ROLE OF NANOTECHNOLOGY IN PHARMACOLOGICAL AND BIOTECHNOLOGY RESEARCH: AN OVERVEIW

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Summary

Nanotechnology is expected to be the basis of many of the main technological innovations of the 21st century. It is a new area of science that involves working with materials and devices that are at the nanoscale level research and development in this field is growing rapidly throughout the world. For the past few decades, there has been a considerable research interest in the area of pharmacology, toxicology and biotechnology using particulate delivery systems as carriers for small and large molecules. Nanotechnologies may have properties of self-assembly, stability, specificity, drug encapsulation and biocompatibility as a result of their material composition .So this technology has been used as a therapeutic approach to alter and improve the pharmacokinetic and pharmacodynamics properties of various types of drug molecules and for treating the many disease conditions. Nanotechnology is being applied to biomarker-based proteomics and genomics technologies. This article aims at providing an updated knowledge regarding the usage of nanotechnology in the fields of pharmacology and biotechnology .We review various aspects of nanoparticles and their applications in chemotherapy and nanobiotechnology.

Key words: Nanotechnology, Pharmacology, Biomarkers, Biosensors

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Introduction

Nanotechnology refers to a field of applied science whose theme is the control of matter on an atomic and molecular scale. Nanotechnology is expected to be the basis of many of the main technological innovations of the 21st century. Research and development in this field is growing rapidly throughout the world. A major output of this activity is the development of new materials in the nanometer scale, including nanoparticles.

The need for the development of new medicines is pressing, and given the inherent nanoscale functions of the biological components of living cells, nanotechnology has been applied to diverse medical fields such as oncology and cardiovascular medicine. Indeed, nanotechnology is being used to refine discovery of biomarkers, molecular diagnostics, and drug discovery and drug delivery, which could be applicable to management of these patients. The National Institutes of Health (USA) reviewing the use of nanotechnology in human diseases introduced the term of 'nanomedicine' to describe such applications. To achieve these aims, nanotechnology strives to develop and combine new materials by precisely engineering atoms and molecules to yield new molecular assemblies on the scale of individual cells, organelles or even smaller components, providing a personalized medicine. Personalized medicine is individualized or individual-based-therapy which allows the prescription of precise treatments best suited for a single patient (1). In the last few years, several pharmacological companies won approval from the food and drug administration (FDA) in the US for the use and development of nanotechnology based drugs.

Recent development and advance on nanotechnology has brought new insight into the area of biotechnology. The detection and formulation of various chemical and biological agents using nanostructured materials is a hot topic under discussion. Due to the comparable size of biomolecule such as antibodies, peptides and DNA with nanoparticles, the understanding of the self assembly of these materials and the cause and cure of the related disease is relying on the understanding of nanoparticle formation and utilizations. A main focus of biotechnology is to discover the cause of genetic disease, to develop a cure and to develop an effective delivery device of the cure.

This review article aims at providing a balanced update of these exciting pharmacological and biotechnology developments. The classes of nanoparticles, the current status of nanoparticle use in pharmacology and nanobiotechnology will be discussed.

NANOTECHNOLOGY IN CANCER RESEARCH:

Nanotechnology has the potential to offer solutions to these current obstacles in cancer therapies, because of its unique size (1-100nm) and large surfaceto- volume ratios (2). Nanotechnologies may have properties of self-assembly, stability, specificity, drug encapsulation and biocompatibility as a result of their material composition (3).

Kommareddy and Amiji were used poly ethylene glycol (PEG) surface modified thiolated gelatin nanoparticles to test drug and gene efficacy on breast cancer cells. Studies demonstrated prolonged circulation due to the hydrophilic PEG-modified surfaces. The PEG-modified thiolated gelatin nanoparticles had a high half-life of 37.8 h in the tumor mass. Also, drug release was administrated through thiolated gelatin nanoparticles that are highly sensitive to reducing environments, similar to those found in tumor cells (4). An article by sathe et al.,(5) demonstrated the use of nanotechnology in cancer detection. Nie team developed dual-functioning beads comprised of quantum dots and iron oxide nanocrystals embedded in silica beads. These particles were able to target specific cells, due to the iron oxide crystals, and have high imaging qualities, due to the quantum dot component. Currently, they are determining ideal combinations of iron oxides and quantum dots that would give the best imaging qualities (5). The study by Ramchandra reddy *et al.,*(6) reveals the versatility and efficacy of the multifunctional nanoparticle for the targeted detection and treatment of brain tumors. The nanoparticles specifically bound to the surface of MDA-435 cells in vitro and were internalized conferring photosensitivity to the cells. Significant magnetic resonance imaging contrast enhancement was achieved in i.c. rat 9L gliomas following i.v. Nanoparticle administration.

NANOPARTICLE DRUG DELIVERY SYSTEMS IN CHEMOTHERAPY OF TUBERCULOSIS:

One of the major problems is noncompliance to prescribed regimens, primarily because treatment of TB involves continuous, frequent multiple drug dosing. Adherence to treatment and the outcome of therapy could be improved with the introduction of long-duration drug formulations releasing the antimicrobial agents in a slow and sustained manner, which would

allow reduction in frequency and dosing numbers. One way to solve this problem is the development of colloidal drug delivery systems. Liposomes are a well-known example of this strategy (7). Other drug carriers (such as nanoparticles) represent an attractive alternative to liposomes. Pandey and colleagues (8) demonstrated that the Nanoparticles provided sustained release of the anti-TB drugs and considerably enhanced their efficacy after oral administration. Three frontline drugs, rifampin (RMP), isoniazid (INH), and pyrazinamide (PZA) were coencapsulated in poly(lactide-co-glycolide) (PLG) nanoparticles. After a single oral administration of this formulation to mice, the drugs could be detected in the circulation for 4 d (RMP) and 9 d (INH and PZA); therapeutic concentrations in the tissues were maintained for 9 to 11 d. In contrast, free (unbound) drugs were cleared from the plasma within 12 to 24 h after administration. Treatment of *M. tuberculosis*–infected mice with the nanoparticle-bound drugs (five oral doses every 10th day) resulted in complete bacterial clearance from the organs.

Free drugs were able to produce bacterial clearance only after daily administration of 46 doses. Similar efficacy of the nanoparticle-bound drugs was also observed in guinea pigs (9). The pharmacokinetics and antibacterial effect of the nanoparticle bound anti-TB drugs administered via respiratory route was investigated in guinea pigs (8). Preferential uptake of nanoparticles by macrophages (mainly by Kupffer cells in the liver) is achieved by the physicochemical properties of the carrier and by physiologic opportunity, thus representing an example of passive delivery. This technology improves drug delivery to macrophages, increasing the amount of the drug reaching this target site, which allows reduction of the overall therapeutic dose and decrease of the adverse effects. Although identifying novel anti-TB agents remains a priority, the development of the nanoparticle-based delivery systems for currently used agents may represent a cost-effective and promising alternative.

NANOTECHONOLOGY IN DIAGNOSTICS

Nanotechnology is being applied to biomarker-based proteomics and genomics technologies. Nanoparticles can be used for qualitative or quantitative *in vivo or ex vivo* diagnosis by concentrating, amplifying and protecting a biomarker from degradation, in order to provide more sensitive analysis (10). *In vitro* streptadivin-coated fluorescent polystyrene Nanoparticles have been used to detect the epidermal growth factor receptor (EGFR) in human epidermoid carcinoma cells by flow cytometry (11). These results were really successful as nanoparticles

enhanced the sensitivity to detect EGFR compared to the conjugate streptadivin– fluorescein. In addition, a nanoparticle oligonucleotide bio-barcode assay has been used to detect small levels of the cancer marker prostate-specific antigen (PSA) in serum (12). The use of this new technique offers a high ratio of PCR-amplifiable DNA to labelling antibodies that can considerably enhance assay sensitivity. Therefore, a low amount of free serum PSA could be detected in patients suffering from prostate cancer or even women suffering from breast cancer with a great improvement in tumour screening and diagnosis (12). Nanoparticles are currently being tested for molecular imaging in order to achieve a more precise diagnosis with high-quality images. In fact, contrast agents have been loaded onto nanoparticles for tumour and atherosclerosis diagnosis Different nanoparticles have been used for molecular imaging with magnetic resonance images (MRI), ultrasound, fluorescence, nuclear and computed tomography imaging (13).

NANOPARTICLE DRUG DELIVERY SYSTEMS

The use of pharmacological agents developed using classical strategies of pharmacological development is frequently limited by pharmacodynamics and pharmacokinetics problems such as low efficacy or lack of selectivity. Moreover, drug resistance at the target level owing to physiological barriers or cellular mechanisms is also encountered. In addition, many drugs have a poor solubility, low bioavailability and they can be quickly cleared in the body by the reticuloendothelial system. Furthermore, the efficacy of different drugs such as chemotherapeutical agents is often limited by dose-dependent side effects. Indeed, anticancer drugs, which usually have large volume of distribution, are toxic to both normal and cancer cells. Therefore, precise drug release into highly specified target involves miniaturizing the delivery systems to become much smaller than their targets. With the use of nanotechnology, targeting drug molecules to the site of action is becoming a reality resulting in a personalized medicine, which reduces the effect of the drug on other sites while maximizing the therapeutic effect. This goal is mainly achieved by the small size of these particles, which can penetrate across different barriers through small capillaries into individual cells. Several anticancer drugs including paclitaxel (14), 5-fluorouracil (15) and doxorubicin (16) have been successfully formulated using polymers and liposomes as drug delivery systems. However, further investigation is needed to control the drug release profile and to guide nanoparticle delivery systems to the specific target.

In addition, *in vivo* studies are needed to study plausible toxicological effects derived from body accumulation of non-biodegradable nanoparticles. Biodegradable nanoparticle-based vaccines, for oral vaccination, are also in development and may allow targeting of antigens to specific dendritic cell receptors (17).

TOXICITY OF NANOPARTICLES

Epidemiological studies have shown that urban pollution with airborne PM deriving from combustion sources such as motor vehicle and industrial emissions contributes to respiratory and cardiovascular morbidity and mortality (18,19,20) One of the main mechanisms of lung injury caused by combustion-derived nanoparticles is via oxidative stress leading to activation of different transcription factors with up regulation of proinflammatory protein synthesis (21). In fact, activation of mitogen-activated protein kinase and nuclear factor-kappa B signal pathways by combustion-derived nanoparticles can culminate in transcription of a number of pro-inflammatory genes such as IL-8, IL-6 and TNF-a (22,23).

The translocation of nanoparticles to CNS may not only take place as a result of systemic distribution. The other mechanism involves the uptake of nanoparticles by sensory nerve endings embedded in airway epithelia, followed by axonal translocation to ganglionic and CNS structures. In addition, nanoparticles can be taken up by the nerve endings of the olfactory bulb and translocated to the CNS. It has been found that C60 fullerenes can induce oxidative stress in the brain of largemouth bass via the olfactory bulb (24).

Nanoparticles can be ingested into the gut by many ways. For example, nanoparticles can be ingested directly from the food, water, drugs and cosmetics, but inhaled nanoparticles can also be ingested by GI tract once they are cleared by respiratory tract (25). Following uptake by GI tract nanoparticles can translocate to the blood stream and distribute all over the body (26). Recently, it has been shown that Cu nanoparticles administered via oral gavage can induce adverse effects and heavy injuries in the kidney, liver and spleen of experimental mice compared to micro-Cu particles (27).

NANO-DNA TECHNOLOGY

The discovery of the polymerase chain reaction (PCR) (28) paved the way to a new era of biological research. The impact can be felt not only in the field of molecular biology, but also in other allied fields of science. Novel classes of semi-synthetic DNA-protein conjugates, self-

assembled oligomeric networks consisting of streptavidin and double-stranded DNA, which can be converted into well-defined supramolecular nanocircles have been developed (29).

The DNA-streptavidin conjugates are applicable as modular building blocks for the production of new immunological reagents for the ultrasensitive trace analysis of proteins and other antigens by means of immuno-PCR methodology (30). Immuno-PCR is a combination of the specificity of an antibody-based immuno-assay with the exponential power of the amplification of PCR, hence resulting in a 1000-fold degree of sensitivity as compared with standard ELISA (Enzyme-linked immunosorbent assay) methods.

Self-assembled DNA-streptavidin conjugates have also been applied in the field of nanotechnology. For example, the conjugates are used as model systems for ion-switchable nanoparticle networks, as nanometre-scale 'soft material' calibration standards for scanning probe microscopy (31), or as programmed building blocks for the rational construction of complex biomolecular architecture, which may be used as templates for the growth of nanometre-scale inorganic devices (32).

NANOBIOTECHNOLOGY IN HIGH-THROUGHPUT SINGLE NUCLEOTIDE POLYMORPHISM ANALYSIS

Following the publication of a map of variation in the human genome sequence containing over two million single nucleotide polymorphisms (SNPs) (The International SNP Map Working Group, 2001), the next challenge is the development of the technologies to use this information in a cost-effective manner. Genotyping methods have to be improved in order to increase throughput by at least two orders of magnitude to enable pharmaceutical, biotechnological and academic research to uncover associations between genetic variants and diseases, with consequent potential for the development of novel diagnostics and therapies.

The potential for nanotechnology to contribute to rapid high-throughput SNP analysis is most evident with smart biochip platforms. The development of an electronically addressable microarray platform as described by Heller *et al.,*(33) has given rise to Nanogen Inc. (San Diego, California, USA). The challenge of providing one or more technology platforms capable of SNP screening throughput of the order of 107 genotypes per day will need to be achieved, to allow significant associations between genes and diseases to be established.

NANOPARTICLES AS BIOMARKERS

Nanoparticles can be used for both quantitative and qualitative in vitro detection of tumour cells. They enhance the detection process by concentrating and protecting a marker from degradation, in order to render the analysis more sensitive. For instance, streptavidin-coated fluorescent polystyrene nanospheres Fluospheres® (green fluorescence) and TransFluospheres® (red fluorescence) were applied in single colour flow cytometry to detect the epidermal growth factor receptor (EGFR) on A431 cells (human epidermoid carcinoma cells) (11). The results have shown that the fluorescent nanospheres provided a sensitivity of 25 times more than that of the conjugate streptavidin-fluorescein.

New tools can now be developed, designed at the intersection of proteomics and nanotechnology, whereby nanoharvesting agents can be instilled into the circulation (e.g. derivatized gold particles) or into the blood collection devices to act as ″molecular mops″ that soak up and amplify the bound and complexed biomarkers that exist (34,35). These nanoparticles, with their bound diagnostic cargo, can be directly queried via mass spectrometry to reveal the low molecular weight and enriched biomarker signatures. Ultimately, utility of any approach for detecting disease is assessed on its clinical impact to patient outcome and disease-free survival (36). What is urgently required in the study of diseases in general, is the development of biomarkers that can detect curable diseases earlier, and not detecting advanced disease better.

Most disease begins at the cellular and molecular levels. However, the tools of modern medicine are too large and cumbersome to reach disease at this stage. With nanotechnology, we will be able to have computer-controlled machines that are much smaller than a human cell that can address disease at the cellular and molecular levels. No one is sure how long these innovations will take-it could be years or decades-but at some point nanotechnology will likely allow us to remove obstructions in the circulatory system, kill cancer cells, repair organs, create artificial mitochondrion and view tissue samples with extraordinary detail.

Within a couple of years, scientists hope to use nanotechnology to detect the location of viruses in the body. The process would involve injecting magnetic nanoparticles into the bloodstream and would potentially allow more precise virus treatments to be developed. Although it is largely still in the experimental stages, nanotechnology is growing fast.

The next step in the nanotechnology adventure is in the realm of nanomedicine, where nanotechnology is expected to contribute to significant developments improving the health of human beings. Nanomedicine is an offshoot of nanotechnology, which refers to a highly specific medical intervention at the molecular scale for curing disease, or repairing damaged tissue, such as bone, muscle, or nerve.

Conclusions

The advance in nanotechnology has opened up various opportunities, especially in the area of biotechnology. In this review, we focus different research areas pharmacology and biotechnology where the nanotechnology has been used. We discussed various bioapplications of nanotechnology have been discussed. Future step is needed to increase the application of nanotechnology in the research of pharmacology and biotechnology.

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