Prostate cancer is the second leading cause of male cancer mortality in the United States [1]. While androgen ablation therapy delivers the first line of treatment for metastatic PCa, effective therapy is limited for advanced castration-resistant (CR) PCa patients. The challenge is finding therapy that is effective after these patients have relapsed. This study focuses on ribonucleotide reductase inhibitors (RRi), AMD and DME, as potential treatments for CR PCa in clinic. The compounds AMD and DME belong to the fused heterocyclic family, where pyridine and imidazole are fused together. (Fig.1) These compounds possess the ability to deprive iron from cells. Since, iron is one of the essential nutrients for cell growth, the depletion of iron may inhibit the RR, stopping DNA synthesis and hence inhibiting cell proliferation [2].

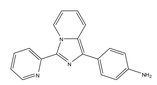
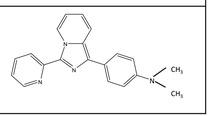
IMP-AMD	Formula Weight 286.33	
IMP-DME	Formula Weight 314. 38	

Fig. 1 Structure of AMD and DME

Hypothesis

Ribonucleotide reductase inhibitors, AMD and its derivative DME, will inhibit the growth of LNCaP C-81 cells under androgen-deprived conditions.

Methods & Materials

Briefly, LNCaP-C-81 cells were maintained as described previously [3,4]. For cell growth and immunoblot analysis, subconfluent cells were harvested and the experiments were performed as described [5,6]. All experiments were repeated in at least two sets of independent experiments in duplicates. The protein level by western blotting was semiquantified by densitometric analysis of autoradiograms. The relative protein level was then normalized to the corresponding loading control protein level.

Results

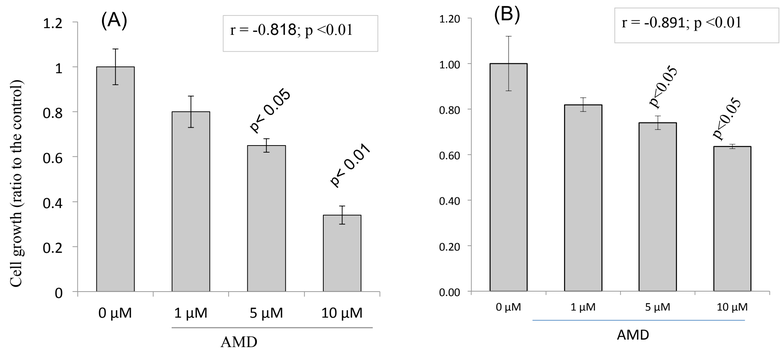


Fig. 2. The effect of AMD on LNCaP C-81 cell growth in regular culture medium (A) and steroid-reduced medium (B). The results presented were mean ± SE. $p<0.05$ is statistically significant with controls.

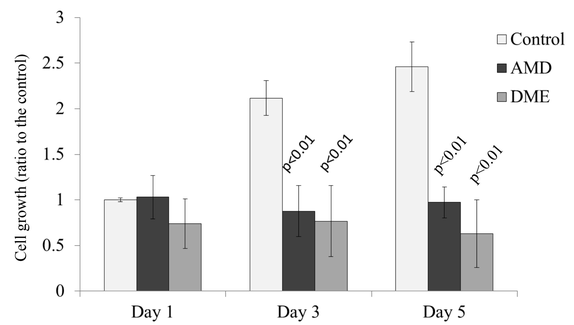


Fig. 3. The effects of AMD & DME on LNCaP C-81 cell growth in a steroid-reduced condition on a time dependent manner. The cells were plated in regular medium for three days, then steroid starved for 48 hours followed by treatment with AMD or DME at 10 µM. Cell numbers were determined at different time points. The results presented were mean ± SE. * $p<0.05$ is statistically significant to controls.

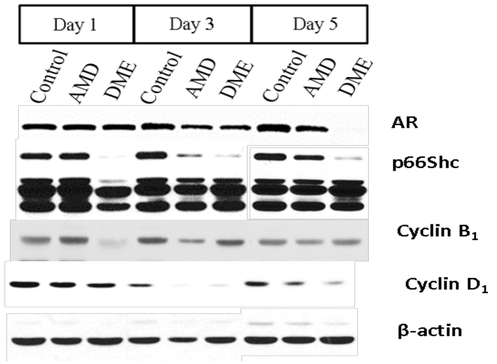


Fig. 4. The effects of AMD and DME on AR, p66Shc and cell cycle protein expression. β-Actin was detected as a loading control. The data shown is a representative from three sets of independent experiments.

Conclusion

Both AMD and DME showed an inhibitory effect on the growth of LNCaP C-81 cells following the dosage and kinetic responses in regular medium and androgen-deprived conditions. Western blot analysis confirmed the inhibitory effect. AMD serves as an important lead compound for improving CR PCa therapy. Further studies are necessary in order to determine their mode of action in cell growth inhibition as well as the mechanism for these compounds.

References

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