# EFFECTS OF A-IFN –SINGLEWALLED CARBON NANOTUBE AND A-IFN-PLGA ENCAPSULATED 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 nanotube (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 compounds in human.

**Key words-** breast cancer, nano drug, tumor markers, CA15-3, α-IFN-PLGA, -IFN –SWNT

## I. INTRODUCTION

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 more likely to succumb to this disease [1]. 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) [2].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). 1 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[3].The use of nanotechnology for drug delivery systems is an actual subject today. A lot ofresearch is taking place at different universities in the world in order to find new formulationscapable of delivering drugs to specific areas of the body[4].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].

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) [7]. 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 months 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 cutoff 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 markers. The aims of the present investigation were to evaluate the association between serum CA15.3 levels in the 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).

## II. MATERIAL AND METHODS

### Statistical analysis

Drugs were prepared in four concentrations (drug concentrations: 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 weeks 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 emotion; W1/O/W2). The final emulsion was continuously 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**

| Nano capsule | Initial concentration of drug( µg/ml) | Nano cap drug content (µg/ml) | Nano cap weight (mg) | capsulation % |
|---|---|---|---|---|
| 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:

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

**Table 2)the protein release rate from PLGA nano polymer**

| nocapsules | 1&2 lays(µg/ml) | | &4days(µg/ml) | | days( µg/ml) | |
|---|---|---|---|---|---|---|
| N1 | 12 | 24 | 22 | 19 | 20 | 21 |
| N2 | 7 | 18 | 18 | 17 | 18 | 17 |
| N3 | 5 | 12.5 | 12 | 13 | 14 | 14 |

*Encapsulation efficacy measurement The drug entrapped in the NPs was determined in triplicates by spectrophotometer (protein assay: coomassie brilliant blue G250 reagent)

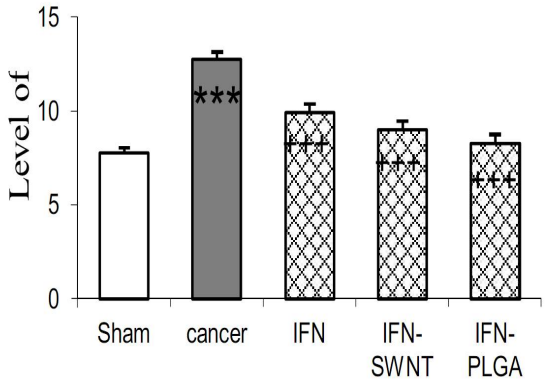



fig1)Average blood levels of CA15-3 in breast cancer rats induced by DMBA(case and control)
+++shows the difference between the groups with cancer groups(p<0.01)
*** shows the difference between the groups with control groups(p<0.01)This current study was investigated the toxicity of three type drugs 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 drug 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 slowlydiffuse 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(8). 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].

## 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 compounds 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 nano materials 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.

## AKNOWLEDGMENT

We would like to thank research deputy director of IAU Saveh Branch .

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