The effect of Nanoliposomal and PEGylated Nanoliposomal Forms of 6-Gingerol on Breast Cancer Cells

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Abstract

This study has been devoted to preparing the nanoliposomal and PEGylated nanoliposomal forms of 6-gingerol and investigating their effect on MCF-7 cell line of breast cancer. The formulations of nanoliposomal and PEGylated nanoliposomal forms of 6-gingerol were prepared through reverse-phase evaporation method and using a defined proportion of phosphatidylcholine, cholesterol, 6-gingerol, dextrano-polyethylene glycol 2000 (for PEGylated nanoliposomal form). The encapsulation rate was measured by ultracentrifugation and the drug release rate within 48-hour interval was assessed using dialysis. The MTT assay was used to determine the effect of cytotoxicity rate of formulations on the growth of breast cancer cells, and the viability and IC50 values were calculated for both formulations using the Pharm program. The rate of drug encapsulation was obtained 85% and 92% for nanoliposomal and PEGylated nanoliposomal formulations, respectively. In addition, the diameter and surface potential values were obtained 345.5 nm and -24.5 mV for nanoliposomal formulation, and 310.4 nm and -8.54 mV for PEGylated nanoliposomal form, respectively. The IC50 value for nanoliposomal and PEGylated nanoliposomal forms was obtained to be 11 and 13 µg/ml, respectively. Obtained results show that the PEGylated nanoliposomal form of 6-gingerol increases its cytotoxic effect and makes the drug release slower. Therefore, the use of PEGylated nanoliposomal form of 6-gingerol helps efficiently increase its therapeutic index and decrease its side effects in treatment of breast cancer.

Keywords: Gingerol, cytotoxic effect, nanoliposome; breast cancer.

Introduction

Breast cancer is one of the most important health problems among women, such that there is no cancer disease as concerning as the breast cancer1. This disease is the result of malignant and unrestrained proliferation of epithelial cells covering the ducts or lobules in the breast tissue. This type of cancer is the second cause of mortality among women after lung cancer and it has the highest prevalence among cancer patients2. According to the World Health Organization report on February 2009, the breast cancer kills 519,000 people worldwide annually2. The total count of breast cancer patients in Iran is 40,000 people, incrementing by 7,000 people annually3. Although in comparison with other countries, prevalence of breast cancer in Iran is low, the increase of these patients in recent years has made it the most common type of malignancy among the Iranian women. The age of onset of this disease in Iran is one decade lower than that in developed countries and more than 30 percent of patients are below 30 years old3,4. When referring to the physician, almost 70% of patients are in the advanced phases of the disease, and the treatment is impossible5. In 2004, almost 216,000 cancer patients and 40,000 deaths caused by it have been reported in the United States6. In Iran, based on the last report by government, the breast cancer is the most prevalent cancer type among women. The heterogeneous nature of breast tumor tissues and their internal pressure decreases the desired response to anticancer drugs and the increased cytotoxicity of administered drugs limits their dose8.

The use of liposomes as drug vehicles have recently become common in drug therapy and drug delivery5,7,8. Liposomes are small globules composed of lipid molecules especially phospholipids. Phospholipids are molecules with amphipathic property, so other substances like cholesterol and dicetyl phosphate amine could also be used in the structure of liposome membranes7,9,10,11. Drug delivery systems are transporting systems which convey the drug to defined targets within body at specific time and with controlled dose. This is remarkably safer and more efficient than spreading the drug to the whole body. One of the common problems is the small size and the outspread dispersal of targets within body. Appropriate use of drug delivery systems could make it possible to use the novel therapeutic methods, for example, it can be used in case of drugs which are highly toxic if consumed incorrectly. To deliver the required dose of drug to the target surface at a specific time, drug delivery systems apply active or inactive nanometer-scale designed systems. Ten years since the discovery of liposomes in early 1960s, the idea of
using the liposomes as drug vehicles was posed. In addition, because of the high similarity of liposome bilayer membrane to the cell membranes, it was used as an artificial membrane in the membrane studies.

Liposomes are vesicular and colloidal structures which are composed of one or more lipid bilayers surrounding water molecules. Because of their small size and high encapsulation efficiency, liposomes are very suitable vectors for drug delivery. Through encapsulating the drug and controlled release of it, these vesicular structures play a significant role in increasing the therapeutic index and reducing the side effects of drugs.

It has been shown that ginger (Zingiber officinale) possesses anti-cancer properties. Gingerol, as the most important constituent of ginger that accounts for its spicy flavor, inhibits the growth of cancerous cells especially in human colon cancer. This characteristic may be originated from its main constituents including starch and essences like zingiberene which gives ginger its specific smell. Most of therapeutic properties of ginger seems to be the result of its spicy components, i.e. the gingerols. The anti-oxidant property of ginger is of a great importance because the anti-oxidant substances help greatly in removing free radicals. Free radicals are chemical agents which are produced through metabolic pathways, chemicals and/or the sunlight rays and cause some dangerous diseases including cancers.

Material and Methods

Phosphatidylcholine, cholesterol, polyethylene glycol 2000 (PEG-2000), 6-Gingerol and MTT (0.5 mg/ml) were purchased from Sigma, ethanol and isopropanol from Merck, RPMI-1640 culture medium from Invitrogen, and MCF7 from cell library of the Pasteur Institute of Iran.

Nanoliposome construction, 6-Gingerol loading and its PEGylation: Nanoliposomes were produced by reverse-phase evaporation method, through the following steps. Phosphatidylcholine and cholesterol (with 15:1 proportion) were solvated in 100 ml of 98% ethanol (300 rpm, room temperature) until the suspension became yellow and transparent. 3 mg of standard 6-Gingerol was added and mixed by magnetic stirrer (300 rpm, 30 min, room temperature). The solution phase was then evaporated using a rotary evaporator (Heidolph Instruments). The obtained gelose was solvated in 80 ml of physiologic serum and divided in four 20-ml vials used as control for both drug formulations. To produce the PEGylated liposome formulation, 56 mg of PEG-2000 was also added to the above-mentioned mixture. Both formulations were sonicated for 5 min with 60 Watt power (Bandelin Sonorex Digitec, 60 Hz) in order to obtain homogenous liposomes and entrap the drug within the vesicles.

Measuring the diameter and surface charge of liposomes: The average diameter and the surface charge of non-PEGylated and PEGylated liposomes were measured using a Zeta-sizer device (Malvern Instruments Ltd).

Standard curve of 6-Gingerol: Standard Gingerol with 0.5, 1, 2, 4, 6, and 8 µg/µl concentrations were prepared and their standard curve of absorption in 282 nm wavelength was plotted. The curve equation was determined to be used in later analyses.

Encapsulation efficiency of liposomes: 1500 mg of each drug formulation was centrifuged in 4°C in 50,000 rpm and the untrapped drug content of the supernatant was calculated by applying its 282 nm absorption and using the standard curve equation. Using the total drug volume applied in formulations, the encapsulation efficiency (EE) was obtained through the following equation:

\[ EE = \frac{\text{Entrapped drug volume}}{\text{Total drug volume}} \times 100 \]

Liposomal formulation stability: By calculating this parameter, the lifespan and persistency of nanoliposomal and PEGylated nanoliposomal drug formulations in room temperature was assessed. The assay was done in the first, the 15th and the 30th days. Each time the released drug volume was measured in 1500 mg of the formulation and the values of different formulations were compared.

Drug release: To determine the pattern of drug release from liposomes, 1000 mg of each formulation was tipped in a dialysis bag. Then, the bag was soaked in 10 mg of phosphate buffer and put on a stirrer with 300 rpm in 37°C. After 12 and 24 hours, the absorption in 282 nm wavelength for 5 ml of the phosphate buffer was read. The concentrations of released drug after 12 and 24 hours are calculated by putting the obtained absorption value in the standard curve equation.

Cytotoxicity of formulations: After culturing the breast cancer cell line MCF7, an appropriate number (i.e. 1000) of cells were tipped into the wells on a 96-well plate. The cells were incubated in 37°C with 5% CO₂ for 24 hours, in order for the cells to adhere to the bottom of the plate. The control wells and test wells were chosen and different concentrations of both formulations were added to the test wells. The plate was incubated for 24 hours for the drug to take effect. The culture medium above the cells was then decanted, MTT was added to the wells and the medium was incubated in 37°C within a CO₂ incubator for 2–4 hours. 100 µl of isopropanol was added to each well. The intensity of produced purple color has a direct relation with the number of cells which are metabolically active. Absorption in 570 nm wavelength was measured using an ELISA reader device. The viability an IC₅₀ values were calculated using the Pharm program and compared between the two formulations.

Result and Discussion

Measuring the diameter and surface charge of nanoliposomes: The average diameter and surface charge were 345.5 nm and -24.9 mV for the nanoliposomal drug and 310.4 nm and -8.54 mV for the nanoliposomal PEGylated drug, respectively.
Encapsulation efficiency of liposomes: The encapsulation efficiency (EE%) was calculated for nanoliposomal and PEGylated nanoliposomal formulations and the values were obtained 85% and 92%, respectively.

Formulation stability: The stability of nanoliposomal drug and the PEGylated nanoliposomal drug was assessed for one month at room temperature. Results showed that the rate of drug release from the PEGylated nanoliposomal formulation is slower than that from the nanoliposomal formulation. In other words, by releasing a smaller amount of drug, the PEGylated nanoliposomal formulation can maintain its stability in releasing the drug for a longer time. This explicitly indicates the advantage of PEGylation in stabilizing the liposomes.

Drug release: Figure 1 shows the trend of drug release in the PBS buffer, within 48-hour time periods. As can be seen, PEG plays a significant role in the gradual and continuing release of drug from the liposome.

Cytotoxicity of formulations: The viability of cancer cells after application of the two drug formulations are compared in figure 2. This comparison shows that the PEGylated form of 6-gingerol decreases the percent of viability further than the non-PEGylated form, in identical conditions of drug concentration. This indicates the larger cytotoxic effect of PEGylated nanoliposomal drug on the cancer cells, which may be due to the advantages of PEGylation in drug delivery tasks.

Determining the IC$_{50}$ of formulations: After identifying the effect of each formulation on viability of cells, the IC$_{50}$ value of each formulation was calculated using the Pharm software. Calculated values are shown in figure 3. Obtained results show that the PEGylated nanoliposomal formulation of Gingerol can inhibit the cancer cells, in a lower concentration when compared with liposomal and standard Gingerol. Therefore, this form of the drug can inhibit the cells more efficiently.

Targeted drug delivery is a novel method for treatment of different diseases. This method is, in part, based on lipid nanovectors. Liposomes are one of the lipid nanovector types. They can be targeted, so the drug delivery to cancer tissues can be increased and the toxicity can be decreased by use of liposomes. Promising results of liposomal drugs in prevention or therapy of a vast variety of diseases, in lab animals and humans, display that in roughly near future, liposome-based products will be produced for clinical uses.

For the first time, Philips and Tsoukas applied liposomes to transfer azidothymidine, an anti-HIV drug, and showed that this method reduces hematopoietic toxicity and increases the drug activity against AIDS in mice$^{12}$. Sharma and colleagues studied taxol-containing polyvinylpyrrolidone liposomes produced by reverse micro-emulsion method. The liposomes were 50-60 nm in diameter. The anti-tumor effect of taxol was studied on B16F10 melanoma grafted in C57B1/6 mice. In vitro efficiency of liposomal taxol was measured in terms of the decrease in tumor volume and increase in mice life period. The results indicated the higher efficiency of liposomal taxol in comparison with its non-liposomal form$^{13}$. 

![Figure-1](image_url)

Comparison of the drug release rate from nanoliposomal (●) and PEGylated nanoliposomal (○) formulations.
Figure-2
Comparison of viability percent between nanoliposomal and PEGylated nanoliposomal formulations in different drug concentrations

Figure-3
Comparison of IC_{50} in different drug formulations
Conclusion

One of the most important side effects of chemotherapy is ovary disorders and infertility, especially in young women affected with the breast cancer. This issue is even more important in Iran, because 40% of breast cancer cases occur in <30, 25% between 30-39, and 20% between 40-49 years old women. Chemotherapy has found many applications in extending the life of breast cancer patients. It is necessary to discover new drugs which are more efficient and have less side effects. Regarding that Gingerol in zingiber is a natural product with no negative effect on normal cells, it can reduce the undesired effects of chemotherapy. PEGylated liposomal Gingerol at nanometer dimensions can hide from macrophages which clear the waste substances from blood in the liver and spleen. Its PEGylation helps control the drug release, hide the liposome against the immune system and protect it against lipase enzymes which cause the lipid solvation. Thanks to all these advantages, the liposome can keep in blood circulation system for a longer period and has enough time to reach the target tumor cells, thus the need to taking medicines will be decreased. Therefore, application of the proposed new formulation, not only lowers the therapy costs and the drug side effects, but also improves the treatment efficiency.

References

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