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ORIGINAL ARTICLE

Effect of Dabrafenib Loaded Nanobubbles on Cancer Cell Lines

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ABSTRACT
The objective of this research is to develop, evaluate, and assess the cytotoxic effects of nanobubbles loaded with dabrafenib for the treatment of cancer. Dabrafenib-loaded nanobubbles were obtained by synthesizing a poly D,L-lactic-co-glycolic acid using a modified water-in-oil-in-water double emulsion, solvent-diffusion-evaporation method. The resulting nanobubbles were evaluated for both qualitative and quantitative criteria. The cytotoxicity of the nanobubbles and their ability to inhibit cell proliferation were evaluated through the MTT experiment, which was conducted spectrophotometrically based on mitochondrial activity in the SK-MEL-37 cell lines. The qualitative and quantitative parameters that were evaluated were within the permissible range, and the dabrafenib-loaded nanobubbles were synthesized effectively. Significant anticancer efficacy was demonstrated by the IC50 value of 83.05 µg/ml obtained from the in vitro anticancer activity assessment of the nanobubbles produced on SK-MEL-37 cell lines. This result suggests that the dabrafenib-loaded nanobubbles that were formulated were thoroughly characterized and exhibited minimal cytotoxicity toward the SK-MEL-37 cell lines.
Keywords: *Nanobubbles, dabrafenib, toxicity, cell line, cancer, IC_{50}*

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INTRODUCTION

Nanobubbles are concavities in an aqueous solution that are capable of transporting gas and have size series of <1 µm [1]. These are round, globular elements that possess a gas occupied center & shell, which contribute to their distinct active characteristics. Shell primarily has lipid, polymer, protein, surfactant, and polyelectrolyte multilayer, while central part can be charged by other gases, like air, CO_2, sulfurhexafluoride, perfluorocarbon, and sulfurdioxide [2]. Half-life of bubble can be significantly influenced by the composition of its shell, which regulates the exchange of gas from the shell core to the surrounding medium. Shell thickness and elasticity are, in fact, the characteristics that contribute to the overall stability of nanobubbles [3]. These nanobubbles, which are sub-micron in size, are being created for improvement of drug's biodistribution at intended diseased spot, as well as its bio-availability and stability. They improve the efficiency and location of delivery by facilitating the extravasation of blood vessels into the adjacent tissues [4].

These nanoparticles accumulate and congregate at the site of a tumor, where they combine to produce microbubbles. Additionally, the release and accumulation of medication within the targeted cells are enhanced with greater efficacy and reduced toxicity as a result of the effective distortion of these microbubbles at the target region by forceful ultrasound [5]. Additional research is required to ascertain the effectiveness of these methods in the treatment of a diverse array of malignancies. In both in vitro and in vivo contexts, nanobubbles could be employed to deliver anticancer treatments, including dabrafenib [6].

The morphology, rate of cell growth, cell mortality, and cell disintegration of cells that are exposed to nanobubbles are altered. Consequently, it is imperative to conduct cell viability surveillance for each compound that has the potential to be of interest in experimental pharmacology. Alterations in

mitochondrial activity can be monitored through MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide). Dabrafenib-loaded nanobubbles are the subject of an in vitro cell toxicity study [7]. Dabrafenib (Tafinlar) is a BRAF inhibitor, a signal transduction inhibitor, a form of targeted therapy that reduces tumor size and increases survival in patients with advanced melanoma [7]. The average response time increases from 5.6 to 9.5 months when a MEK inhibitor is used in conjunction with a BRAFi. Dabrafenib mesylate salt (DBF.MS) is a BCS Class II pharmaceutical that is available for purchase and has high permeability and low solubility [8, 9].

Advancements in cancer-targeted nanotechnologies benefit clinical diagnosis and treatment. NBs are advanced drug carriers with a gas-filled core and a protein, polymeric, or phospholipid coating. When exposed to high-pressure ultrasound, NBs undergo inertia cavitation, causing shock waves and microjets. This process has significant bioeffects, exerting mechanical impacts on nearby tissues or cells (10).

The objective of the present investigation is to articulate, estimate, &measure cellular noxiousness of nanobubbles full with dabrafenib through the dealing of cancer.

MATERIAL AND METHODS

Materials

Dabrafenib was procured from Hetero Drugs Ltd. in Hyderabad, India. PLGA 50:50 (viscosity of 0.22 dl/g & mw 25,000) was supplied by Sigma Aldrich, US. Polyvinyl alcohol was from Sigma Aldrich. Solvents procured from S.D. Fine Chemicals, Hyderabad. D-PBS [#TL1006], Fetal Bovine Serum [#RM10432], DMEM [#AL007A], and EMEM [#AL047S] were obtained from HiMedia. The MTT Reagent [# M5655] and DMSO [#PHR1309] available from Sigma. The 96-well plate used for cell culture got sourced from Corning, USA. Cell lines got cultivated on Eagle's minimum essential medium and obtained from National Center for Cell Sciences in Pune, India.

Methods

Preparation of drug loaded nanobubbles

Dabrafenib got loaded into poly D,L-lactic-co-glycolic acid (PLGA) nanobubbles by a modified water-in-oil-in-water (W/O/W) dual emulsion, solvent diffusion & evaporation technique. The initial emulsion was generated by sonicating PLGA into dichloromethane. Subsequently, a double emulsion was generated by ultrasonifying polyvinyl alcohol. Drug-loaded bubbles that got generated were filtered & freeze-dried for subsequent examination [11].

Description & assessment of drug filled nanobubbles

The sound structure of plain & dabrafenib-loaded nanobubbles observed with field emission scanning electron microscopy. Drug & excipient compatibility examinations got conducted using fourier transformed infrared spectroscopy & differential scanning calorimetry. *In* vitro release kinetics got determined using the dialysis bag method. Nanobubble stability was evaluated over a one-month period, and the haemolytic potential of drug-loaded nanobubbles was evaluated using human blood cell suspension.

In-vitro cytotoxic study

The dabrafenib-loaded nanobubbles were assessed for their cytotoxicity and their capacity to spectrophotometrically inhibit cell proliferation in living SK-MEL-37 cell lines in relation to mitochondrial activity using the MTT assay [13].

Preparing cll line

Each cell line was extracted from liquid nitrogen packing& swiftly defrosted to room heat in a vial. Contents of receptacles got combined with 9ml of whole medium & centrifuged at 125g (5min). Upon centrifugation, supernatant gotcastoff, & particle got combined to 10ml of whole medium. The mixture was then suspended in T-25 flask &nurtured at 37°C (5% CO2). Upon reaching approximately 80% confluence, cells got centrifuged of 125g (5min). The particle got then combined by 15mL of whole medium & transferred to T-75 flasks. Cells in flask were employed for the assay when the cell confluence attained approximately 80-90% [14, 12].

MTT ASSAY

A 96-well plate was seeded with 20,000 cells per well and a 200µl cell suspension in whole culture medium by 10% FBS. The test agent did not present, and the cells were grown for 24 hours. The exhausted media in wells gotsubstituted with Dabrafenib test formulation at the appropriate concentrations after 24 hours of incubation. Plate then incubated at 37°C in (5% CO_2) for 48 hours. Plates got removed from the incubator after incubation, and exhausted media was detached. Subsequently, MTT reagent was added to final point of 0.5mg per ml (0.2 µm filter purified). To prevent exposure to light, plates got wrapped in aluminum foil & set in incubator for three hours. The MTT reagent got removed

after incubation, & 100 µl of DMSO got added. The absorbance determined at 570 nm using a Tecan™ Infinite 200Pro spectrophotometer [14, 15].
The untreated group assumed to have a 100% cell viability, while the treated groups were estimated to have a cell viability that was comparative to -ve control [16]. Concentration was designedin contradiction of percentage viability, & the dose response was assessed. Asuitable model got fitted to presummitImax & IC_{50} based on the dose response relationships.
Percentage viability formula:

$$\%\ Viability = \frac{100\ X\ OD570e}{OD570b}$$

OD570e= Optical Density test item dilution;
OD570b=Optical Density of -ve control

RESULTS AND DISCUSSION

Drug loaded nanobubbles

In current years, numerous researchers have finished numerous endeavors to create innovative drug distribution systems that utilize nanobubble to reduce noxiousness, enhance stability & specificity, upsurge bioavailability, and accomplish continued release of a variety of hydrophilic & hydrophobic drugs. Various techniques have been employed to prepare nanobubbles, reliant on physicochemical properties of polymer & drug to be filled. The W/O/W method was selected for formation of dabrafenib-loaded nanobubbles.

Characterization and evaluation ofdrug loaded nanobubbles

Dabrafenib-loaded nanobubbles were assessed. The dabrafenib-loaded nanobubbles were measured at 190.6 ± 18.4 nm, 0.397 ± 0.096 nm, and -21.4 ± 4.2 mV, with a drug payload of 26.29 ± 4.01. Particle size, zeta potential, & polydispersity index were all measured. Electron microscopy measurements of both dabrafenib-loaded nanobubbles are effectively correlated with the dynamic light scattering approach. The nonappearance of an endothermic peak of dabrafenib-loaded nanobubbles and nonappearance of distinctive peaks of the drug in Fourier transformed infrared spectra indicate that drug may be encapsulated within the PLGA. Nanobubbles with acoustic assistance released more than 95% of their contents within 24 hours. The stability investigations have shown that the nanobubbles loaded with dabrafenib are a stable formulation. The aqueous solutions of PLGA nanobubbles exhibited no haemolytic activity, while the drug-loaded nanobubbles exhibited exceptional safety when used with erythrocytes.

In-vitro cytotoxic analysis

These experiments offer valuable insights into the model's advantages and disadvantages for the mechanistic evaluation and screening of possibly harmful compound. Anextensive variety of biochemical & morphological indicators are accessible to obtain data at cellular & molecular level in order to identify chemical-induced disturbance [17]. Under laboratory conditions, in vitro cell line models are relatively straightforward to implement. Human tumor cell lines are the primary focus of cellular screening in oncological research. This system is the most effective in terms of reproducibility and management [18]. Cell viability assays were conducted on the SK-MEL-37 cell lines (Table 1; Figure 1) to assess toxicity of dabrafenib-loaded nanobubbles. The IC_{50} values were determined using the MTT method.

Table 1: Cytotoxicity of dabrafenib pure drug &dabrafenib nanobubbles on SK-MEL-37 cell line

Concentrations (µg/ml)	Dabrafenib Pure Drug		Dabrafenib Loaded Nanobubbles	
	% Mean Viability	IC50 value (µg/ml)	% Mean Viability	IC50 value (µg/ml)
Blank	-	72.45	-	83.05
Vehicle Control	100.00		100.00±0.00	
5.00	97.33±2.54		97.33±1.26	
10.00	83.74±1.54		83.74±2.14	
20.00	64.23±2.76		74.23±2.95	
30.00	54.25±2.45		57.25±1.74	
50.00	45.43±2.36		34.43±1.84	
100.00	31.35±1.84		21.35±1.24	

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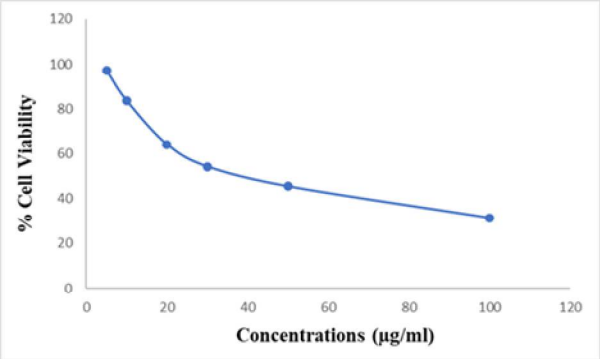
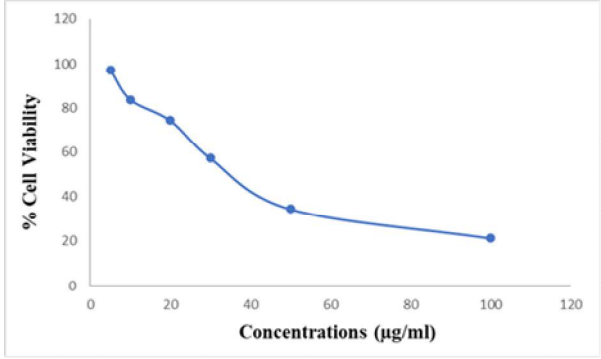


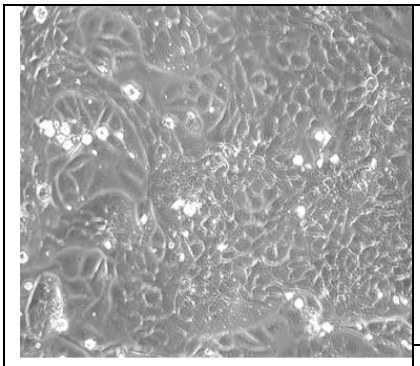
Cytotoxicity of Dabrafenib Pure Drug on SK-MEL37 cell line



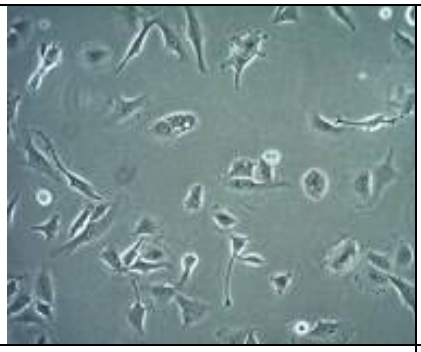
Cytotoxicity of Dabrafenib nanobubbleson SK-MEL37 cell line

Figure 1: Cytotoxicity of Dabrafenib Pure Drug and Dabrafenib nanobubbles on SK-MEL37 cell line

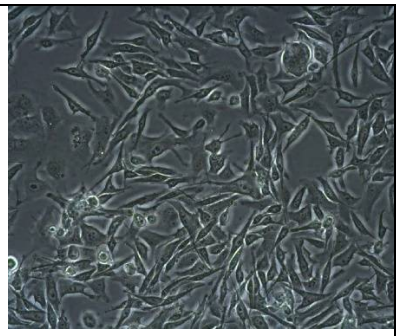
The morphology of the SK-MEL37 cell lines got analyzed after treatment with an assortment of concentrations of dabrafenib and dabrafenib-loaded nanobubbles (Figure 2). The cell surface exhibited substantial deformations and modifications, which are likely due to the influence of the nanobubbles that were examined. The impact of pure medicines on cell morphology was significantly reduced. The concentration of dabrafenib-loaded nanobubbles increased in conjunction with alterations in cell morphology and apoptotic processes. The anticancer properties of dabrafenib-loaded nanobubbles are associated with their ability to induce apoptosis and disrupt membranes [19].



Vehicle control



Dabrafenib test formulation agaainst SK-MEL-37 cellline



Dabrafenib Pure Drug on SK-MEL-37 cellline

Figure 2: Morphological changes of SK-MEL-37cancer cells when treated with Dabrafenib nanobubbles

The MTT assess is colorimetric method that evaluates metabolic action of cells. Cytotoxic potential of a test medication was assessed for preliminary screening of necrosis or apoptosis using the SK-MEL-37 cell lines. MTT assay's machinery includes the NAD(P)H-dependent cellular oxidoreductase enzyme, which converts yellow tetrazolium MTT [3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] into insoluble (E, Z)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan (formazan). In a protection assessment conducted by MTT assess, the formulation that contained aforementioned safe compounds demonstrated minimal cytotoxicity. We confirmed that the engineered formulation's pretreatment media had a cell viability (%) of over 97% (less than 3% cell mortality at each concentration). This outcome designates that microemulsion that has been formulated has minimal cytotoxicity toward the SK-MEL-37 cell lines.

CONCLUSION

The targeted delivery of a variety of anticancer pharmaceuticals, including synthetic compounds and natural products, has recently garnered significant interest in nanoparticulated drug delivery systems. Tumor accumulation was optimal for particle size in the optimized formulations. The formulation's uniform monodispersed was suggested by the nanobubbles' limited size distribution. The formulation's significant cytotoxic effect was demonstrated by the MTT assay. Each of these demonstrated the clinical importance of the nanobubbles that were laden with dabrafenib. This finding implies that the dabrafenib-loaded nanobubbles that have been prepared have minimal cytotoxic effects on the SK-MEL-37 cell lines.

CONFLICTS OF INTEREST
No conflict of interest.

AUTHOR CONTRIBUTION
The investigation work, execution, and writing were completed by B.V.L., P.S.R., and DB, who also conducted the work plan, review, and corrections. The submission and publication are approved by all authors. The published version of manuscript was read & accepted.

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