m-TOR inhibitors: Potential anti-neoplastic agents for Prostate cancer?

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Abstract

Introduction: m-TOR (mammalian target of Rapamycin; a kinase enzyme) inhibitors (For example Rapamycin(R), Everolimus(E), Temsirolimus(T) which were used in this study) act by inhibiting the various growth and survival signals mediated via this kinase enzyme. In this in-vitro study, their role as chemotherapeutic agents for prostate cancer is being studied.Methodology: Using LNCAP M. Luc (Prostate cancer) cell line, time and dose dependent effect of m-TOR inhibitors on cell viability was seen. The cell line was repeatedly subcultured in T150 cell culture flasks for 2 weeks before. Cells were grown in RPMI1640 media and appropriate Antibiotics (Ciprofloxacin and Gentamycin) were used to prevent contamination. Different concentrations of m-TOR inhibitors (R – 6.25µM (max conc.) and obtained 5, 2-fold dilutions and same for 'E' and 'T'; E - 125µM (max conc.); T – 12.5µM (max conc.) 5E4 cells (2mL volume media) were counted and plated in 6 well plates along with appropriate control. Additional 2mL of drugs (of different concentration) plus media were added. A chemiluminiscence assay was performed using Luciferin as substrate. Metridia Luciferase, a bioluminescent reporter gene has been used to measure real time viability of mammalian cells. Luminescence was measure using Chemi-luminescence enzyme assay with for Metridia Luciferase enzyme (incorporated in genetic code of LNCAP cells). Cell viability was measured at 37 and 61hr. Data was collected in RLU (Relative Light Unit).Results: 6.25µM of Rapamycin killed 44.7% cells in 37 hrs which increased to 58.8% in 61hrs. The least concentration of Rapamycin used, i.e. 0.390625µM also managed to kill 29.5% in 37 hrs and 50.8% in 61hrs. Similar results were obtained for Everolimus (125µM(max conc.) – 47.7% in 37hr and 53.1% in 61hr; 7.8125µM(min. conc) – 52.6% in 37hr and 58% in 61hr) and Temsirolimus (12.5µM (max. conc.) – 41.7% in 37hr and 50.2% in 61hr; 0.78125µM (min. conc.) – 6.7% in 37 hr and 23.4% in 61hr). IC 50 of Rapamycin was calculated to be 1.5625 µM; Everolimus had IC 50 83µM and that of Temsirolimus is 12.5µM.Conclusion: Plotting of RLU (Relative Light Unit) versus concentration of m-TOR inhibitor used showed an inverse relationship which proves that there is dose dependent action of drug on LNCAP cells. Also, variation in percentage cell death was observed for the two time pints (37hr and 61hr) . These preliminary results prove the sensitivity of LNCAP M. Luc prostate cancer cell line to m-TOR inhibitors.