Research Article

The Effect of Nano-Transferosomal Hydroxyurea on 4T1 animal Breast Cancer Cell Line: An in Vitro Study

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ABSTRACT

Breast cancer considered as a major health problem in women worldwide. Despite the various chemotherapeutic strategies, complete and effective therapy with lowest side effects didn't reach until now. So, in this regard, evaluation of the new treatments based on nanotechnology chemotherapeutic agent's drug delivery gained a much attention. The aim of the present study was to formulate, optimize and investigate the potential of novel nano-transferosomal hydroxyl urea (HU) effect on 4T1 mouse breast cancer cell line. Nano-transfersomal HU were prepared using reverse-phase evaporation method. Then evaluated for size, encapsulation& loading yield and drug release pattern. Cytotoxic effects of prepared Nano-drug in compared to free standard form evaluated by MTT assay. The optimized nano-transfersomes HU formulation showed vesicles size of 52.09±4.6 nm. Also, Encapsulation and loading yield were estimated 97.09±4.4 and 4.2±0.3 respectively. The optimized nano-transfersomes HU shows a higher cytotoxic effect in the dose dependent manner in compared to standard HU. The present study confirmed the higher cytotoxic effects of HU due to encapsulation (nano-transferosomal form). It was concluded that the nano-transfersomes provides an improved drug delivery in 4 T1 breast cancer cell line.

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Introduction

Cancer has become as the second most common cause of death in developing countries [1]. Breast cancer is a major cause of mortality due to cancer among women worldwide [2]. Despite the advances in breast cancer therapy including surgery, radiotherapy, chemotherapy, hormone therapy, but incompletely successful treatment still remained [3]. Failure in effective cancer treatments could be associated to high levels of toxicity, chemotherapy resistance and severe side effects. So, the ability to choose drugs with high specificity against cancer could be considered as new promising approach to achieve successful treatment [3]. Definitely, HU as chemotherapeutic anti-cancer agent has been used in breast cancer therapy. Nevertheless, of HU usage in breast cancer treatment, some problems including severe side effects have been reported in this regard, diverse techniques have been introducing to decrease the side effects and improve treatment efficacy [4]. Indeed, the major goal of the targeted drug delivery describes as organized drug delivery to the target organ with optimal therapeutic dose with minimal toxicity [5]. Liposomes are bilayer phospholipid vesicles that can encapsulate hydrophilic and lipophilic drugs to promote drug stability [6]. Nano-based drug delivery by liposomes are advanced technologies for the optimal anticancer drug delivery. These drug carriers offered effective pharmacokinetic trait, optimal and constant rate of drug release with minimal toxicity [7]. The novel drug carriers contain deformable vesicles, such as transferosomes have potential efficacy in molecules transport across mammalian skin to the targeted organ. Recently, the application of lipid vesicles-based drug delivery system across skin became a much popular, but their efficacy still not completely approved [8]. In a recent survey, 5-Fluroauracile-as anti-cancer drug loaded transferosomal gel led to increase the drug skin absorption and improved treatments outcome in skin cancer [9]. In previous studies, HU urea drug delivery using liposomes has been done. Liposomation show higher cytotoxicity effect in nanoliposomal drug form in compared to standard HU drug [4]. Also, in another study by Mehrabi et al, evaluate the efficacy of PEGylated liposomal etoposide nanoparticles on breast cancer cell lines. Their results show the higher antitumor activity in compared to free drug [10]. So, with considering the efficiency of liposomal-based drug delivery, in this study we evaluate the nano-transferosomal effects on the HU therapeutic index.

Materials and Methods

HU, Phosphatidyl choline, twin 80 and sodium deoxy cholate, Cholesterol and MTT kit were prepared from Sigma Company (Germany). The RPMI-1640 culture medium was obtained from Invitrogen (Invitrogen, USA). 4T1 mouse cell line was supplied by Pasteur Institute of Iran.

Preparation of nano-transferosomal HU

Nanotransferosomal-HU were prepared using reverse-phase evaporation method. Briefly, approximately 200 mg of Phosphatidyl choline, 40 mg of cholesterol, 75 mg of twin 80 and 75 mg of sodium deoxy cholate with 5 mg of HU were dissolved in 20 ml of ethanol-chloroform (1:1 ratio) at room temperature (37°C) and stirring for 1 hour at 120 rpm. After perfect dissolving, the solvent was removed to make the thin lipid layer by rotary evaporator for 1 hour at 37 °C, 60 rpm (Company Heidolph, Germany). In following, 10 ml of phosphate buffer (PH 7.4, 10 M) was added to obtain nano-transferosomal HU. In this lipid layer, the final concentrations of Phosphatidyl choline, cholesterol, twin 80, sodium deoxy cholate and HU were estimated 20 mg/ml, 4 mg/ml, 7.5 mg/ml, 7.5 mg/ml and 0.5 mg/ml, respectively. The solution was sonicated for 6 min (model Bandelin Sonorex Digitec, Germany, 60 Hz) and then passed from whatman filter paper to obtain a more homogeneous formulation. The formulations were stored in at 4°C until usage.

Characterization of Nano-carriers

The particle size and zeta potential of nano carriers, were measured by Zetasizer (Nano ZS3600, Malvern Instruments, UK). In this way, the nano carrier's suspension was diluted in PBS (1:10 ratio), and its absorbance was estimated using Zetasizer instrument (630 nm).

Encapsulation and loading yield

To estimate the rate of the entrapped HU, 78 mg of the nano-transferosomal HU was centrifuged (21000 rpm, 4°C for 30 min) and drug supernatant absorbance was estimated according to the standard curve at 214 nm by using a spectrophotometer (UV1800, Shimadzu Co). Encapsulation and loading yield were measured based on the below formulae:

Drug loading yield (%) = the amounts of HU loaded into nanoparticle (mg/ml) ×100
Nanoparticle weight (mg/ml)

Drug encapsulation (%) = HU preliminary concentration (mg/ml)-drug concentration in the supernatant (mg/ml)
HU preliminary concentration (mg/ml)

Drug release evaluation

In order to evaluate the nano-transferosomal HU release, dialysis membrane technique was used. The sediment of HU-loaded nano carriers was prepared as mentioned above. Then the sediment was resuspended into 5 ml fresh PBS (PH: 7.4, 10M). In following, nano-transfrosomal HU, standard free HU were poured into separate dialysis bags (Sigma, cut off 10000 Da) while it was placed on the magnetic stirrer for 48 hours (50 ml, 150 rpm, 37°C). Then, 2 ml of PBS buffer was removed and replaced with 2 ml of fresh PBS buffer. The amount of released drug in the PBS was calculated using spectrophotometry technique (214 nm).

Cell culture and cytotoxicity test

The T41 mouse breast cancer cell line was cultured in humidified atmosphere containing 5% CO2 in RPMI-1640 cell culture supplemented with 10% Fetal Bovine Serum (FBS), penicillin/streptomycin antibiotics (0.1 and 0.06 mg/ml respectively) at the density of 10^4 per in 96 well plates. After 24 h, cell culture was removed and free HU and nano-transferosomal HU at the same concentrations was treated into the cells (0.01, 0.03, 0.07, 0.15, 0.3, 0.6,

1.2, 2.4, and 4.8 mg/ml). After 24 h of incubation, the media was removed, and MTT solution (0.5 mg/ml PBS) was added to each well and incubated for 2h. The formazan crystals formed were dissolved in isopropanol 100%, and the absorbance was read at 540 nm using a microplate scanning spectrophotometer (ELISA reader, Organon Teknika, Netherlands). Cell viability was evaluated by following formula:

$$\text{Viability} = \frac{\text{ODaverage of the sample}}{\text{OD average of the control}} \times 100$$

The half maximal inhibitory concentration (IC50) was calculated using Pharm program. For statistical examination, one-way ANOVA analysis by SPSS software version 18 was used, and P values >0.05 were considered as significant level. Results expressed as the Mean±SD from separate tests that examined duplicates.

Results

I Characterization of nano-carriers

After preparing the transferosomal nano-carriers suspension containing the HU and control, their particle size and zeta potential was measured by Zeta Sizer. The average particle size of control Transferosomal nano-carriers was 78.4±6.4 nm (Fig.1), and the zeta potential was -10.3±0.17.
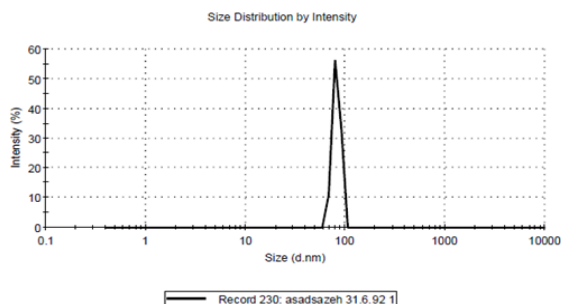

Figure 1: The average size of control Transferosomal nano-carriers. Particle size was measured by Zeta Sizer. The average size of Nano-Transferosomal HU was 52.09±4.6 nm (Fig. 2), and the zeta potential was -8.07±0.7.
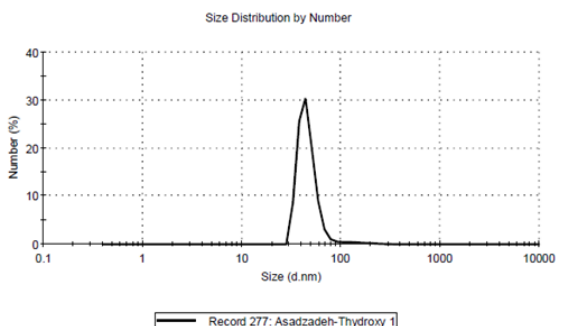

Figure 2: The average size of Nano-Transferosomal HU. Particle size was measured by Zeta Sizer.

II Encapsulation and loading yield

The results of encapsulation and loading yield were estimated 97.09±4.4 and 4.2±0.3, respectively (based on standard curve). Indeed, 97% of used HU become associated with nano-carriers and HU accounts for 4% of nano-conjugated HU weight.

III Drug release

The amount of nano-transferosomal HU release according to presented curve initiated with a burst release (33% of encapsulated drug) followed by the mild ascending slope with the maximal release of 12% after 48 h. The drug release study showed an initial burst during the second 2 hours and after that the release amount was not in a regular pattern (Fig3).
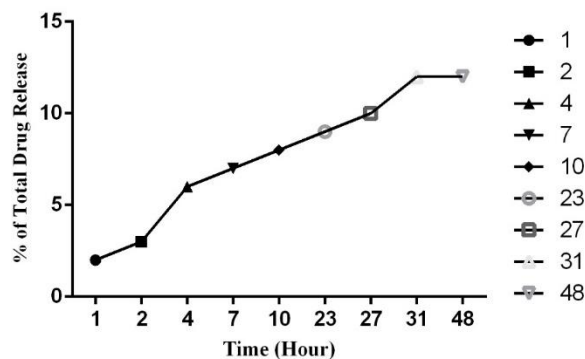

Figure 3: Drug release rate of nanotransferosomal HU. Results were expressed as mean ± 5% values. Curve initiated with a burst release, in following by the mild ascending slope with the maximal release after 48 hours.

IV Cytotoxicity effects of drug and nanodrug

According to our findings, cell viability was decreased in a dose dependent manner after exposure of breast cell line to HU and nano-transferosomal HU using the MTT assay. In comparison between these two treated cases, higher cytotoxic effects were obtained in all nano-transferosomal HU concentrations toward free HU (Fig. 4). Also, IC50 of nano-transferosomal HU and free HU was found to be 49±4.4 and 97±8.6 µg/ml respectively. In other words, transferosomes Nano-carriers enhance the efficacy of standard HU by 50%.
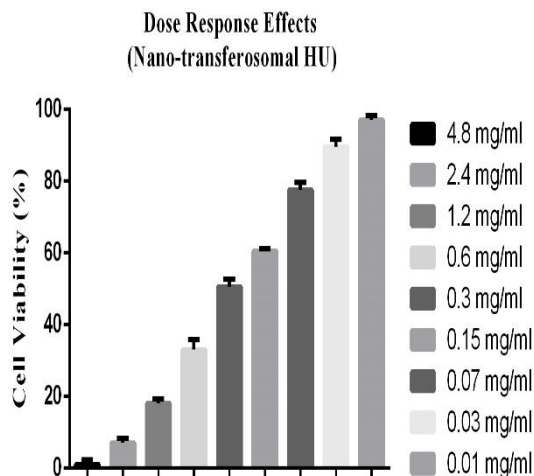
Dose Response Effects (Nano-transferosomal HU)
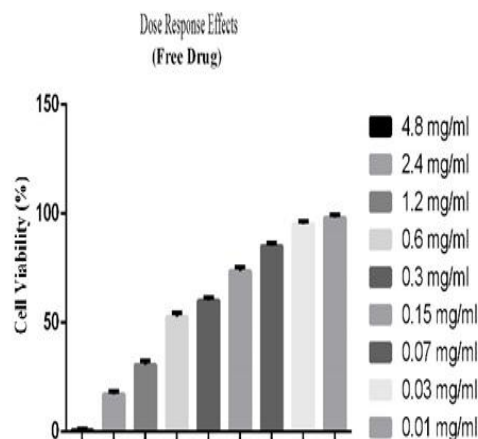
Figure 4: Cytotoxic effects of nanotransferosomal HU and standard HU on the viability (MTT assay) of mouse breast cancer cell line 4T1. Results were expressed as mean ± SD values of two independent experiments.

Discussion

Transfersomes as new vesicular drug carrier systems led to improved transdermal drug delivery. Indeed, it seems that this method is more effective in skin drug delivery in wide range of molecular weight. Based on previous studies, transfersomes displayed higher anti edema activity in compared to liposomes that is related to enhanced infusion across the skin [11]. On the other hands, breast cancer therapy is faced to drug resistance phenomena and high level of toxicity in clinic [12, 13, 5]. With considering HU as anti-neoplastic in breast cancer treatment that is related to severe side effects and related stigma, we aimed to prepare nano-transferosomal HU to gain a higher anti-cancer effects in breast cancer cells.

In this presented survey, we evaluate the cytotoxicity effects of standard HU in compared to nano-transferosomal HU. According to our findings, prepared NPs had optimal particle size, Zeta potential, and balanced drug release pattern. Nano-conjugated HU had low IC50 level in compared to standard HU which displays that nano-transferosomal HU had higher cytotoxic effects to free HU in 4T1 mouse breast cancer cell line. indeed, nano-transferosomal formulation improves the anti-neoplastic effect of HU.

In this regard, in a similar study, fadel et al evaluate the effects of encapsulated indocyanine green (ICG) in a vesicular colloidal nanocarrier (transfersomes). According to this study, transfersomal nanoencapsulated ICG is an effective treatment procedure for basal cell carcinoma that is parallel to our results [14].

In another study, a new hyaluronic acid altered transfersome, produced to lymphatics drugs delivery. Definitely, Doxorubicin loaded HA-GMS-T powerfully penetrate into the skin that related to higher absorption lymphatics rate. They indicated that, in cancer cells hyaluronic acid led to increased drug loaded nanocarriers absorbtion [15].

In a recent survey, 5-Fluroauracile-as anti-cancer drug loaded transfersomal gel led to increase the drug skin absorption and provides improved treatment outcome in skin cancer. Also, transferosomal form of other anti-cancer agent's efficacy has been reported previously [9]. Based on author knowledge we couldn't find any study related to preparation and evaluation of nano-transferosomal HU on breast cancer cell lines or animal and human subjects. Despite in a similar study, that investigate the effects on liposomal HU on breast cancer cell line, the higher cytotoxic effects of liposomal-HU has been confirmed in compared to free drug (5), that this result was similar to our presented findings. Overall, further studies are needed to evaluate the effectiveness of this new drug for effective treatment in breast cancer.

Conclusion

According to our findings, increased cytotoxic effects in the case of nano-transferosomal HU in compared to standard form were obtained. This higher efficacy supposed the new drug delivery approach in breast cancer therapeutic methods. Thus, this formulation can be an alternative chemotherapeutic candidate for breast cancer in the future with more evaluation in further cell lines and animal methods.

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