Formulation and Evaluation of Hybrid Onconase Nanoparticles for treatment of Mesothelioma

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ABSTRACT SUMMARY

A novel hybrid nanoparticulate strategy is presented herewith to potentially deliver Onconase (ONC), a potent cytotoxic ribonuclease, extracted from the oocytes of Rana pipiens.¹ This biocompatible Albumin and Chitosan polymer based hybrid nanoparticle (HNP) system approach assured improved stability and enhanced potency of ONC.

INTRODUCTION

Mesothelioma is a complex malignant disease that develops from transformed cells originating in the mesothelium, the protective lining that covers many of the internal organs of the body.¹,² An estimated 3000 new cases per year are diagnosed in the US. Most patients die within a year, even when intensive combined therapeutic strategies are used.¹,³

Treatment of mesothelioma chiefly relies on radiation therapy, chemotherapy and surgery, with chemotherapy being the mainstay of treatment. Owing to challenges associated with many conventional chemotherapy (high systemic toxicity, multiple drug resistance etc), new therapies and antitumor drugs are constantly under exploration. ONC is a potent cytotoxic ribonuclease (RNase) that enters the cell-cytosol, degrades RNA, causing cell death and is a drug of choice used against mesothelioma.⁴ The unique mechanism of action of ONC together with its lack of immunogenicity even after its repeated administration have raised immense expectation in ONC as a promising alternative tool to currently employed DNA/RNA targeted chemotherapeutics.²

However, recently published reports on phase III clinical trial of ONC for the treatment of malignant mesothelioma have failed to display therapeutic benefits. The reports infer this lack of therapeutic usefulness was primarily due to the unfavorable pharmacokinetics and biodistribution of ONC owing to its high glomerular filtration rate, which ultimately resulting into its short plasma half life (t₁/₂<1 h) and sub-optimal tumor uptake.⁵ Moreover, after intravenous injection of ONC, 50% ONC is found in kidneys within 3 h, causing renal toxicity and limiting its efficacy. Hence, there is an urgent need to improve cell specificity, cell uptake and cytotoxicity of ONC to improve the outcome of mesothelioma treatment.

To overcome these problems and fulfill the promising therapeutic potential of ONC, we designed a novel hybrid nanoparticulate carrier for the efficient delivery of ONC. The ultimate goal of this project is to develop RGD peptide specific hybrid nanoparticles; however, the current abstract/poster reports the formulation and characterization of parent HNP system.

EXPERIMENTAL METHODS

ONC loaded hybrid nanoformulation (ONC-HNP) was prepared by desolvation method employing 3³ factorial design using different dependent and independent variables. Dependent variables were particle size, zeta potential, and entrapment efficiency while concentration of albumin (2, 4, 6 %w/v), ethanol dilution (20, 30, 40 %v/v) and total volume of non-solvent (ethanol; 70, 90, 110 %v/v) were evaluated as independent variables. In total 27 ONC-HNPs batches were taken.

The ONC-HNP was evaluated for particle size, charge, entrapment efficiency, in vitro release and cytotoxicity assay. The size and charge of ONC-HNP was measured using NICOMP 380 ZLS, USA. In vitro release studies were performed using cellulose membrane (MW cut off 100KDa) and PBS (pH 7.4) as dissolution medium to evaluate release pattern. Optimized ONC-HNP and appropriate controls were evaluated for...
cytotoxicity effect on REN malignant mesothelioma cells.

**RESULTS AND DISCUSSION**

The ONC loaded albumin nanoparticles (ONC-ANP) by desolvation technique. The ONC-ANP made of albumin, 4 %w/v; total ethanol, 90 %v/v with ethanol added as 30 %v/v dilution was found to be optimum. This optimized ONC-ANP were found to be of 28.1±2.15 nm size and showed charge of -24.34±0.80 mV. The entrapment efficiency was found to be 45.81±2.63% and released ONC in a controlled manner up to 24 h. These nanoparticles were then coated with Chitosan (0.125% w/v) to produce hybrid nanoparticles and the formulations were stabilized in presence of TPP to generate ONC-HNP. The hybrid nanoparticles elicited improved release behavior in compared to ONC-ANP. ONC-HNP showed entrapment of 48.18±3.05% and size was found to be 51.9±4.36 nm, while charge to be of 10.45±0.23 mV. The ONC-HNP showed more sustained release of ONC (upto 48 h) as compared to ONC-ANP, which released almost 80% ONC with in 24 h.

The dose dependent ex vivo efficacy of these formulations was assessed in REN mesothelioma cells against untreated cells as controls. ONC-HNP showed significantly (p<0.05) higher cytotoxicity in a dose dependant manner compared to plain ONC and ONC-ANP. The IC\textsubscript{50} of ONC, ONC-ANP and ONC-HNP were found to be 12.5±1.10, 19.9±2.19 and 5.11±0.23 µM, respectively. The increased cytotoxicity with ONC-HNP can be attributed to the tumor specific accumulation of positively charged NP by uptake through electrochemical diffusion.

The developed hybrid nanocarriers showed enhanced activity against mesothelioma cells compared to conventional albumin NP. This innovative delivery approach will help to overcome the limitations and adverse side effects associated with current delivery approaches. We are currently investigating ONC-HNP conjugation with RGD peptide to enhance the tumor specific delivery via receptor mediated endocytosis. This will spare normal cells and will increase efficacy against tumor cells.

**CONCLUSION**

From our studies it was concluded that ONC-HNP efficiently enhanced the anticancer activity of ONC in vitro. Hence, the utility of hybrid nanocarrier approach for enhanced therapeutic effect of ONC was confirmed from this study. Therefore, the ONC delivery via hybrid nanocarriers has potential for improving the anticancer activity and specificity to tumors compared to native ONC. We are further investigating the anticancer activity of developed HNP by optimizing the formulation characteristics. The results from these investigations will be important for enhancing the efficacy and clinical potential of ONC.

**REFERENCES**


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