INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 5, p. 142-148, 2013

RESEARCH PAPER OPEN ACCESS

Cell cytotoxicity of alpha interferon alone and in combination with nano particles against breast cancer cell line

Eghdami A[1*], Monajjemi M[2], Eidi A[3], Hashemi M[4]

[1]*Basic Science Faculty, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[2]*Department of Chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[3]*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[4]*Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran*

Key words: Cell cytotoxicity, alpha interferon, breast cancer cell line.

doi: http://dx.doi.org/10.12692/ijb/3.5.142-148 Article published on May 22, 2013

Abstract

Despite extensive research, most anticancer drugs have nonspecific toxicity. Pharmacological experiments, using dozens of animals for every new compound, most often needed more time for biological characterization than for chemical synthesis. This situation started to change about thirty years ago. Slowly rational approaches developed, like QSAR and molecular modeling. Such cytotoxic drugs have a narrow therapeutic window, which limits their efficacy and results in severe side effects. The use of biological response modifiers and immunotherapy is one of the newer approach to the treatment of cancer and other diseases .Alpha interferon (α-IFN) , the first biologic agent tested ,is now widely available and used to treat a growing number of illnesses. The cytotoxicities of SWNT-IFN , IFN and peg-PLGA -IFN with different concentrations against 4T1after 24 ,48,72hours were evaluated using the MTT assay. The aim of this study was to establish an in vitro model for culturing 4T1 mice breast cancer cell line by MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5dipheny tetrazolium bromide] assay.MTT assay was used to assess the viability percent of 4T1 cell culture after 24,48,72 h with different concentration of treatment . the higher toxicity was observed at all concentration of Peg-PLGA –IFN in all time (24,48,72 h) In an attempt to reduce the side effects associated human leukocyte IFN, we investigated physicochemical properties of alpha-interferon subunits with computational study. The structure of protein α-interferon was selected from the protein data bank (PDB code 1RH2). We choose Monte Carlo and on the compute menu and Amber force fields for molecular mechanics calculations. Temperatures of calculation were kept 288-315 K. Moreover, additional parameters were calculated using the QSAR Properties Module of HyperChem 8.4 software. we measured partial derivative of each subunit in constant and variable volume and temperature .we obtained curves and equations of each subunits , at last we administered differential equation of alpha- interferon subunits by using thermodynamic data of energy and QSAR parameters. this survey results will help us find the best sub units for binding to nano-tubes and the best active subunits to cross the cell membrane.

* **Corresponding Author:** Eghdami A ✉ eghdami_a@yahoo.com

Introduction

In many cases, carrier materials are used in particulate forms. Various types of microparticle colloidal carrier systems, together with ranges of particle diameters in nanometers. Microspheres and nanoparticles have continuous matrices containing dispersed or dissolved drug whilst microcapsules and nanocapsules are composed of a drug core surrounded by a layer acting as a coating or barrier to drug division or dissolution. The types of carrier materials used, the drug substance and the biological environment for drug delivery all influence the mechanisms of drug release.Cytokines in the tumor microenvironment can profoundly influence cancer pathogenesis. Cytokines participate in complex interactions with cells and molecules to promote tumor growth, metastasis and invasion or, alternatively, engender a robust immune response that inhibits cancer progression. The balance of immunostimulatory and immuno-suppressive cytokines may determine the outcome of a developing cancer (Finn, 2008).

IFN-α actually comprises a family of proteins, but other than a limited number of phase I trials using IFN-a1 that showed equivalent biological response to reduced side-effects, only IFN-a2 has been broadly assessed (Borden *et al.,* 2000) ; as such, it is this latter variant to which we will henceforth refer. IFN-a has the longest history of therapeutic use of any cytokine in clinical oncology and is perhaps the most effective agent yet identified (Dranoff, 2004). It promotes cytotoxicity by enhancing tumor-antigen presentation, the expression of death receptors and adhesion molecules, and bypromoting B cell, T cell, macrophage and DC activity. Besides acting directly on cancer cells to induce apoptosis, IFN-a also exhibits anti-angiogenic properties .the anti-cancer activity of IFN-a has been well-established using *in vitro* assays and preclinical model systems (Borden *et al.,* 2000) that provided the rationale for numerous subsequent clinical trials.Cancer is a leading cause of death world-wide and accounted for a total of 7.4 million deaths in 2004 (~13% of all deaths); a number which is expected to rise rapidly with the increase in global ageing1. Cancer is a generic term for a large group of diseases that can arise from all nucleated cells in our body. The hallmarks of cancer is a transformation of normal cells by a series of inherited and acquired genetic mutations, which provide growth and survival advantages, and eventually generate malignant neoplasms able to invade adjacent tissues and spread to distant organs (Hanahan and Weinberg, 2000). The spreading of cancer cells known as metastasis is a defining feature of the disease and is the major cause of death from cancer (Hanahan *et al.,* 2000). Chemotherapy is still the first line treatment of most disseminated cancers, but despite the arrival of more than 20 new compounds in the last decade, chemotherapy is only curative in very few cases. Daily, patients and physicians are faced with the shortcomings of these conventional treatments and clearly new approaches are needed.Cancer immunotherapy is a novel approach aiming to harness our immune system to combat cancer, and has the potential to specifically target cancer cells with limited systemic toxicity (Savage *et al.,* 2009).

Our immune system is a tremendously potent defense system, which protects us from a large and versatile array of microbial intruders, and the idea of using its inherent strengths to fight cancer is appealing. In 1970, the concept of cancer immunosurveillance was conceived, which proposed the existence of immunological mechanisms that eliminate potentially dangerous mutant cells (Burnet, 1970).Since then, this concept has been substantiated by evidence that both the innate and adaptive parts of the immune system indeed recognize, shape and partly inhibit cancer development (Dunn *et al.,* 2002).

Still, cancers clearly develop in the presence of a competent immune system, showing that the immune system alone is not equipped to protect against all cancers.The traditional strategies for cancer treatment, includes surgery, radiation, and chemotherapy or combined strategies of these treatments. These are supplemented by some more specialized therapies such as immunotherapy or

hormone therapy which can be applied only some tumor types .Interferons (IFNs) are a family of related cytokines that mediate a range of diverse functions including antiviral, anti-proliferative, anti-tumor, and immunomodulatory activities (Stark *et al.,* 1998). IFNs bind to cell surface receptors which, after dimerization, initiate a cascade of phosphorylation reactions in the Janus Kinase – Signal Transducer and Activator of Transcription (JAK–STAT) signaling pathway, eventually activating transcription of IFNs timulated genes, known as ISGs (Jonasch, 2001). Treatment with interferon-alfa is associated with a significant number of side effects that require close monitoring. 19-21 These side-effects may hamper reaching and maintaining the dose needed for maximal therapeutic effect (Sleijfer *et al.,* 2005). Carbon nanotubes (CNTs) are rolled up seamless cylinders of graphene sheets, exhibiting unparalleled physical, mechanical, and chemical properties which have attracted tremendous interest in the past decade . Depending on the number of graphene layers from which a single nanotube is composed, CNTs are classified as single-walled carbon nanotubes (SWNTs) or multi-walled carbon nanotubes (MWNTs). Applications of CNTs span many fields and applications, including composite materials , nanoelectronics , field-effect emitters , and hydrogen storage (Becktel *et al.,* 1983). In recent years, efforts have also been devoted to exploring the potential biological applications of CNTs, motivated by their interesting size, shape, and structure, as well as attractive and unique physical properties (Zhuang *et al.,* 2009) .Pharmacological experiments, using dozens of animals for every new compound, most often needed more time for biological characterization than for chemical synthesis. This situation started to change about thirty years ago. Slowly rational approaches developed, like QSAR and molecular modeling. The consequence was a lower output in such projects, when certain chemical structures had to be synthesized that were proposed by these methods. On the other hand, *in vitro* test systems like enzyme inhibition or the displacement of radio-labeled ligands in membrane preparations enabled a much faster investigation of new analogs . Molecular volume is a property fundamental to understanding the structure and physical properties of biological molecules. The relevance of calculations of molecular volume can be seen in the correlation of the volume, or change in volume, with other experimental observable factors. These include energetics and heat capacities in biological molecules (Privalov *et al.,* 1988) factors influencing drug design (Becktel *et al.,* 1983) and packing and general geometric considerations at an atomic level. Correlations between calculated volumes and other thermodynamic values depend upon the accuracy with which the experimentally determined volumes are reproduced. In spite of its importance, methods for calculating molecular volumes have been plagued by errors. Some are avoidable, molecular "surface" (McGowan *et al.,* 1986).

Many pharmaceutical agents, including various large molecules (proteins, enzymes, antibodies) and even drug-loaded pharmaceutical nanocarriers, need to be delivered intracellularly to exert their therapeutic action inside cytoplasm or onto nucleus or other specific organelles, such as lysosomes, mitochondria, or endoplasmic reticulum. This group includes preparations for gene and antisense therapy, which have toreach cell nuclei; proapoptotic drugs, which target mitochondria; lysosomal enzymes, which have to reach the lysosomal compartment; and some others Intracellular transport of different biologically active molecules is one of the key problems in drug delivery in general. In addition, the introcytoplasmic drug delivery in cancer treatment may overcome such important obstacles in anticancer chemotherapy as multidrug resistance. However, the lipophilic nature of the biological membranes restricts the direct intracellular deliveryof such compounds. The cell membrane prevents big molecules, such as peptides, proteins,and DNA, from spontaneously entering cells unless there is an active transport mechanism, as in the case of some short peptides (Vladimir *et al.,* 2006).

Over the next few years it will be important to establish the optimal biological doses of the

interferons, so that we can maximize their usefulness in therapy and avoid the trap of thinking of them as purely cytotoxic agents.In an attempt to reduce the side effects associated human leukocyte IFN, we investigated physicochemical properties of alpha-interferon subunits with computational study.

Material and methods

Computational methods

During modeling ,the thermodynamic significance (energetic)of stability ,is to make the energy of the system as low as possible.Structure of proteins and nucleic acid are available in PDB files .these structures are derived from physical studies of molecules (for example, X ray diffraction or nuclear magnetic resonance (NMR) analysis .we choose Molecular Mechanics to use a classical Newtonian calculation method instead of a quantum mechanical (semi-empirical or abinitio) method.The crystal structures of proteins were from the Brookhaven protein data bank. The structure of protein α-interferon was selected from the protein data bank (PDB code 1RH2).All modeling procedures, including energy minimization and molecular dynamics, were performed using the HyperChem 8.6 software. Energy calculations were carried out using the MM+, Amber, Bio+ and OPLS force fields for molecular mechanics calculations. We could use any of these methods for single point, Geometry optimization and molecular Dynamics calculation. We choose Monte Carlo on the compute menu. The 1RH2 molecule is composed of six subunits (A-F) , residues of each chain are shown in table 1. The entire molecules contain 13128atoms.The 1RH2 molecules were sectioned into constituent subunits (6 subunits) by Chem 3D. These studies provided insights into the steric, electrostatic, hydrophobic and hydrogen bonding properties and Monte Carlo, with 100 ps step and without any constraints. Temperatures of calculation were kept 288-310 K. Moreover, additional parameters were calculated using the QSAR Properties Module of HyperChem 8.4 software and include the following: surface area of the molecule available for solvent (SA), volume of the molecule (V), hydratation energy (HE), the logarithms of the *n*-octanol-water partition coefficient (LOG_P), refraction (R) and polarizability (P).

Cell toxicity assay

MTT assay: For cytotoxicity assessment, a direct colorimetric assay, adapted from the Mossman MTT method was employed for the tumor cell lines. This cell viability assay is based on living cells' property to transform the MTT dye tetrazolium ring into a purple-colored formazan structure due to the action of mitochondrial and other dehydrogenases inside the cell The color intensity yielded by the cell population is directly proportional to the number of viable cells, and one can quantify the absorbance measurements using mathematical parameters. For the normal human lymphocytes, which are in suspension, the cytotoxicity was evaluated using the water-soluble MTT dye (provided by Promega).

From this cell suspension, 100 µl were pipetted into 96 well microtiter plates and these wells were incubated for 24 h in 5% CO_2 incubator at 37°C. The diluted range of test treatment being 0.1, 0.1, 0.01, 0.001, µg /ml. After adding the treatments(IFN,SWNT-IFN, peg –PLGA –IFN) samples, new medium were added to make up the final volume of 200 µl each well. The plate was incubated in 5% CO_2 incubator at 37°C for24,48 and 72h. Then, 20 µl of MTT reagent was added into each well. This plate was incubated again for 4 h in a CO_2 incubator at 37°C. After incubation, 200 µl solubilization solution (Roche, USA) was added into each well. The cell was then left overnight at 37°C, 5% CO_2 incubator. Finally, the absorbance was read by the ELISA reader in 570 nm.

Results and discussion

Geometry optimization is one of the most often applied techniques in computational drug discovery. Although geometry optimization routines are generally deterministic, the minimization trajectories can be extremely sensitive to initial conditions, especially in case of larger systems such as proteins. Quantitative Structure –Activity (QSAR) are attempting to correlate molecular structure , or

properties derived from molecular structure , with a particular kind of chemical or biochemical activity . The kind of activity is a function of the interest of the user: QSAR is widely used in pharmaceutical ,environmental, and agricultural chemistry in the search for particular properties . The calculations are empirical , and so , generally , are fast.

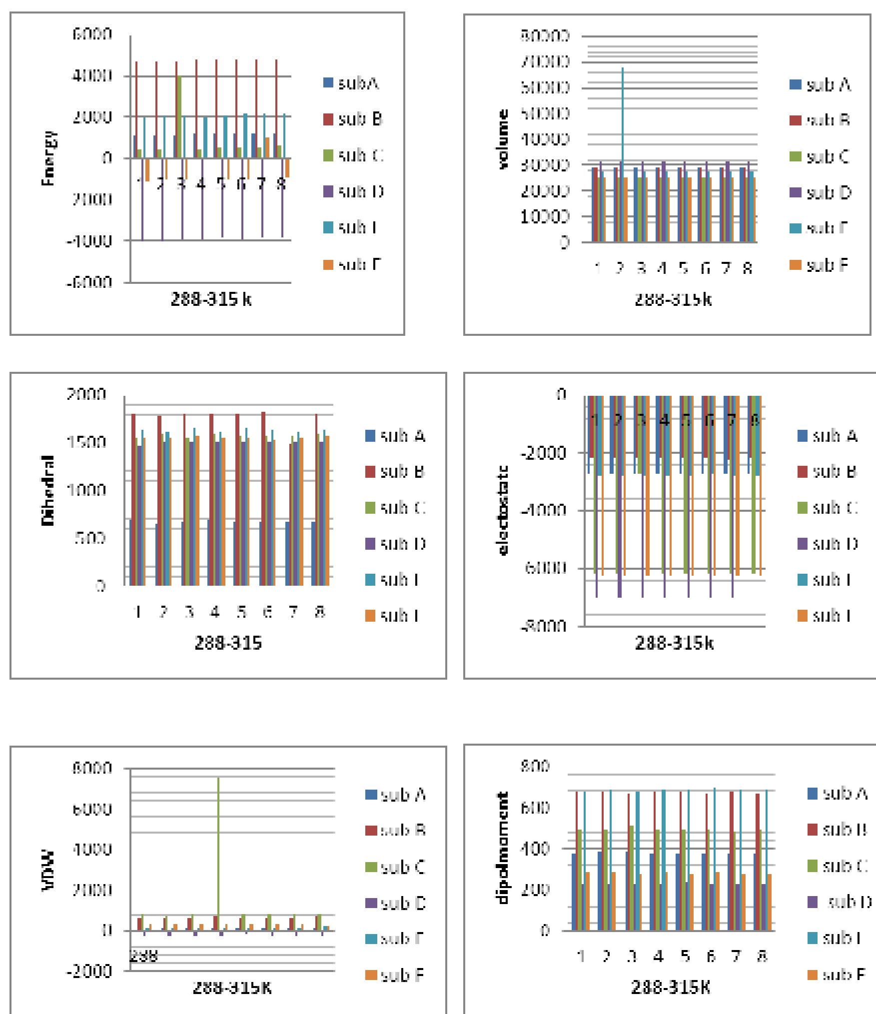

Fig. 1. Energy and QSAR parameters of alpha-interferon subunits.

Cytotoxicity of IFN- nano particle conjugates

In most case ,drugs used for treatment of cancer are not effective or have unpleasant side effects.this has forced scientists to find more effective drugs with less toxicity.alpha interferon nano conjugated was prepared.caltivated 4T1 (mice breast cancer) cell line were incubated with different concentration of treatment for 72 hours and cell growth inhibition was determined using MTT assay.The cytotoxicities of SWNT-IFN , IFN and peg-PLGA -IFN with different concentrations against 4T1(the 4T1 is the mice breast cancer cell line was purchased from Pasteur institute of Iran) after 24 ,48,72hours were evaluated using the MTT assay. As shown in Table 1, peg-PLGA -IFN exhibited stronger cytotoxicity than that of peg-PLGA -IFN and IFN conjugate at all tested concentrations to 4T1 cells. Of note, SWNT-IFN themselves at the concentrations used for formulations did not affect

the survival of 4T1cells after exposure 24 and 48hours (data not shown), but was reduced viability of 4t1 cells after 72 .Peg-PLGA –IFN have higher cytotoxicity in all times because this nano particle released alpha interferon gradually.The aim of this study was to establish an in vitro model for culturing 4T1 mice breast cancer cell line by MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5dipheny tetrazolium bromide] assay.MTT assay was used to assess the viability percent of 4T1 cell culture after 24,48,72 h with different concentration of treatment . after 24 hours treatment of cells with the three types of alpha interferon the cell viability was evaluated by MTT colorimetric assay . the higher toxicity was observed at all concentration of Peg-PLGA –IFN in all time (24,48,72 h) and SWNT-IFN after 24 an48 h .the most cytotoxicity effects were for Peg-PLGA –IFN on breast cancer cells after 72h.

Table 1. Cytotoxicity effect of nano particles on 4T1line cell.

24 hours

sample	Concentration (μg/ml)	Ave abs 570nm	Viability%	Cytotoxicity%
IFN-α	1	0.212	72.36	27.64
	0.1	0.216	73.72	26.28
	0.01	0.220	75.09	24.91
	0.001	0.230	78.50	21.50
IFN-α +SWNT	1	0.163	55.64	44.36
	0.1	0.165	56.32	43.68
	0.01	0.173	59.05	40.95
	0.001	0.179	61.10	38.90
PEG-IFN-α	1	0.162	55.29	44.71
	0.1	0.165	56.32	43.68
	0.01	0.167	57.58	43
	0.001	0.177	60.41	39.59
Control		0.293		

48hours

sample	Concentration μg/ml	Ave. abs.570nm	Viability%	Cytoto xicity%
IFN-α	1	0.182	85.05	14.95
	0.1	0.186	86.92	13.08
	0.01	0.200	93.46	6.54
	0.001	0.208	97.20	2.80
IFN-α +SWNT	1	0.145	67.76	32.24
	0.1	0.148	69.16	30.84
	0.01	0.152	71.03	28.97
	0.001	0.157	73.37	26.63
PEG-IFN-α	1	0.127	59.35	40.65
	0.1	0.130	60.75	39.25
	0.01	0.133	62.15	37.85
	0.001	0.136	63.56	36.44
control		0.214		

72 hours

sample	Concentration μg/ml	Ave. abs.570nm	Viability%	Cytoto xicity%
IFN-α	1	0.175	85.79	14.21
	0.1	0.179	87.75	12.25
	0.01	0.185	90.69	9.31
	0.001	0.192	94.12	5.88
IFN-α +SWNT	1	0.128	62.75	37.25
	0.1	0.130	63.73	36.27
	0.01	0.134	65.67	34.31
	0.001	0.141	69.12	30.88
PEG-IFN-α	1	0.088	43.14	56.86
	0.1	0.091	44.61	55.39
	0.01	0.097	47.55	52.45
	0.001	0.103	50.49	49.51
control		0.204		

References

Becktel WJ, Rellick LM. 1983. Techniques in Protein Chemistry IV, Academic Press, San Diego, California **491,** 14-36.

Borden EC, Lindner D, Dreicer R. 2000. Second-generation interferons for cancer: clinical targets. Seminars in Cancer Biology **10,** 125–144.

Burnet FM. 1970. The concept of immunological surveillance. Progress in experimental tumor research **13,** 1-27.

Dranoff G. 2004. Cytokines in cancer pathogenesis and cancer therapy. Nature Reviews Cancer **4,** 11–22.

Dunn GP, Brucen AT, Ikeda H, Old LJ, Schreiber RD. 2002. Cancer immunoediting: from immunosurveillance to tumor escape. Nature Immunology **3,** 991-998.

Finn OJ. 2008. Cancer immunology. New England Journal of Medicine **385,** 2704–2715.

Hanahan D, Weinberg RA. 2000. The hallmarks of cancer. Cell **100,** 57-70.

Jonasch E. 2001. Interferon in oncological practice: review of interferon biology, clinical applications, and toxicities. Oncologist **6(1),** 34-55.

McGowan JC, Mellors A. Molecular Volumes in Chemistry and Biology: Applications In cluding Partitioning and Toxicity, Ellis Horwood, Ltd., Chichester, UK,1986.

Privalov PL, Gill SJ. 1988. Adv ProteinChem. **39,** 191–234.

Savage P, Stebbing J, Bower M, CrookT. 2009. Why does cytotoxic chemotherapy cure only some cancers? Nature Clinical Practice Oncology **6,** 43-52.

Sleijfer S, Bannink M, Van Gool A, Kruit W, Stoter G. 2005. Side effects of interferon α therapy. Pharmacy World and Science **27(6),** 423-431.

Stark GR, Kerr BR, Williams RH. 1998. How cells respond to interferons. Annual Review of Biochemistry **67,** 227-64.

Vladimir P, Torchilin. 2006. Recent Approaches to Intracellular Delivery of Drugs and DNA and Organelle Targeting. Annual Review of Biomedical Engineering **8,** 343-375.

Zhuang L, Scott T, Kevin W, Hongjie D. 2009. Carbon Nanotubes in Biology and Medicine: In vitro and in vivo Detection, Imaging and Drug Delivery. Nano Research **2(2),** 85–120.