Drug delivery system by micro-encapsulation of a radio-protective inclusion complex

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Statement of Purpose: Seleno-proteins like glutathione peroxidases act as important antioxidant enzymes preventing cellular damage from free radicals [1]. The antioxidant bioactive molecule 9-Phenyl *symm* octahydro selenoxanthene (POSX) protects tissue from cellular damage, and inhibits the differentiation of hematopoietic progenitor cells under radiation and chemotherapeutic conditions [2]. POSX forms with hydroxypropyl betacyclodextrin (HPCD) water-soluble inclusion complexes improving bioavailability, as well as the stability against atmospheric oxygen. Formulations such as suspensions, ointments or pastes are useful especially for brachy therapy in order to protect the healthy tissue areas against radio-generated radicals. The POSX-HPCD complex was encapsulated in biodegradable matrices such as poly D,L-lactic-*co*-glycolic acid (PLGA) and gelatine. The degradation kinetic PLGA depends on the average molecular weight. Both formulation routes provide tailored protection effects over extended therapeutic periods.

Methods: POSX was supplied by *medbiopharm Ltd*., Obninsk (Russia), as medical grade quality. The source of HPCD (CAVASOL®W7HP PHARMA) was *WACKER Chemie AG, Germany.* PLGA (50:50) as RESOMER® 502 H (MW 13.000) and 503 H (MW 35.000) were purchased from *Boehringer Ingelheim* (Ingelheim, Germany; now: EVONIK Roehm). Gelatine GELITA®VacciPro was delivered by *GELITA AG*, 32423 Minden, Germany). Avoiding any organic solvents, the water-solubilization of POSX was executed by dry micro-milling with HPCD (mol ratio 1:5) using a planetary micro mill (PULVERISETTE 7 PREMIUM LINE, *Fritsch GmbH*, Idar-Oberstein, Germany). The preparation of the POSX-HPCD complex loaded PLGA micro spheres were prepared from acetone solutions (2,5g PLGA, 0,5g POSX-HPCD in 90mL acetone) by spray drying in nitrogen atmosphere at 55°C (flow rate: 0,6 L/h, nozzle size: 1µm) using Mini Spray Drier B-20 (*BUECHI Labortechnik AG*, Flawil, Switzerland). The encapsulation with gelatine by spray-drying was executed with mass ratios of 1:1 and 2:1 (gelatine : POSX-HPCD). The microspheres and the entrapped POSX-HPCD were studied by SEM (ZEISS Leo 1530 VP, Germany), the particle size distribution with Delsa ™Nano C Counter (BECKMAN COULTER). The spray dried particles were dispersed in water (10 w%) using an ultrasonic homogenizer (UP200S, HIELSCHER GmbH, Germany), and stabilized against agglomeration adding 1 w% PLURONIC F-127 (SIGMA-ALDRICH). The radio-protective effect of the suspensions and the pasty mixtures of 5 w% micro spheres in polyethylene glycol gel (Macrogol DAC, CAESAR & LORENTZ GmbH, Germany) were investigated *in-vivo* in C57BL/6 mice and Wistar rats using the irradiation apparatus Luch-I endowed with a ^{60}Co -ray source (NII NPO LUCH. Podolsk, Russia).

Results: The micro-milling process at 100 r.p.m. (bowl volume: 50 mL) over a period of 5 hours achieved water soluble POSX-HPCD inclusion complex (50g/L). Shapes and particles sizes of the simultaneously spray dried PLGA and POSX-HPCD depend strongly on the content of solid in acetone and the spray conditions. Almost uniform micro spheres of 2-3 µm were obtained at 55°C, the flow rate of 0,6 L/h, 1µm nozzle size and adding 1 w% PLURONIC F-127. SEM investigations disclosed POSX-HPCD particles within the PLGA micro spheres of about 300 nm. The micro-spheres on the base of GELITA®VacciPro showed generally a lower average particle size of about 150 nm. The radio-protective investigations in mice (130 animals. 22-24g) and rats (100 animals, 110-150g) were carried out with suspensions and gels of Marcogol containing 1 w% drug delivery system. It has been found that administration of 5 mg/kg (calculated as POSX) to mice and rats seven days before irradiation (0,54 and 1,5 Gy/min; dose: 1,5 Gy) inhibits significantly the development of tumorous cells and blocks the differentiation of haemopoietic cells. Treatment with PGLA/POSX-HPCD should be started 7 days before irradiation which corresponds with the degradation kinetic of PLGA (RESOMER® 502 H). In contrast, the release of the POSX-HPCD from GELITA®VacciPro provides after about 30 minutes within interstitial medium an efficient level of protection.

Conclusions: The novel antioxidant and radio-protective 9-Phenyl *symm* octahydro selenoxanthene forms water soluble inclusions complexes with HP-cyclodextrin and can be encapsulated with biodegradable materials such as PLGA, gelatine, or alternative retarding or biodegradable matrix materials. Formulations as suspensions, ointments and pasty gels have demonstrated the inhibition of tissue damages by radiation generated radicals. The developed drug delivery system is highly recommended for brachy therapy.

References:

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