RESEARCH ARTICLE

Niosome encapsulated fluorouracil as drug delivery system to basal-cell skin cancer

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Basal-cell skin cancer is one of the most common diseases in countries which are in contact with sunshine more. Hence, it can be considered as a sophisticated problem that researcher are faced to that. Although, a few drugs such as fluorouracil have been suggested and accepted for this purpose, but still it's necessary to design a system to deliver this drug to deep part of skin. Hence, with this system the drug can pass easily through the Stratum Corneum (SC) and reach to desire site of action. The aim of this research is to assemble and evaluate a nano-carrier to carry fluorouracil to desired site of action without any side effects as enhanced permeability system. For this purpose, after encapsulation of noisome as a vehicle the size and stability of that, was measured by Malvern Mastersizer. The result showed that the size was between 200 to 400 nm and the zeta potential was about 60 that is good stability.

Key Words: *Niosome; Vehicle; Enhanced permeability; Fluorouracil; Basal-cell skin cancer*

INTRODUCTION

With the growth of nanotechnology, nanomedicine is developed for various application of treatment through delivery of the drug to the desired site of action. To this regards using a system based on nano size concept is well recognized [1-3]. As a matter of fact, this idea has derived from the nature that most of the activities in the living organism like human bodies are based on the design of bioactive systems with a concept of polymeric drugs delivery. Some well-known bioactive compounds for these systems can be counted as heparin, insulin, growth hormone and others which are designed and fabricated in our body within an accurate architecture [4]. The design and composition of cells and the extracellular matrix in the human organism are created to have interactions with nano molecules through specific properties [5,6]. Niosome as a complete system can be a suitable vehicle for delivery of drug to desire cells such as cancer cells or tumor. This process is possible due to the amphiphilic nature of niosome's structure, containing both hydrophobic and hydrophilic domains [7-9]. The structural characteristics of niosome have several advantages to improve the effectiveness and safety of cancer therapy in case of clinical use [10-13]. For instance, the encapsulation of drug in the core of this system improves their aqueous diffusion and transport, as well as bioavailability and decreasing their toxic side effects [14,15]. This system also allows the drugs to protect from degradation and produces their controlled release to the cancer cell due to the Enhanced Permeability and Retention (EPR) effect [16-18]. On the other hand, advances in engineering suggests a wide range of possibilities to control the most influential properties of the polymeric assemblies, such as the particle size, stability or loading capacity [7,19]. In fact affinity to receptors over expressed in skin cancer cells, are the most important parameters to control and enhance mechanism of incorporation bioactive nano particle systems which are endocytosis or pinocytosis. The application of this system brings novel and advanced possibilities including the lowering toxicity, without a noticeable decrease of the drug activity that can be considered the first generation of nanomedicine. It includes polymers with characteristics biological activity, that form multicomponent designed for intracellular delivery of drug [20,21]. The self-assembled nano particle contains the Fluorouracil (5-FU) in the core and a sensitizer located in shell layer targeting ligand for interaction and selective linking to the cytoplasmatic membrane [22]. The design of this system protects the fluorouracil until the cytoplasmatic membrane is reached [23,24].Hence, skin drug delivery represents a promising approach that aims to address the disease from the molecular point of view. This type of treatment is based on modified or normal functioning of drug that is delivered into the cell nucleus to prevent DNA replication. Fluorouracil (5-FU) acts in several ways, but principally as a Thymidylate Synthase (TS) inhibitor. Interrupting the action of this enzyme blocks synthesis of the pyrimidine thymidine, which is a nucleoside required for DNA replication. Thymidylate synthase methylates deoxyuridinemonophosphate (dUMP) to form thymidine monophosphate. Administration of fluorouracil causes a scarcity in dTMP, so rapidly dividing cancerous cells undergo cell death via thymineless death. Calcium folinate provides an exogenous source of reduced folinates and hence, stabilises the 5-FU-TS complex, hence enhancing 5-FU's cytotoxicity [25]. It is necessary the use of capable vehicle to deliver efficiently the drug inside the cells that are mainly based on cationic polymers and helper lipids [26]. Niosomes are drug carrier systems similar to liposomes with a bilayer structure, where the phospholipids of the liposomes have been substituted by non-ionic surfactants. Compared to liposomes, niosomes show some significant advantages, such as low cost and high chemical and storage stabilities. Even though the application of niosomes in skin drug delivery has been poorly studied, some optimistic results have been recently reported in the literature that highlights the satisfactory properties of niosomes for drug delivery purposes [27,28]. Niosome as a vehicle is commonly based on non-ionic surfactants, cationic polymers and lipids. Over the years, several researchers have studied these components and their effect on the niosome formulations. Such studies have shown that non-ionic surfactants make niosome formulations stable, and prevent the aggregates of the particles [29,30]. Cationic lipids handle the interaction with the negatively charged fluorouracil and its condensation to form nio-flu by electrostatic interactions [31]. Additionally, it has been observed that cationic lipid chemical structures influence on the niosomes charge, toxicity, biodegradability, and transfection efficiencies [32,33]. Regarding to helper lipids, it has been described that they are responsible for enhancing the physicochemical properties of the emulsion and the improvement of drug delivery [34,35]. However, the mechanisms that involve these improvements in cationic niosome formulations for drug delivery applications have not been completely surveyed, and more detailed studies are required. The final impact on fluorouracil expression, among many other factors, clearly depends on the cell to be transfected and on the capacity of the vehicle to enter the cell and the posterior pathway employed to deliver its cargo into the nucleus [36]. Different endocytic routes can mediate the cellular uptake

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and the final cargo delivery. Among these endocytic routes Clathrin-Mediated Endocytosis (CME), Caveolae-Mediated Endocytosis (CvME) and macropinocytosis are among the most studied [37-39]. Additionally to the uptake pathways, the particle transport mechanisms can determine the final intracellular destiny of the vehicle, e.g., lysosomal degradation [40]. Such pathways have their particular characteristics and their intervention in the cellular uptake and further internal processing will depend on many factors related with the drug delivery vehicle such as the size, surface charge, morphology and composition [41-43].

EXPERIMENTAL PROCEDURE

Niosomes prepared by oil-in-water emulsion technique (o/w). Dicetyl phosphate used to improve encapsulation. Chloroform in its solvent form and distilled water used for the hydration process. Moreover, labra sol used instead of cholesterol, because labra sol has better flowed and permeability and as a result more effective for delivery applications in compare to cholesterol. Niosome prepared based on the protocol of Arora and Sharma (2010) and Bhaskaran and Lakshmi (2009) with minor modification. In this method a mixture of the vesicle forming agents such as the surfactant and labra sol, dissolve in chloroform (a volatile organic solvent) in a round bottom flask. The organic solvent removed using a rotary evaporator, which leaved a thin film of solid mixture deposited on the walls of the flask. The thin film thus formed was then hydrated with buffer containing fluorouracil to form large multilamellar vesicles that were transformed to small unilamellar vesicles by extrusion. The resulting niosomal suspension mixed by vortex and sonicate. The niosomal suspension leaved overnight at 4°C and stored at refrigerator then sonicate by probe sonicator to yield nio-flu. Niosome characterized in terms of size, zeta potential and polydispersity index. The capacity of the niosomes to condense, release and protect the fluorouracil against enzymatic degradation evaluated by agarose gel electrophoresis. Moreover, cell uptake studies at 60 minutes after the addition of the niosome. To comprehend the internalization process, this analyzed cell trafficking of formulations in different entry pathways (CME, CvME, macropinocytosis) and lysosomal compartment. The factors influencing delivery of a drug mainly formulated composition, physicochemical properties of the drug and experimental parameters. Zeta potential and size distribution of niosomes studied by Dynamic Light Scattering (DLS). The method modified from online user manual Malvern Instruments Ltd., Worcestershire, UK. Particle size analysis determined by light scattering based on laser diffraction using the Malvern Mastersizer (Malvern Instruments Ltd., Worcestershire,UK). For zeta potential there was especial cuvette by disposable and reusable capillary cuvette with 0.5 mL capacity of sample and two gold electrodes in two side of sample. Zeta potential widely used for quantification of the magnitude of the electrical charge at the double layer. Manufacturer software used for analysis of particle size distributions niosome. Samples for DLS measurements were prepared by diluting 1% (w/v) aqueous niosome suspensions with the desired phosphate buffer to a concentration of 5 μg/mL. The intensity of light scatter optimized by optical adjustment of the instrument. The samples were allowed to equilibrate at room temperature for 1 h prior to the initiation of particle size measurement. A period of 5 minutes allowed in the measurements that performed using a 45 mm focus objective and a beam length of 2.4 mm. The zeta-potentials (ζ) of the niosomes determined from the electrophoretic mobility (μ) of niosomes measured in Distilled and Deionized Water (DDIW) using the Malvern Mastersizer. For measurements of the temperature and pH-dependence of (ζ), the niosomes suspensions prepared in 1 mM KC1 using 0.1 N HC1 and 0.1 N NaOH to adjust the suspension to the desired pH. The Malvern Mastersizer equipped with a Pelletier block to maintain temperatures of the niosome suspensions 0.1°C.

RESULTS AND DISCUSSION

The values reported represent the average of three independent measurements of each sample. The higher the concentration of lipids in the formulation, the more peaks registered on the Zetasizer, indicating numerous niosomes sizes. Log-normal size distribution observed for all prepared noisome formulations. Niosome size affects encapsulation efficiency (percentage of fluorouracil initially added to the preparation that becomes entrapped in the niosome), which generally increased with size. Size of the niosomes depends on the preparation method.

TABLE 1
Mean vesicle size (z-ave) and % entrapment efficiency of niosome

Before Filteration			After Filteration				EE (%) Day 0 for 4°C
Z-ave (nm)	PDI	Size	Z-ave (nm)	PDI	Size	Zeta Potential (MV)	
420 ± 5	0.463	528.33	264 ± 4	0.27	283.72	63	99.62 ± 1.02
548 ± 9	0.465	612.117	359 ± 2	0.273	291.26	58	99.50 ± 3.05
610 ± 6	0.471	685.492	392 ± 5	0.281	298.41	51	98.87 ± 1.67

Table 1 shows 3 selected niosome encapsulating fluorouracil 10% from 3 different ratios (50:50, 60:40, and 70:30) of span 60/labra sol that were measured before and after extrusion by Malvern zeta. The mean particle size (z-ave) (nm), Polydispersity Index (PDI) values, size, zeta potential (MV), and EE (%) (99.62, 99.50, and 98.87) were at zero days stored in 4°C.

Release of fluorouracil best fitted by diffusion based model, i.e. Higuchi equation. In 1983, Higuchi published the probably most famous and most often used mathematical equation to describe the release rate of drugs from matrix systems. It was later modified and extended to consider different matrix characteristics including porous structures. The basic equation of the Higuchi model is: $\frac{Mt}{M\infty} = K\sqrt{t}$ where Mt is the cumulative absolute amount of drug released at time t, $M\infty$ is the absolute cumulative amount of drug released at infinite time (which should be equal to the absolute amount of drug incorporated within the system at time t=0), and K is a constant reflecting the design variables of the system. Thus, the fraction of drug released is proportional to the square root of time. Alternatively, the drug release rate is proportional to the reciprocal of the square root of time. Unencapsulated fluorouracil solution gathered by using column chromatography equipment in G-50 gel Sephadex. After separation of the formed vesicles, encapsulation efficiency calculated from this formulation:

Encapsulation efficiency % =[1-(encapsulated fluorouracil/ total fluorouracil)] × 100

Photomicroscopy and Scanning Electron Microscopy (SEM) use to study the formation, morphology and size of the fluorouracil loaded niosomes. Before observation of vesicle formation and morphology analysis by SEM, the samples prepared and coated by specimens with cool sputter coater equipment (Bo-Rad brand) from UK. Optical micrographs obtained with a Nikon TE-2000 inverted light microscope (magnification900X). For SEM, a drop of vesicle dispersion applied to a 200 meshcopper grid and stained with a 1% Phosphotungstic acid. Then samples observed under a Scanning electron microscope (Hitachi Model H-7000, Tokyo, Japan).

Figure1 shows the morphology of niosome which encapsulates fluorouracil 10% provided by span 60. Strong evidence relating to fluorouracil encapsulated by niosome was found when the morphological behavior of niosome encapsulating fluorouracil was observed through images taken by SEM.

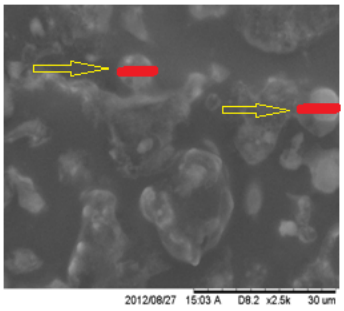

Figure 1) *Scanning Electron Microscopy (SEM) of niosome made from span 60. The red line size is 10 nm*

Morphology evaluation of niosome encapsulating fluorouracil 10% carried out by Transmission Electron Microscopy (TEM). Figure 2 (A1, B1) shows the spherical shape and multilamellar niosomes under optical microscope with a magnification of 300X to confirm formulation success (high specification life science microscopes Olympus BX51).

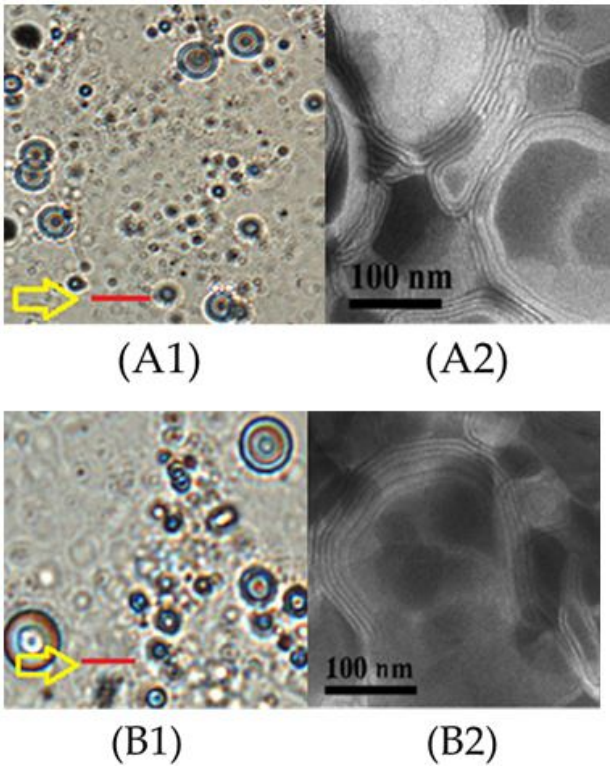

Figure 2) *Photomicrographs of niosome encapsulating fluorouracil 10% (A1, B1) optical microscope (red line=10 μm) and (A2, B2) Transmission Electron Microscopy (TEM)*

The vesicle sizes of all niosomes were in the range of more than 100 nm in Figure 2 (A2, B2). After fluorouracil was entrapped into the niosomes, the vesicle size of the niosomes significantly increased to 100-300 nm. In addition, after submitted to the extrusion process, the niosomes of small and homogeneous size were obtained from span 60 with the mean vesicle size of 300 nm.

The niosome encapsulated fluorouracil considered the unit for statistical comparison among different variations. Quantified data expressed as means at each point. T-test used for different treatments. Data presented as means standard error of triplicate samples.

CONCLUSION

This study was carried out with the aim of assessing the importance of niosome-encapsulated fluorouracil in the elimination of skin cancer cell. A statistical analysis of niosome-encapsulated fluorouracil showed a significant difference when compared to other treatments with niosome. According to the results that were obtained, it could be suggested that niosome affected the permeation of fluorouracil into the skin. This finding is identical to that reported by previous researchers. In summary, this work described the novel action of niosome-encapsulated fluorouracil in stimulating an innate anti-cancer response.

CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest.

DATA AVAILABILITY

The owner of the data set where requests may be sent to.

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