DIRNANO

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Green chemical synthesis and testing of novel steroidal complement inhibitors

Nanomedicine involves the usage of nanoscale materials as biocompatible systems for diagnosis, delivery, sensing or actuation process in the living organism. The new advances in protein engineering and materials science have provided a pathway to the novel nanoscale targeting approaches affording new opportunities in the treatment of cancer *(1,2,3).*

The present biology and pathology data reveals that many diseases originated from malfunctioned cells. These molecules or infectious agents (bacteria, viruses) are nanometers in scale and located in biological systems that are protected by physiological barriers. The nanovehicle chemical properties, size, and shape are important factors for the transport of molecules to specific biologic compartments such as tumour sites *(4,5,6).*

Doxil® an anti-cancer chemotherapy drug delivery system based on a liposomal vehicle containing doxorubicin is intravenously administered and used in the treatment of Kaposi's sarcoma, breast cancer, ovarian cancer, and other solid tumours *(7).* This delivery system has been demonstrated to induce anaphylactic reactions in some patients that have been so severe that they have resulted in patient death. Experimental studies in pigs revealed that the Pegylated liposomal doxorubicin (Doxil®) causes hypersensitivity reactions (HSRs) due to the activation of Complement, a part of the innate immune system. Symptoms include cardiopulmonary distress with anaphylactoid shock. A clinical study also revealed a great and frequent C activation in patients *(8,9)*. So, the clinical evidence suggests that these issues caused in patients'needs to be addressed.

Complement activation by Liposomes: what constituents of liposomes are effecting the activation?

The interaction between nanomaterials and the complement system is complex and is affected by a range of carrier characteristics including nanoscale size, morphology, and surface characteristics *(10).* Anti-polyethylene glycol (PEG) antibodies are already present in the healthy individuals and as well as in individuals with active disease. Antibodies against the PEG-coated nanocarriers induces clearance of these from the systemic circulation. Rofflers' group *(11)* has demonstrated that anti-PEG IgG and IgM antibodies bind to the PEG molecules on the surface of the PEG-Coated liposomal Doxorubicin, Resulting the complement activation, formation of the membrane attack complex (C5b-9) in the liposomal membrane and rapid release of the encapsulated drug.

Is cholesterol activating complement? Why do we need to focus on the cholesterol composition in liposomes?

Cholesterol crystals (CC) activate the complement (C) system, resulting in sequestration of the particles by cells such as macrophages. The CC activate Complement through the classical pathway and this is then amplified by the alternative pathway, resulting in the complement-dependent phagocytosis *(12,13).*

Thus activation of the complement system by Cholesterol-rich liposomes *(14)* and the elevated serum level of the lipoproteins (HDL and LDL) seems to modulate the complement activation and associated adverse responses. Increase in cholesterol content to 45 mol% activated the complement enormously, resulting in a significant increase in the terminal pathway SC5b-9 levels.

The activation of the complement with 22 or 33 mol% cholesterol has demonstrated activation of the C system via classical pathway and 44 mol% cholesterol via alternative pathway by calcium (Ca^{2+}) in a dependent and independent manner respectively *(15)*.

Various approaches to reduce the recognition of complement by liposomes:

To address the clinical issues which are caused following intravenous administration of liposomes in patients we are focusing on the design on the liposomal architectures to reduce this recognition and subsequent complement activation by susceptible individuals.

This project aims on modifying the composition of the liposomes with development of a range of new cholesterol analogues using cholesterol using green chemistry. Here different fungi are used to chemically alter the structure of cholesterol. The optimised formulations containing the cholesterol analogues in the liposomal bilayers of the drug delivery vehicle are subjected to ELISA assays to see if they achieve reduced recognition of the Complement system resulting in decreased activation in the liposomes. The different combinations of

the substituted cholesterol behaviours in lipid bilayers are studied at the atomic level using *in silico* Molecular dynamics simulations and NMR studies. The combination of these will produce a predictive model system for improved design of liposomal formulations for avoidance of recognition by the Complement system.

News in the Consortium (DIRNANO):

1. Hajira Banu Haroon (ESR 4, UNEW), Prof. Moein Moghimi (UNEW), Dr. Alan Christy Hunter (UOL), Prof. Emanuele Papini (UNIPD), and Dr. Shadi Farhangrazi (SMDG) has published an article - ***Perspectives on complement and phagocytic cell responses to nanoparticles: From fundamentals to adverse reactions.*** You can find the article here.

2. Hajira Banu Haroon (ESR 4, UNEW) has started her secondment at the University of Padova in Prof. Emanuele Papini's group to perform proteomics and secondary immunological testing of Nanoparticles.

3. Carlos Pavon Regana (ESR 5, UNIPD) recently attended a 24hour poster session on twitter organised by the Royal Society of chemistry entitled - ***Dispersity within brushes as a tuning parameter for interfacial physicochemical properties***. Please, find here for more information.

4. Foivos Sokratis Lazaris (ESR 14, UR) recently attended the BIENAL VI congress of the RSEQ association in Chemical Biology in Valencia, Spain, with a poster contribution, named: ***"Methyl-to-Ethyl replacement makes the difference: Structure-Guided design of a new cancer vaccine based on a Tn Antigen Surrogate."***

News in the Field:

1. The 18th annual event of ETPN & the 5th ENM conference merged into a unique event: Nano Med Europe23 at Liverpool, UK at June 19-23,2023.

2. 4th International Future Materials Conference on Materials Science & Nanotechnology at Valencia, Spain, October 23-27, 2023.

3. Jiang et al., in their recent study, showed that Improved therapeutic effects in osteosarcoma patients due to the combination of a chemotherapeutic drug and gene therapy *(16).*

4. Sydor et al., recently published in their study that Selective manipulation of lysosomal cholesterol may be a way of attenuating lysosomal disruption and preventing silica-induced chronic inflammatory disease progression *(17).*

5. Prakash et al., Engineered "tail-flipping" nanoliposomes in a pre-clinical model, which paves the way to their development as cancer immunotherapeutics in humans *(18).*

6. Bubeck et al., recently provided the evidence of the Structural and molecular dynamics simulations which explains the membrane attack complex inhibition by CD59 *(19).*

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