editorial

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Toward improving drug delivery research in Egypt: Cancer Nanotechnology Research Laboratory (CNRL) as a role model

Pharmaceutical market and drug delivery research in Egypt

Egypt's pharmaceutical market is considered one of the top markets in the Middle East and Africa region. By 2020, it is anticipated to grow by ~US$5.3 billion because of the increased incidence of chronic diseases [1]. For long time, the pharmaceutical industry in Egypt has only been concerned with the manufacturing of conventional pharmaceutical formulations. However, in recent years, the progress in nanomedicine research globally has been translated into several nanomedicines being approved in the pharmaceutical market. In parallel, drug delivery research in Egypt has witnessed rapid progress in the past years with a special focus on the use of nanomedicines for drug delivery purposes.

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Cancer Nanotechnology Research Laboratory (CNRL) as a role model

Cancer incidence and mortality in Egypt has been progressively increasing with breast, liver and lung cancer among the most common cancer types. However, the research institutions in Egypt lack specialized centers for cancer nanomedicines. In 2014, inspired by the successful translation of some leading cancer nanomedicines into clinics, for example Doxil® and Abraxane®, Dr Elzoghby established the CNRL at the Faculty of Pharmacy, Alexandria University, Egypt, using the fund provided by the Science and Technology Development Fund (STDF). The research focus of CNRL is directed at the design of novel drug-loaded nanoparticles, mainly fabricated from proteins and polysaccharides as natural polymers, for combined cancer therapy and/or imaging with a special emphasis on lung, breast and liver cancer. We were able to pick several highly motivated, hard-working and talented researchers to constitute the backbone of CNRL in the past four years. CNRL researchers have been divided mainly into four research groups, we will spotlight some of our research in this editorial.

Inhalable nanocomposites for localized lung cancer therapy

Localized delivery of anticancer therapeutics to lung via inhalable nanoparticles holds great promise by increasing drug accumulation in lung tissues and, hence, minimizing systemic toxicity. Therefore, the first group of CNRL researchers developed inhalable spray-dried nanocomposites for targeted drug delivery to lung cancer cells. Elgohary *et al.* developed boronate-targeted albumin nanoparticles for co-delivery of etoposide and berberine (BER) for treatment of lung cancer [2]. Etoposide was transformed into a water-soluble form by phospholipid complexation which enhanced its encapsulation into albumin nanoparticles. Albumin nanoparticles were decorated with phenylboronic acid resulting in enhanced antitumor efficacy via targeting sialic acid overexpressed by lung cancer cells.

In another approach, the electrostatic interaction between the anionic polysaccharide chondroitin sulfate (ChS) and the cationic protein lactoferrin (LF) was utilized to form a nanocomplex (192.3 nm) for co-delivery of doxorubicin (DOX) and ellagic acid (EA) to lung cancer cells [3]. The high uptake and cytotoxicity of nanocomplexes against A549 lung cancer cells was attributed to preferential binding of LF and ChS to transferrin and CD44 receptors of those cells, respectively. Pre-formulation of EA into water-soluble nanocrystals enabled sequential release demonstrated as fast EA release followed by slow release of DOX from the nanocomplex. Such a release pattern helped sensitize lung cancer cells to the cytotoxic action of DOX.

In a novel approach, rapamycin (RAP) and BER showed extremely different release patterns from lipid nanoparticles owing to their differential solubility. Therefore, pre-formulation approaches were attempted to reduce the release gap between the quickly released BER and slowly released RAP [4]. Complexation of BER with the negatively charged sodium lauryl sulfate increased its lipid solubility and hence reduced its release from the lipid core nanoparticles. By contrast, the release of RAP was enhanced by its complexation with phoshatidylcholine forming a bilayer encapsulating the BER lipid core nanoparticles.

Finally, to avoid rapid exhalation of those previously mentioned (albumin, LF, ChS and lipid) nanocarriers and facilitate effective lung localization, the nanoparticles were converted to microparticles by spray-drying with inert carriers [2–4]. Using mannitol, maltodextrin or leucine as carriers, the resultant inhalable nanocomposites displayed optimal aerodynamic properties (MMAD: Mass median aerodynamic diameter ~2–3 μm, FPF: Fine particle fraction ~63–89%), as well as a low re-dispersibility index (~1). Thus, the nanocomposites would be readily reconstituted into their primary nanoparticles in the lung aqueous environment. In lung cancer animal models, the inhalable nanocomposites showed enhanced antitumor efficacy over the inhaled free drugs and the intravenous nanocarrier suspension. This was manifested as significant reduction in lung weight, tumor angiogenic markers, as well as number and diameter of metastatic lung foci.

Dual-targeted green nanomedicine for synergistic phytotherapy of hepatocellular carcinoma

The second group of CNRL researchers focused to develop green nanomedicines for cancer therapy based on natural biocompatible polymers and herbal drugs. We have developed two types of amphiphilic nanomicelles based on the milk protein casein and the polysaccharide maltodextrin to entrap hydrophobic drugs. In the study by Abdelmoneem *et al.*, two herbal medicines: BER and diosmin (DSN), were co-loaded into generally recognized as safe (GRAS) casein micelles (CAS-MCs) [5]. The poorly soluble DSN was solubilized within the hydrophobic core of CAS-MCs based on a pH-modulation strategy, thus enabling its parenteral delivery. By contrast, combined hydrophobic ion pairing with sodium deoxycholate and genipin, crosslinking of CAS-MCs was successfully utilized to reduce the initial burst effect of BER.

In the second approach, the water-insoluble ursodeoxycholic acid (UDCA) was coupled to the natural hydrophilic oligosaccharide maltodextrin (MD) resulting in amphiphilic MDCA nanomicelles [6]. The water-insoluble drug, sulfasalazine (SSZ), with potential anticancer effect, could be chemically coupled to MD via an ester bond to ensure its release only at tumor sites, and hence reducing its systemic toxicity. For synergistic activity, the herbal compound resveratrol (RSV) was encapsulated into the core of MDCA micelles, to increase its chemical stability and enabling its injection. Dual functionalization of CAS and MDCA micelles by lactobionic acid (LA) and folic acid (FA) significantly improved their tumor targetability via binding to asialoglycoprotein (ASGP) and FA receptors overexpressed by HepG2 cells [5,6]. The powerful anticancer effect of dual-targeted CAS- and MDCA-MCs was confirmed in HCC-bearing mice by decreasing liver weights and tumor growth biomarkers. Moreover, the enhanced accumulation of fluorescent-labeled CAS micelles in hepatic tumors was visualized by *ex vivo* imaging.

Multicompartmental nanocarriers for combined therapy of breast cancer

Recent approaches focus on the development of multicompartmental nanocarriers for combined delivery of anticancer drugs to promote synergism and suppress drug resistance. Therefore, the third group of CNRL researchers are engaged with the development of multireservoir nanoparticles for combined breast cancer therapy. Protamine-coated oily core nanocapsules (PMN-NCs) were prepared for combined delivery of the aromatase inhibitor letrozole (LTZ) and cyclooxygenase-2 inhibitor celecoxib (CXB) [7]. PMN-NCs were coated with PEGylated CXB-phospholipid complex bilayer. This multicompartmental nanocarrier conferred biphasic CXB delivery characterized by a relatively faster CXB release from the outer phospholipid corona followed by slow release of CXB and LTZ from the oily core.

Other multireservoir nanocarriers were fabricated for combined delivery of Monascus yellow pigments (MYPs) isolated from red mold rice and RSV [8]. The water-insoluble MYPs, monascin and ankaflavin were incorporated within the hydrophobic core of CAS-MCs. Then, MYP-loaded CAS-MCs were encapsulated within the PEGylated RSV-phytosomal bilayer. Both multicompartmental nanocarriers: PEGylated phospholipid bilayer enveloped PRM-NCs and CAS-MCs, showed superior *in vivo* antitumor efficacy in Ehrlich ascite tumor-bearing mice [7,8].

Hybrid natural polymer inorganic nanotheranostics for breast cancer therapy and imaging

The focus of the fourth group of CNRL researchers was not only to improve the delivery of therapeutic and imaging contrast agents but also to track their distribution within the breast tumor. Abdelhamid *et al.* fabricated two types of hybrid nanotheranostics by combining the merits of highly fluorescent quantum dots (QDs) with the favorable drug delivery characteristics of polymeric-shell oily-core nanocapsules. The oily core of the nanocapsules enabled solubilization and parenteral administration of CXB and rapamycin (RAP) or CXB and honokiol, respectively, for synergistic growth inhibition of breast cancer cells [9,10]. To enable tumor targeting, a layer of ChS was deposited onto the positively charged core to enhance the cellular internalization via CD44-mediated endocytosis. In the first approach, to overcome the nonspecific binding of ChS-NCs with normal cells during systemic circulation, a cationic gelatin layer was electrostatically deposited onto the surface of the negatively charged ChS-NCs. Cadmium telluride (CdTe) QDs were conjugated to the gelatin shell via a tumor-cleavable bond to offer image-guided tumor therapy. At the tumor microenvironment, the gelatin layer was degraded by the overexpressed matrix metalloproteinases (MMPs), thus enabling ChS-CD44-receptor-mediated internalization into cancer cells [9]. In the other approach, a positively charged layer of LF glycoprotein was electrostatically deposited onto the anionic ChS-NCs to enhance tumor targeting via binding to low-density lipoprotein (LDL) receptors. The fluorescence of QDs was quenched upon coupling to LF via a tumor-cleavable bond (OFF state) as a result of an electron/energy transfer mechanism [10]. Upon intracellular uptake, the fluorescence was restored (ON state), which enabled imaging of cancer cells. Both types of NCs demonstrated high cytotoxicity against breast cancer cells as well as superior *in vivo* antitumor efficacy. After four years of extensive research at CNRL, the output was very encouraging: four PhD and 15 Master's theses, 32 published articles in high impact journals, four book chapters, 13 submitted patent applications and 34 conference posters. The government should aim to replicate this experience by establishing specialized drug delivery research centers and laboratories to provide novel drug delivery systems and advanced treatment strategies for chronic diseases in Egypt.

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