

Radiolabelled Microspheres as a Delivery System for Targeted Radiotherapy

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ABSTRACT:

Re-188(V)Dimercaptosuccinic acid microspheres were prepared by solvent evaporation technique using varied concentration of PVA. Microspheres of (0.2-20 micron) were prepared. Drug loading and release was evaluated by I.R. spectroscopy and spectrophotometric analysis. Re-188(V)DMSA-PLGA microspheres were incubated with cultured Glioma cells (U87MG) for the assessment of in-vitro cell survival.

Keywords: Microspheres, Glioma, Dimercaptosuccinic acid, Targeted radiotherapy.

INTRODUCTION:

Radioactive microspheres are able to deliver high radiation doses to a target area without damaging the normal surrounding tissues. The effective treatment range in tissue is not more than 12 mm for β -emitters. The emitted β -electrons interact mainly with water, lose energy and lead to activate atoms. The activated species (e.g., radicals) are responsible for therapeutic effects, damage DNA of cancer cells. Radionuclide-based tumor therapy has been performed mostly with β -particle emitting isotopes due to availability and favorable characteristics (1-3). The radioactive microparticles could replace the use of isotopes alone for sustained and targeted therapy (4,5). The target size should match with the radiation range of the radioisotope to maximize the therapeutic effect and minimize the toxicity (6).

The biological system can not distinguish Tc (technetium) and Re (rhenium) and handle them in similar manner as these share the same group in periodic table (7). The pharmacokinetic of $^{188}\text{Re(V)DMSA}$ (Dimercaptosuccinic acid) have shown similar to that of $^{99\text{m}}\text{Tc(V)DMSA}$ (8). At pentavalent state, $^{99\text{m}}\text{Tc}/^{188}\text{Re(V)DMSA}$, both $-\text{SH}$ groups of DMSA are bound with $^{99\text{m}}\text{Tc}$ or ^{188}Re forming a stable complex $^{99\text{m}}\text{Tc(V)DMSA}$ had been used for the detection of neurogenic and neuroendocrine tumors. The arresting or slowing down the growth and spread of tumor is a major challenge. The radiotherapy from external sources is

restricted due to the harm imposed on normal cells. $^{188}\text{Re(V)DMSA}$ is a therapeutic analogue of imaging $^{99\text{m}}\text{Tc(V)DMSA}$. The attachment of a β -emitter / higher energy γ - emitter in lieu of positioned Tc-99m of DMSA molecule, loaded in a polymeric microspheric delivery system may be utilized theoretically as therapeutic agent for neurogenic/ neuroendocrine tumors.

EXPERIMENTAL METHODS:

Radiolabelled microspheres, $^{99\text{m}}\text{Tc}/^{188}\text{Re(V)DMSA-PLGA}$, of different sizes were synthesized with different polyvinyl alcohol concentration (14%-0.4% PVA). Primary emulsion, (w_1/o), was formed by mixing the oil phase with an aqueous DMSA solution (w_1 , (unlabeled or $^{99\text{m}}\text{Tc(V)DMSA}$; 50mCi), followed by homogenization at 10,000 rpm for 3 min (9,10). To the primary emulsion 10 ml of varying concentration of aqueous solution of PVA (10%, and 0.4% w/w) was added (w_2) and homogenized for 4 min at 10,000 rpm to form secondary emulsion ($w_1/o/w_2$). Emulsion was stirred using magnetic stirrer at room temperature for 3 h to evaporate DCM. The effect of solvent evaporation speed on microspheres was studied by using different speeds of stirring (400 or 1000 rpm). Microspheres were collected by centrifugation at 12,000 rpm for 30 min and were washed thrice with water. The amount of PLGA, DMSA, and temperature were kept constant while varied PVA (surfactant) concentrations were used for preparing microspheres. Microspheres characterization was done by scanning electron microscope (LEO 435 VP, Cambridge, U.K). The size determination and counting was done using Lieca Q-win software (Cambridge, U.K). Initial burst and release kinetics were studied using different copolymer concentration and PEG. Cells were subjected to MTT assay. The formazan crystal formation correlates well with the proportion of live cells in the plate. 3×10^3 cells/well were plated onto 96 well plates. $^{188}\text{Re(V)DMSA}$ microspheres (doses 0 mGy-200 mGy) were added to wells in triplets at an increment of 20 mGy in triplet and kept for 24h, 48h and 120h (5 days). The wells without adding $^{188}\text{Re(V)DMSA}$ microspheres were treated as control. 100 μl of DMSO was added to each well for dissolution of crystals and was immediately read spectrophotometrically at 550 nm with 690 nm as reference in an Anthos ELISA

Reader (H-L 1) with untreated cells as blank. The intensity of color developed is directly proportional to the number of viable cells. Percentage survival, for each dose, was calculated.

RESULTS AND DISCUSSION:

Microspheres of varied size-range (0.2-20micron) were fabricated using different PVA concentration. However, the microspheres size decreased as the PVA concentration was increased. Use of PLGA (75:25) and coating of microspheres with PEG decreases the initial burst. The microspheres had the tendency to accumulate in the tumor due to enhanced permeability and retention property of the tumor (11) The amount of viable cells is correlated to the absorption of the dissolved formazan salt in the MTT assay. At 24 h the percent survival of glioma cell line treated with different doses of $^{188}\text{Re(V)DMSA}$ microspheres (0-200 mGy) was between 95%-68%. The lowest survival was observed at 140 mGy and highest survival was observed at 180 mGy. The percent survival of glioma cell treated with different doses of $^{188}\text{Re(V)DMSA}$ microspheres(20-200 mGy) was 100% -162%, showing the overall increased growth as compared to untreated control cells (Figure-2). Highest growth was observed at 120 mGy and lowest growth was observed at 200 mGy. The behavior pattern of cells at 120h was similar as observed at 24h. The percent survivals of glioma cells, treated with different doses of $^{188}\text{Re(V)DMSA}$ microspheres (20-200 mGy), were 75%-48% with lowest survival at 120-140 mGy (Figure-2). From the repeated measures it was found that there was significant difference in cell survival (%) between each time point i.e. 24h with 48h, 48h with 120h and 24h with 120h ($p < 0.001$). Encapsulation of $^{188}\text{Re(V)DMSA}$ within the microspheres increased the effective half-life of $^{188}\text{Re(V)DMSA}$ in vivo however the dose-rate/total electron flux was decreased with time. After irradiated with $^{188}\text{Re(V)DMSA}$ microspheres, the cells at radiosensitive (G_2/M) phase responded to irradiation resulted declined cell survival at 24 h. Some cells might have repairable damage, survived and repaired tumor cells followed by uncontrolled growth. Additionally, tumor cell that were at G_0 phase of cell division might have entered G_1 phase resulted in overall increase in number of cell at 48 h. With time, there was sufficient amount of energy electrons available for cell destruction at their checkpoints (G_2/M) (12). This explains the cytotoxic response observed at 120 h. The cell response at higher dose (>160 Gy) might be due to a prolonged cell cycle arrest.

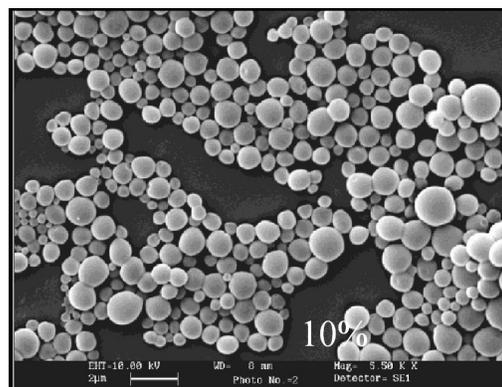


Figure-1 Electron Micrograph (SEM) of $^{188}\text{Re(V)DMSA}$ Microspheres

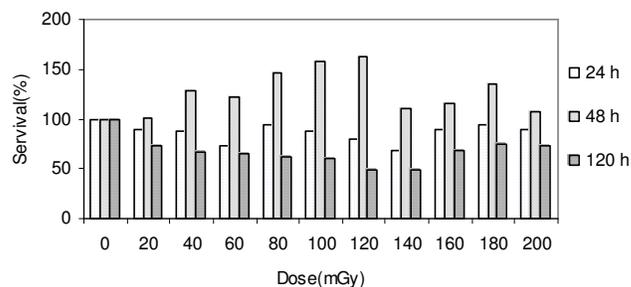


Figure-2 In vitro Cell Survival of U87 MG Glioma cells Treated With $^{188}\text{Re(V)DMSA}$

CONCLUSION:

The results indicated that $^{188}\text{Re(V)DMSA}$ loaded in PLGA microsphere, has capability to kill the Glioma cells and can be utilized as an alternative to external beam radiotherapy by manipulating the size and surface property.

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