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Investigation of α-IFN –SWNT and α-IFN-PLGA Effects on Breast Cancer in Rats induced by DMBA by using CA15-3 Tumor Marker

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ABSTRACT
Conventional anticancer drugs display significant shortcomings which limit their use in cancer therapy. For this reason, important progress has been achieved in the field of nanotechnology to solve these problems and offer a promising and effective alternative for cancer treatment. Tumor markers may also be measured periodically during cancer therapy. Tumor markers may also be measured after treatment has ended to check for recurrence the return of cancer. The aim of this study was to evaluate the effect of nano drug delivery in induced breast cancer with DMBA by using CA15-3 tumor marker. The rats were divided into five groups .the first group (control n=15) were fed only sesame oil as a gavage. In the second group $^{n=15}$, 10 mg DMBA was dissolved in 5ml of sesame oil and were fed as a gavage. In addition to DMBA treatment as the second group, in the 3,4and 5 groups after cancer creation, respectively affected by alpha interferon (α-IFN), alpha interferon conjugated with single walled carbon nano tube (α-IFN-SWNT) and encapsulated in poly lactic poly glycolic acid (α-IFN-PLGA). Tumor marker was measured in recent three groups. The ANOVA test was used to determine the differences among the groups. Cancer inducing in rats (group 2) caused a significant increase in blood levels of CA15-3 (P<0.05). Administration of α-IFN ,α-IFN –SWNT and α-IFN-PLGA in 3 groups of cancerous rats caused a significant decrease in blood levels of CA15-3 only the group that treated with α-IFN-PLGA (p<0.05). The results of this study indicate that nano drugs more effective than traditional drug in cancer treatment, although further work is needed to elucidate the safety and side effect of these compound in human.
Key words: breast cancer, nano drug, tumor markers, CA15-3, α-IFN-PLGA, -IFN –SWNT

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INTRODUCTION

Cancer development occurs when cells in a part of the body begin an 'out-of-control' growth of abnormal cells, and instead of dying, they outlive normal cells and continue to form new abnormal cells. Hanahan and Weinberg have highlighted six hallmarks of most cancer, if not all. Cancer cells acquire autonomy from growth signals, evasion of growth inhibitor signals, evasion of apoptotic cell death, unlimited replication potential, angiogenesis, and invasion andmetastasis, of which all are essential for carcinogenesis [1].

Breast cancer is the most common malignant tumor among women, and of all cancers, it is the second leading cause of mortality in women in the US. Estimates for 2013 predicted that 232,340 women were likely to be diagnosed with invasive breast cancer and that 39,620 women were likely to succumb to this disease [2].

Several serum markers have been developed in different types of cancer as tools for non-invasive assessment of the tumor burden, mostly in metastatic patients. Quantitative variations of serum markers are, therefore, often used in several cancer types as noninvasive tools to assess treatment efficiency in metastatic patients. However, the use of serum tumor markers faces several issues and unanswered questions: their specificity and sensitivity are considered as low and no clear consensus exists on what threshold and/or variation should be considered clinically significant and which serum marker to follow. In breast cancer, the commonly used serum markers are carcinoembryonic antigen (CEA), cancer antigen 27.29 (CA 27.29), and cancer antigen 15.3 (CA 15-3) [3].

For many malignancies, serum tumor markers play an important role in patient management. In breast cancer, however, the role of serum markers is less well established. The most widely used serum markers in breast cancer are CA 15-3 and carcinoembryonic antigen (CEA). Less widely used markers include BR 27.29 (also known as CA27.29), tissue polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS) and the shed

form of HER-2 .The potential uses of serum markers in breast cancer include aiding early diagnosis, determining prognosis, prospectively predicting response or resistance to specific therapies, surveillance after primary surgery, and monitoring therapy in patients with advanced disease. The aim of this review is to examine the role of serum tumor markers in the detection and management of patients with breast cancer. As CA 15-3 is the most widely used serum marker in breast cancer, most of the review will focus on it [4].

The use of nanotechnology for drug delivery systems is an actual subject today. A lot of research is taking place at different universities in the world in order to find new formulations capable of delivering drugs to specific areas of the body[5].

The extensive research on carbon nanotube done to their structural characterization and discovered by Iijima in1991 year. Among the numerous delivery systems currently under investigations, single-walled carbon nanotubes seem to embody a promising option . Single-walled carbon nanotubes are made up of carbon atoms arranged in a series of condensed benzene rings and wrapped into a tubular form [5].

SWNTs have been explored as novel drug delivery vehicles in vitro. SWNTs can effectively shuttle various bio-molecules into cells including drugs, peptide, proteins,plasmid DNA and small interfering RNA ,siRNA,via endocytosis [6].

For the past few decades, there has been a considerable research interest in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. Particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. They have been used in vivo to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing side effects [7].

Poly(lactic-co-glycolic acid) (PLGA) is another biocompatible and biodegradable polymer, composed of lactid acid (left repetitive structure in Figure 5) and of glycolic acid (right repetitive structure in Figure 5). Indeed, PLGA hydrolyzes into lactid acid and glycolic acid in the presence of water, two compounds which are commonly present in the body because they are by-products of metabolic reactions in the body. PLGA is preferred to polylactic acid (PLA) and polyglycolic acid (PGA), as it has a better solubility in organic solvents and the degradation time of PLGA can be more easily controlled. This can be done by varying the ratio lactid acid: glycolic acid. The degradation time of polylactide is much longer than for polyglycolide due to a higher hydrophobicity and a month against 5-6 months [8].

Among the multiple experimental animal models employed for analyzing the various aspects of mammary carcinogenesis, the induction of mammary tumors in rats by chemical carcinogens is one of the models most utilized. In vivo experimental animal models provide information not available in human populations; they are adequate for hazard identification, dose-response modeling, exposure assessment, and risk characterization, the four required steps for quantifying the estimated risk of cancer development associated with toxic chemical exposure. Using the DMBA (7, 12 dimethylbenz(a)anthracene) rat mammary model [9].

The prognostic contribution of CA 15.3 was highly significant. Log relative hazard of relapse was constant until approximately 10 (U/ml) of CA15.3 and increased thereafter with increasing marker levels. CA15.3 showed a significant contribution using as a cut-off point a value of 31 U/ml. However, the contribution to the model of the marker as a continuous variable is much greater. From these findings, we can conclude that: (i) CA15.3 is a prognostic marker in node-negative breast cancer; (ii) its relationship with prognosis is continuous, with the risk of relapse increasing progressively from approximately 10 U/ml [10].

In this study, we investigated in vivo anti-tumoral effects of alpha interferon nano derivative compound on breast cancer in rats induced by DMBA by using CA15-3 tumor marker. The aims of the present investigation were to evaluate the association between serum CA15.3 levels in case and control group and after treated by drugs. In an attempt to contribute towards improving cancer therapy nano drug delivery approaches have been explored in the present study. The first approach uses target specific, polymer nano particles. The second novel therapeutic approach for cancer is to use of nanotube (SWNT).

MATERIAL AND METHODS

Statistical analysis

Drugs was prepared in four concentrations (drug cncentration: 0.001<0.01<0.1<1 μmol/ml). All experimental data were given as mean + SD (standard deviation). Statistical analysis was carried out using the Student's unpaired t test and one-way analysis of variances (ANOVA) using SPSS software. Probability values were found to be less than .05 ($p < .05$). The work presented here has been aimed at exploring a polymer nanoparticle based approach to cancer diagnostics and therapy. Cancer is the second leading cause of death in the world. Nanoparticles and polymer science have opened up a new world of opportunities for the development of efficient medical diagnostic methods and of selective cancer therapy.

Tumor induction

DMBA is a carcinogen which is known to induce mice mammary carcinoma from the ductal elements of the mammary gland by increasing substantial oxidative stress. Tumor was induced using DMBA as a carcinogen by a single dose of 20 mg/kg body weight, dissolved in sesame oil (1 mL) given through an oralgavage. The test

samples of the drugs were given daily through an oral gavage. During the experimental period, animals were weighed weekly. Palpation of mammary tumors began 4 week after animals received DMBA. Animals were observed daily to assess their general health.

Nanoparticles preparation

NPs were prepared by Double emulsion technique .The drug solution was added dropwise via a syringe into DCM (2.5 mL) and PLGA (50 mg) by sonicating for two min in an ice bath to form W1/O emulsion. PVA (3% w/v) was added and sonicated for 2 min (secondary emulsion; W1/O/W2). The final emulsion was continually stirred for 18 h to evaporate DCM. The particles were gathered by centrifugation at 1500 rpm, rinsed in deionized water 3 times, and then lyophilized at -75°C and 0.03 Pa.

Preparation of α-IFN-SWNT

SWNT 5 mg was mixed with 5 mg α- IFN in anhydrous ethanol 0.5 ml using an ultrasonic bath for about 15 minutes while drop wise phosphate-buffered solution 3 ml was added. The mixture was ultrasonicated using an ultrasonic probe (400 W, 10 times). The suspension was centrifuged at 10,000 rpm for 15 minutes until the SWNT were fully precipitated, and the remaining solids were thoroughly rinsed with anhydrous ethanol and deionized water to remove excess-IFN.

CA15-3 assay

The CA15-3 ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal antibody, directed against a distinct antigenic determinant on the intact CA15-3 molecule, for the solid phase immobilization (on the micro titer wells). A rabbit anti-CA15-3 antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react sequentially with the two antibodies, resulting in the CA15-3 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After two separate 1-hour incubation steps at 37°C, the wells are washed with wash buffer to remove unbound labeled antibodies. A solution of TMB Reagent is added and incubated for20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution and the color is changed to yellow. The concentration of CA15-3 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

RESULTS AND DISCUSSION

Our results show that, average amount of CA15-3 in cancerous rats induced by DMBA was 12.73±0.44 and 7.14±0.29 in case and control group respectively. There was significant difference between the two groups (P=0.000). It indicates an increase CA15-3tumor marker after induction of cancer. cancerous rats were exposed by α-IFN ,α-IFN –SWNT and α-IFN-PLGA .mean comparison CA15-3levels in the α-IFN ,α-IFN –SWNT groups was not significant(P>0.05) and a significant decrease in blood levels of CA15-3 only the group that treated with α-IFN-PLGA (p<0.05). The mean serum CA15-3 values are shown in fig (1).

Table1) Encapsulation % of alpha interferon in difference concentration of PLGA

no capsule	Initial concentration of drug(μg/ml)	Nano capsule drug content (μg/ml)	Nano capsule weight (mg)	Encapsulation %
N1	24.14	14.23	50	58.9
N2	14.96	10.33	50	69
N3	5.93	4.62	50	77.9

In order to compare the different formulations, the amount of drug incorporated in the nanoparticles was determined for each one. The expression for the encapsulation efficiency if the following:

$$encapsulation\ efficiency(\%) = \frac{Amount\ of\ drug\ in\ the\ nanoparticles}{initial\ amount\ of\ drug} \times 100$$

Table 2): The protein release rate from PLGA nano polymer

Nanocapsules	1&2 days(μg/ml)		3&4days(μg/ml)		5&6 days(μg/ml)	
N1	12	24	22	19	20	21
N2	7	18	18	17	18	17
N3	5	12.5	12	12	14	14

*Encapsulation efficacy measurement. The drug entrapped in the NPs was determined in triplicates by spectrophotometer (protein assay: coomassie brilliant blue G250 reagent)
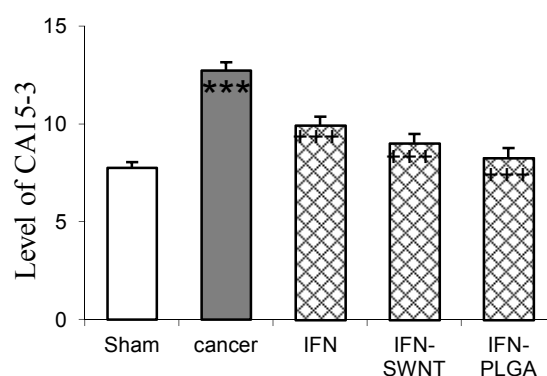
Fig1: Average blood levels of CA15-3 in breast cancer rats induced by DMBA (case and control)
+++shows the difference between the groups with cancer groups (p<0.01)
*** shows the difference between the groups with control groups (p<0.01)

This current study was investigated the toxicity of three type drugs on the breast cancer in rats induced by DMBA. Cancer inducing in rats (group 2) caused a significant increase in blood levels of CA15-3 (P<0.05). Administration of α-IFN ,α-IFN –SWNT and α-IFN-PLGA in 3 groups of cancerous rats caused a significant decrease in blood levels of CA15-3 only the group that treated with α-IFN-PLGA (p<0.05).

Alpha interferon should not be used for breast cancer routinely. But it for different leukemia is used .in this research was used nano product of alpha interferon. Because alpha interferon conjugated with single walled carbon nanotube (α-IFN- SWNT) increases the permeability of α-IFN to cancerous cell membrane (Fig1). The alpha interferon encapsulated with poly lactide-co-glycolic acid (α-IFN-PLGA) will increase the targeted dug delivery to cancer cell .also the drug release was done gradually thus time spent of drug efficacy will be greater(Table 2) . However, far too little attention has been paid to nano compound of α-IFN for breast cancer.

Most drugs which have been used in chemotherapy have rapid blood clearance, low tumor selectivity, and heavy poisonous side effects for normal tissues. Nanoparticless reduce these side effects, improve their distribution in the body, increase their specificity, prolong activity, and improve their in vivo degradation resistance [11].

The use of CNT's in drug delivery and biosensing technology has the potential to revoutionalize medicine. Fictionalization of SWNT's has proven to enhance solubility and allow for efficient tumor targeting/drug delivery. It prevents SWNT's from being cytotoxic and altering the function of immune cells [12].

Drug release is affected by particle size. Smaller particles have larger surface area, therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release.

Whereas, larger particles have large cores which allow more drug to be encapsulated and slowly diffuse out 30. Smaller particles also have greater risk of aggregation of particles during storage and transportation of nanoparticle dispersion. It is always a challenge to formulate nanoparticles with the smallest size possible but maximum stability. Polymer degradation can also be affected by the particle size [7].

In vivo experiment has been conducted to observe the preventive role of α-IFN-PLGA against (DMBA)-induced mammary cancer.

DMBA toxicity is associated with its oxidative metabolism leading to the formation of free radicals, which bind covalently to nucleophillic sites on cellular macromolecules eliciting cancerous responses.Free radicals and their biochemical reactions in each stage of the metabolic process are involved in cancer development. Antioxidants act as the primary line of defense against reactive oxygen species and suggest their usefulness in estimating the risk of oxidative damage induced during carcinogenesis [13].

The advantages of using nanoparticles as a drug delivery system include the following: Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration. They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity .Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance .The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc [8].

CONCLUSION

The results of this study indicate that nano drugs more effective than traditional drug in cancer treatment, although further work is needed to elucidate the safety and side effect of these compound in human. Carbon nanotubes are used as drug delivery vehicles to achieve in vivo tumor treatment efficacy. This opens up further exploration of biomedical applications of novel carbon nanomaterials with animals for potential translation into the clinic in the future. This result attempts to show that formulation nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing side effects.

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