INVESTIGATION OF CYTOTOXICITY EFFECT OF ALPHA INTERFERON AND ITS NANOPARTICLE DERIVATIVES ON CANCEROUS AND HEALTHY CELL LINE: 4T1, HL60 AND L929

*Anoosh Eghdami and Sayed Mehdi Hashemi Sohi

1Department of Biochemistry, Medical faculty, Saveh Branch, Islamic Azad University, Saveh, Iran
2Young Researchers and Elite club, Saveh Branch, Islamic Azad University, Saveh, Iran

*Author for Correspondence

ABSTRACT
Cytokine-based research is revolutionizing the treatment of several diseases including cancer, inflammation, infectious diseases, obesity, haematological disorders and other diseases affecting the immune system. The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immuno pharmacology. Present investigation was carried out to study the anticancer effects of Different nano compound against cell lines 4T1, HL60 and L929.cell lines were used as biological models. Two lines were derived from breast cancer (4T1) and acute myeloid leukaemia (HL60). Another cell line was normal mouse fibroblast cell line (L929). Drugs was prepared in four concentrations (drug concentration: 0.001, 0.01, 0.1, 1 µmol/ml). The difference between groups was determined by analysis of variance (ANOVA). Cell lines incubated with different concentrations of drugs for 24 hours and cell growth inhibition was determined using MTT assay. Inhibitory effect of α-IFN-SWNT conjugated and encapsulated α-IFN in PLGA on three cell line, had shown significant differences (p<0.05). The maximum growth inhibitory effect was on HL60 cell line. All drugs induced a significant growth inhibition and apoptosis in a dose-dependent (drug concentration: 0.001<0.01<0.1<1 µmol/ml). The results of this study indicate that nanoparticles could be a potentially useful delivery system for IFN as an anticancer agent and other anticancer drugs in treatment of leukemia.

Keywords: Cytotoxicity, Alpha Interferon, Nanoparticle, Cell Line, Cancer

INTRODUCTION
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. 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. However, far too little attention has been paid to nano compound of α-IFN for breast cancer.

Interferon-alpha is a highly purified, bacterially synthesized agent with antiviral, anti proliferative and immune modulatory properties. Interferon-alpha has been approved for the treatment of several types of cancer (eg, leukaemia, lymphoma, renal cell carcinoma and melanoma) and of chronic hepatitis (Iulian et al., 1997).

In addition to an antiproliferative or apoptotic effect, interferon treatment is known to also be capable of eliciting a direct cytotoxic effect on some tumor cells. One of the first references to this effect was in work done by Vílick and others 25 years ago, in which he found that of five tumor lines tested, two, an adenocarcinoma line and a rhabdomyosarcoma line, were highly sensitive to either purified natural or recombinant interferon-gamma even at concentrations of 1 unit/mL with the effect being abrogated by the addition of a specific monoclonal anti-interferon gamma antibody (Joseph et al., 2010). Cancer is one of the most serious threats to human health in the world and chemotherapy is still the standard treatment method. Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells and cause immuno toxicity which affects not only tumor development, but also aggravates patient’s recovery (Mary et al., 2012).
The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immuno pharmacology (Xu et al., 2009). With this aim, many attentions have been paid to nano drug delivery for cancer therapy. The predominant methods to deliver drugs are oral and injection, which has limited the progress of drug development. Most drugs have been formulated to accommodate the oral or injection delivery routes, which are not always the most efficient routes for a particular therapy. New biologic drugs such as proteins and nucleic acids require novel delivery technologies that will minimize side effects and lead to better patient compliance (Gareth et al., 2005). Additional benefits of using targeted nanoscale drug carriers are reduced drug toxicity and more efficient drug distribution (Kumar, 2000).

Nanoparticle drug delivery using biodegradable polymers is expected to provide a more efficient way to overcome some of these problems. The pharmacological properties of a polymer-drug conjugate can be manipulated by changing the physical and chemical properties of the drugs based on nanoscale. Another issue with chemotherapy is that the drug may be delivered to tissues other than the tumor, affecting organs such as the heart and liver. Nanoparticles could provide a controlled and targeted means to deliver encapsulated drugs, resulting in lower side effects and higher efficacy (Langer, 2000). Numerous studies attempted to explain effects of biologic compounds on cancer Interferons (IFN) are a group of cytokines which exhibit pleiotropic activities that play major roles in both innate and adaptive immunity. Type I IFNs consist of multiple numbers of IFN-alpha (IFN-α) genes and at least one IFN-beta (IFN-β) gene in most vertebrates, and a few other family members such as limiting in the mouse (4). There are 14 IFN-α subtype in the mouse which shares at least 75% identity in protein sequence. In the human, IFN-α is used to treat viral diseases and cancer therapy (Chevaliez et al., 2009; Antonelli, 2008; Ascierto et al., 2008).

Nanotubes have several properties that make them suitable for use as nanotube-supported drugs. Functionalized CNTs have been shown in many studies to be able to cross cell membranes. The ability of CNTs to cross cell membranes allowing them to be used as carriers is of particular high interest for drug delivery strategies. In targeting the delivery of drugs to cells, the drugs are first attached to the carrier by either covalent or non covalent bonding. The drug carrier conjugates are then directed to the targeted cells via passive targeting methods (ie, a methodology to increase the target/non target ratio of the amounts of drugs delivered primarily by minimizing nonspecific interactions with non target organs, tissues, and cells) or active targeting methods (ie, the method by which the therapeutic agent is delivered to tumors by attaching the agent with a ligand that binds to specific receptors that are over expressed on target cells (Khazaeei et al., 2010).

Polymeric nanoparticles are solid, biodegradable, colloidal systems with submicron sizes where the drug can be dissolved, entrapped, adsorbed, attached or encapsulated into the nanoparticle. Polymeric nanoparticles are fabricated from biodegradable natural or synthetic polymers. Some of the natural polymers include heparin, chitosan, dextran, albumin, gelatin, alginate or collagen; and within the synthetic polymers, polyethylene glycol (PEG), polyglutamic acid, polyglycolic acid (PGA), polylactic acid (PLA), polycaprolactone (PCL), poly (D, Llactide-co-glycolic) acid (PLGA), polyaspartate (PAA) andN-(2-hydroxypropyl)-methacrylamide copolymer (HPMA)have also been intensively investigated (Egusquiuaguirre et al., 2012).

In this study, we investigated in vitro anti-tumoral effects of nano derivative drug of alpha interferon on murine mammary carcinoma cells cell line (4T1), cell line originated from acute myeloid leukaemia (HL60) and normal mouse fibroblast cell line (L929) by MTT assay. For most of cancers there is no treatment and most of them ended in death. Alpha interferon is important anti proliferative drugs. The present investigation was carried out to study the anticancer effects of Different nano compound against cell lines 4T1, HL60 and L929.

MATERIALS AND METHODS
Drugs was prepared in four concentrations (drug concentration: 0.001<0.01<0.1<1 μmol/ml). All values are represented as mean ± SD of triplicate measurements (for example table 1). Statistical analysis was performed with the SPSS software version 15.0. And the difference between groups was determined by
analysis of variance (ANOVA). A p value less than 0.05 was considered statistically significant. Significance was accepted if the null hypothesis was rejected at the p < 0.05 level.

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 α-IFN.

Preparation of α-IFN Encapsulation

A-IFN -PLGA were prepared by Double emulsion technique. The drug solution was added drop wise via a syringe into Dichloromethane (DCM) (2.5 mL) and PLGA (50 mg) by sonicating for two min in an ice bath to form W1/O emulsion. Poly vinyl alcohol (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.

Cell Culture

4T1, HL60 and L929 cells was produced from Pasteur institute of Iran and were cultured in RPMI medium supplemented with 10% fetal bovine serum, 2 mg/ml sodium bicarbonate, 10mM HEPES, 100 unit/ml of penicillin, and 100 µg/ml streptomycin at 37°C in humidified atmosphere with 5% CO₂. Cell suspension (5x10⁵ cells/ml) was plated out into 96-well microtiter plate. Drugs were initially dissolved in DMSO as mentioned earlier, with the final concentration of DMSO being 0.1% (v/v). Serial dilutions of the sample were prepared in RPMI1640.

Cell Viability - MTT Assay

Cell viability was determined using the MTT colorimetric assay. Cytoxicity profiles of the drugs were assessed using 3-[4,5-dimethylthiazol-2-y1]-2,5-diphenyltetrazolium bromide (MTT) micro culture tetrazolium viability assay as described by Mosmann. Thereafter, various concentrations of the α-IFN nano drug were plated out in triplicates. Each plate included untreated cell controls and a blank cell-free control. After 24 h of incubation, MTT (5 µg/ml) was added to each well and re-incubated for further 4 h. Then, the media was removed and DMSO was added into each well to solubilize the formazan crystals. Finally, the absorbance was read at wavelength of 595 nm using a microtiter plate reader (Labsystems iEMS Reader MF) and the percentage cell viability was calculated with the appropriate controls taken into account, where OD is the optical density.

\[
\text{Growth inhibition} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{treated}}}{\text{OD}_{\text{control}}} \times 100
\]

RESULTS AND DISCUSSION

Our results show that, alpha interferon had inhibitory effect on HL60 cell line more than 4T1 and L929, this difference is significant (P<0.05)(figure1). Inhibitory effect of α-IFN-SWNT conjugated and encapsulated α-IFN in PLGA on three cell line the above mentioned, had shown significant differences (p<0.05). The maximum growth inhibitory effect was on HL60 cell line (figure 2 & 3).

All treatment has the inhibitory effect on HL60 cell line. All drugs induced a significant growth inhibition and apoptosis in a dose-dependent (drug concentration: 0.001<0.01<0.1<1 µmol/ml).

Table 1: Effect of α-IFN on breast cancer cell line (4T1)

<table>
<thead>
<tr>
<th>Sample</th>
<th>CONC.(mol/ml)</th>
<th>abs595 nm</th>
<th>ave</th>
<th>SD</th>
<th>Cell Viability %</th>
<th>Cell Cytotoxicity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α</td>
<td>1</td>
<td>0.183</td>
<td>0.182</td>
<td>0.181</td>
<td>0.182</td>
<td>85.05</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.187</td>
<td>0.186</td>
<td>0.184</td>
<td>0.186</td>
<td>86.92</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.202</td>
<td>0.200</td>
<td>0.199</td>
<td>0.200</td>
<td>93.46</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.209</td>
<td>0.209</td>
<td>0.207</td>
<td>0.208</td>
<td>97.20</td>
</tr>
</tbody>
</table>
**Research Article**

**Figure 1:** Comparison the effect of alpha interferon on the three cell lines in 24 hours

**Figure 2:** Comparison the effect of alpha interferon conjugated with carbon nanotube on the three cell lines in 24 hours

**Figure 3:** Comparison the effect of alpha interferon encapsulated in poly lactic, poly glycolic on the three cell lines in 24 hours
This current study was investigated the toxicity of three type drugs on the three type of cell lines. In order to evaluate potential harmful effect on normal mouse fibroblast cell line (L929) and growth inhibitory effect on murine mammary carcinoma cells cell line (4T1), cell line originated from acute myeloid leukaemia (HL60). L929 cell line was more resistant to treatments than two other cancer cell lines. In acute leukaemia, the maturation of the malignant cells is arrested and the cells merely proliferate. In the recent years, beside chemotherapy combination of differential factors cytokins and cytotoxic agents have been used in treatment of acute leukaemia particularly acute promyelocytic leukaemia. The present study was an evaluation of, cytotoxicity above mentioned treatment on HL60 cells individually. All agents in concentration 1µmol/ml moderately reduced growth of cancer cell line but this inhibitory was higher in HL60 culture. It was also shown that nanoparticle and SWNT conjugated IFN was more effective than α-IFN alone. A possible explanation for this might be that the higher permeability of conjugated drug to SWNT or it may be due to gradual liberalization of α-IFN IN cancer cells by PLGA encapsulation.

Slow release is critically significant in drug delivery for minimizing the amount of drug lost before reaching the target. Shell structures and supports can be used for slow delivery of drugs and are usually made from organic materials. For example, liposomes, microspheres, polymeric shells, and polymeric micelles have been well investigated. In constructing a drug delivery system from organic materials, the combinations of shell or support materials, targeting molecules, and drugs are restricted to ensure stability, targeting efficiency, and drug effect. Although, many supporting polymers are expensive, this restriction can be reduced by using carbon nanotubes (Khazaei et al., 2010).

However, Goldstein (1998) points out that, Interferon is likely to be the treatment of choice for hairy cell leukemia and possibly also for symptomatic nodular lymphoma. Interferon is very useful in treating papillomas and condylomas, and its role as a local agent will probably expand. Nevertheless, in both melanoma and renal carcinoma, meaningful responses do occur. Furthermore, because prior failure to respond to chemotherapy does not predict response to interferon, its use as a second-line agent should also be considered. The future of such biological agents, however, clearly lies in combination with other agents (Goldstein D and Laszlo J 1998).

Shokrzadeh (2012) showed strong inhibition of HepG2 cell growth of the nanoparticle compared with DTX. The results showed higher cytotoxicity effect of nanoparticles in comparison with drug alone, against HepG2 cell lines (Shokrzadeh, 2012). Lie wang et al., (2011) argued that SWNT-NGR-DTX showed higher efficacy than docetaxel in suppressing tumor growth in a cultured PC3 cell line in vitro and in a murine S180 cancer model. Tumor volumes in the S180 mouse model decreased considerably under near-infrared radiation compared with the control group (Wang et al., 2011).

Conclusion
In conclusion that α-IFN has direct inhibitory properties and some synergistic influence as conjugated with SWNT and encapsulated in PLGA determined by MTT assay. Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells and cause immunotoxicity which affects not only tumor development, but also aggravates patient’s recovery. Nanoparticles could be a potentially useful delivery system for IFN as an anticancer agent and other anticancer drugs in treatment of leukemia.

ACKNOWLEDGMENT
The author acknowledges the support from Islamic Azad University Research Council for their work.

REFERENCES

© Copyright 2014 | Centre for Info Bio Technology (CIBTech)


Shokrzadeh M (2012). Cytotoxicity evaluation of docetaxel nanoparticles by culturing HepG2carcinoma cell line. Journal of Mazandaran University of Medical Sciences 22 (90) 2-10 (Persian).