

Minimal-Dose Cancer Chemotherapy: Anti-Cancer Drugs Loaded Nanodevices towards Time, Site & Organ-Specific Releases for Development of Personalized Nanomedicine

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ABSTRACT

An attempt to design a site-directed, organ-targeting, time-manuevered, controlled drug delivery nanodevice was undertaken. The anti-cancer drug methotrexate loaded nanovesicles of human serum protein albumin, surface-functionalized with tumor-affinity protein avidin, for treatment of human mammary gland carcinoma were prepared. The formulation was designed for a minimum dose requirement with predictable span of delivery and fixed-frequency of administration towards a goal for developing personalized nanomedicine suitable for individually defined medication requirements.

Keywords: Personalized nanomedicine, site & organ specific deliveries, Minimal dose cancer chemotherapy, methotrexate, breast cancer

The ever-increasing need for improvements in cancer-care has been the driving force for advanced drug delivery systems development. The drug's poor solubility, high toxicity to healthy tissues, diminished accessibility to tumors, drug refraction and maintenance of regular and constant dose-levels at specific tumor sites are primary concerns in the desired delivery with the conventional chemotherapy used currently for cancer management. The vesicular delivery is among the choicest means for curtailing side-effects and other shortcomings in delivery including site-targeting the therapeutic load. This is getting world-wide attention [1-6].

The introduction of drug loaded micro and nanodevices, liposomes, aerosols, metal-core-protein and metal-core-

polymer entities enclosing or chemically conjugated with drugs or prodrugs are some of the delivery modes tried in this connection [7]. The nano-sized vesicles have received special attention and lately are being used for delivery and vectorization of many pharmacologically active drugs including anti - cancers, genetic and proteomic materials of biological importance [8,9]. Various natural and synthetic polymer materials based nanovesicles have been developed over the years and tried as carrier for different drugs pay-load deliveries in site-specific as well as random delivery modes [10, 11]. However, some of the problems with site-targeting of nano-vesicles still persist. An ideal drug delivery system should be able to deliver only the minimum required payload at desired rate in a sustained manner and at the exact site of action with maintaining the therapeutic concentration at the desired minimum dose levels for requisite period of time. Thus, it will reduce not only the frequency of administration but also the high chances of toxicity related to over-dosing and unwanted distribution of drugs especially in cancer treatments where majority of the drugs are highly cytotoxic to healthy tissues. At this initial stages, our work attempts to develop an integrated controlled drug delivery system to provide site-of-care therapeutic support for different cancers in a tumor-localized and personalized manner based on the individual needs for dose frequency and drug's required concentration level of medication.

A series of different nanodevices from several polymers belonging to synthetic and natural origins loaded with anti-cancer drugs were prepared. The nanodevices were surface-functionalized with for appropriate site-specific molecular recognition and were attached via a conjugating linker or adsorbed on the outer surface of the nanovesicles.

The surface functionalization of nanodevices outer-surface is essential for recognizing the host molecules of proteomic, immunological and/or genomic nature playing crucial part in personalizing the therapeutic needs. The outer-surface with their capacity to physiologically adsorb or chemically bind the designed and site-delivered or blood-stream-delivered loaded nano vesicles with the added feasibility to transmit itself through the tumor-mass areas is dictated by the physiological and biochemical characteristics during transport and at the tumor-site wherein, the outer-surface attached molecular recognition tags of the nanodevice and its compatibility with physiological environment inside the tumour is important for successful delivery of the load deep inside the organ after crossing the peripheral regions as dictated by the tumour characteristics and the reception of the nanovesicles.

The short and long chain fatty acids and intermediate molecular weight range lipid entities, proteins, the peptides of synthetic and biological nature, single amino acid based polypeptide chains, beta-amino acids, amino-sugars and long chain carbohydrates as molecular recognition tags have been tried for functionalizing the drug loaded nanovesicles via multiple-site active N-succinimide ester, sulphur based and, pH-sensitive linkers. The incubation of nanovesicles in appropriate pH based medium with the attachment-desired protein or other macromolecular structures were also tried for obtaining different tumor's permeabilities for facile transmit, site-recognition and load delivery capabilities. Several synthetic, natural and FDA-approved biodegradable and biocompatible polymers including super-para magnetic iron oxide nanoparticle encapsulated dextran, the synthetic homo and hetero polymers e.g., PLGA, PEG, OMe-PEG, PEG-polyamine, polyethylene-*block*-poly-aspartate, sebacic acid-based PCPP anhydride, Sebacic acid and fatty acid copolymer, PCPP {poly [bis (*p*-carboxy phenoxy) propane]}, the natural origins alginate, pectin, chitosan, cellulose and, biological polymers avidin and albumin were tried for effective encapsulation of anti-cancer drug methotrexate and docetaxel for deliveries to various tumors.

The tumor specific nanodevice design in conjunction with physiological criteria and biochemical factors working for the specific tumor in its particular biological stage as well as physiological status is important for determining the drug-load, site and organ specific molecular recognition tags choice for effective drug supply to the site. The transport and permeability possibilities of nanovesicles in affected tissue are crucial in effectively designing the required nanovesicles. The tumor surface receptor details about its structure, functional characteristics and compatibility with the targeting nanovesicles surface-attached recognition tags play crucial role in site based transmit and distribution of the loaded vesicles inside the tumor body. Moreover, the encapsulating polymer and nanovesicles characteristics on electro-activity, cationic, anionic nature, neutrality and partial charge including its reactivity in biological systems and temperature bearing to with stand body physiology is

important. The stipulated-time release (Hr/Days) for patient, biochemical condition of the tumor and nanodevice release nature decides the stability and overall transport behavior. The delivery modes like injectable, blood-stream release, magnetically driven injectable or dermally-released drug-load will suggest the size and physico-chemical characteristics of the final nanodevice and its drug loading concentrations. The choice of the drug also plays an important role in the preparation and function of the nanovesicle. Thus, an in-depth consideration on the tumor specificity varying with individuals and their health-conditions will decide the course of nanovesicles design in terms of its origin, characteristics and delivery capabilities at large. The analysis of quantitative releases based on established protocols for specific anti-cancer drugs in polymer encapsulation or conjugation and their biochemical behavior including any allergic or pyrogenic reactions will have the final say in the design which is developing as a prescriptive delivery mode based on individualized feedback. The designed nanovesicles integrated with safe-carriers and/or transport enhancing molecules based on needs for different organs including brain permeability and, molecular tags for site-specific recognition for tumors at specified location are crucial. The time bound targeted release capabilities, mostly affected by the encapsulating polymer's characteristics and its outer coat for a given biological or simulated medium are also considered. Thus, keeping in view of the patient's tumor physiological conditions, personal choices, therapeutic needs and delivery mode preferences, the consideration and accounting for these design factors will be a step closer to obtaining a personalized nanomedicine.

The other factors and inputs including relevant information from genome or genetic tests, disease causing internal and xenobiotic factors including life-forms, biomarker structure and characteristics details, structural and expression-related proteo-genetics influences in the tissue and other epigenetic connections to disease, personal immunological factors, molecular mechanism and specific receptor specificity, population pharmacogenetics and ecogenetics details suitable for consideration in developing therapy on an individual basis are in future arena needing attention from the personalized nanomedicine viewpoint.

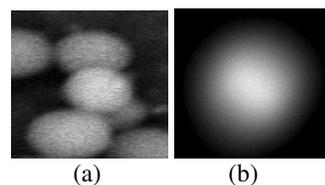


Fig. 1: (a) SEM and, (b) TEM at 5000x resolution of the functionalized nanovesicle

The study presents initial findings with regard to developing a nanomedicine towards a minimal dose, site-specific, fixed administration frequency therapeutic protocol considered for a prototypical situation. The development

was supposed as a typical design of the nanovesicle loaded with required amount of drug for time-specific deliveries and required concentrations to the desired site possessing specified transport feasibilities. The albumin nanoparticles loaded with anti - cancer drug methotrexate and outer surface functionalized with mammalian carcinoma affinity protein avidin for the on-site recognition by biotin high prevalence in the affected mammary glands tumor cells was prepared [12-13]. The effective therapeutic dose availability of the drug with every 10 days administration frequency keeping in view of minimum dose requirement at 20 µg reachable in 3 days for this formulation in simulated situations for a population of about 10⁴ malignant cells, based on previous studies on cultured carcinoma and tumor cell densities, were designed and prepared. The protocol can be changed according to dose needs based on tumor volume and its biological stage requirements for a more densified or diluted dose. A change in dose-administration frequency in conjunction with dose-level concentration can also be considered by changing the stock concentration keeping in view of the required therapeutic levels of the drug to avoid any complications arising out of escalation due to excess drug availability, inoculum effects associated with dose and tumor response. However, a low-dose methotrexate has been observed minimally toxic and effective in heavily pretreated breast cancer patients in a combination therapy with cyclophosphamide [14-15]. Thus, the delivery methodology may be employed for development of multiple drugs loading smaller sized-dose with required transport features and further effective. The multi-drug carrier design is dependent on the characteristics of the drugs and encapsulating polymer, physico-chemical interplay between components present while encapsulating, stability and shelf-life of the formulation and drugs and finally, their effective transport and release.

Finally, the development of stable solid dose-form as a lyophilized matrix resuspendable in water for administration as injectable is among the possibilities having transport and distribution considerations for dispatching the medication to different areas.

EXPERIMENTAL

Preparation of Nanovesicles

To designated biopolymer, albumin (500 mg, Human Serum, purity ca. 97%) in water (25 ml, double distilled, nanofiltered), methotrexate (disodium salt, 55 mg) was charged under slow stirring for 10 minutes. A mixture of pre-cooled *n*-hexane (60 ml), mineral oil (20 ml) and 2 ml of sorbitan sesquioleate were added dropwise to the drug-albumin mixture with stirring at 600 rpm. A pre-cooled, 25% aqueous solution of glutaraldehyde (20 ml), after replacing water by saturating it with toluene under sonication at 40% amplitude for about 3 minute (20 seconds intermittent medium strength pulse), is added dropwise with cooling

at 0°C in an ice-bath and stirred again for about 1 hr at 1000 rpm. A slowly rising temperature in a minimum of 30 minutes from 0°C to RT (25 °C) and later to 30 ± 5 °C under stirring was maintained by warm water. The heating was replaced by a RT water-bath with the slow stirring at 600 rpm until the *internal* temperature of the resultant matrix comes to RT producing the required nanovesicles which were separated by centrifugation after settling and washing twice with petroleum ether and water (25 ml x 2).

Functionalization of Nanovesicles

The albumin nanovesicles were suspended in 50 ml of deionized water, PBS buffer (pH 7.4) was added (50 ml) followed by Sulfo-SMCC (0.25 g) to the dispersion. The thiolated avidin (250 mg) and nanovesicles were reacted together at 25 °C (+2 °C) controlled temperature under a water bath with slow overhead stirring for 1.5 hrs. The dispersion was cooled to <20 °C and washed repeatedly (50 ml X 4) with PBS buffer (pH 7.4) to give the unreacted avidin free HSA-Avidin nanovesicles loaded with methotrexate which were of 60-90 nm size (Fig. 1).

Drug Release

A portion of the prepared nanovesicles (33.7% entrapment efficacy) was stirred at 600 rpm at 35±2 °C temperature and drug release was determined by UV absorbance at 302 nm. The initial results showed a release of ~ 4 % of drug in 10 hrs which stabilized after 3 days to 20 µg levels with release for over 11 days.

CONCLUSION

The surface functionalized albumin nanovesicles loaded with anti-cancer drug methotrexate prepared as an integrated controlled drug delivery system designed for a 10 day periodic injection delivering approx. 100 µg of drug for use as pre-sorted dose-defined nanomedicine which could be multiplied further for higher doses based on requirements.

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