Cytosolic Delivery of Anticancer Drug Using Endosomolytic Peptide Bearing Nanoparticles Against Solid Tumor

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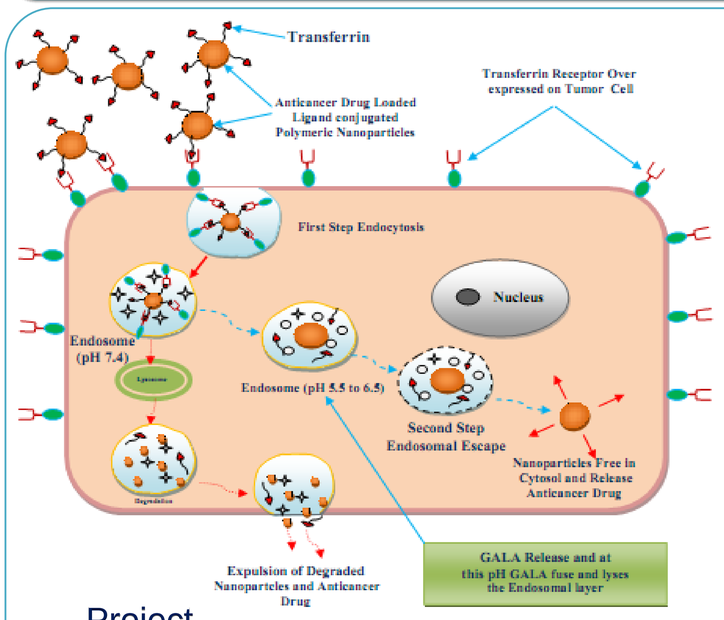
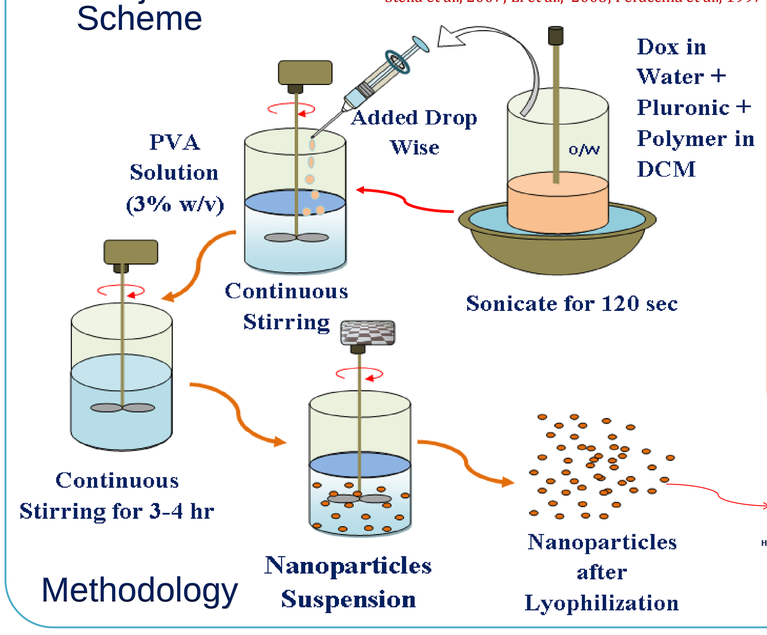
OBJECTIVE

The objective of the present project is to develop transferrin coupled biodegradable nanoparticles (NPs) with high encapsulation efficiency (Doxorubicin). GALA (Glutamic acid-alanine-leucine alanine) a pH sensitive fusogenic endosomolytic peptide is added to the formulation to enhance the endosomal lysis that will further potentiate the delivery of anticancer drug inside tumor cell as well as prevent loss of drug via endosomes. Transferrin receptors are in abundance on the tumorous tissue and the proposed carrier is supposed to bind with the tumor cells specifically and will release the drug directly to the tumor.

INTRODUCTION: Generally in case of targeted drug delivery to specific body organ or tissues, novel drug carriers such as liposomes, microsphere, nanoparticles, polymeric micelles and others are used and their endocytosis occurs by targeting cells. The two situations arise during endocytosis, (a) degradation of both polymeric carrier and drug may occur by endosomal content such as enzymes, pH condition and other related factors (b) expulsion of drug carrier system from target cells without drug release, and it can be prevented by lysing the endosomal layer. Hence, it is proposed to design polycynoacrylate NPs encapsulating anticancer drugs like doxorubicin. Encapsulation will lead higher drug loading in a system and then the NPs conjugated with transferrin on the surface which will target the NPs to tumor expressing transferrin receptors. Nanoparticles also contain a pH sensitive fusogenic peptide GALA which helps in endosomal scape of drug loaded NPs, (Yamada 2005).



Stella et al., 2007; Li et al., 2003; Peracchia et al., 1997



MATERIALS AND METHOD

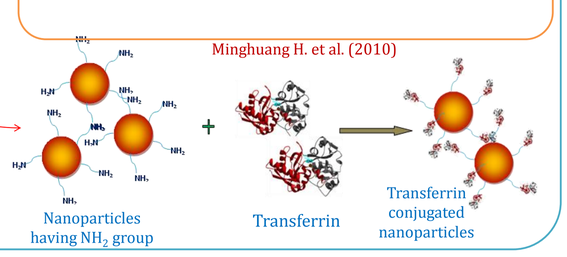
Doxorubicin as a gift sample from: Khandelwal Pvt Ltd Mumbai, India.
Transferrin, MePEG– 5000: Sigma Aldrich, Missouri, USA
DCC, 1-Hexadecanol ,Cyanoacetic Acid, DMAP: HiMedia and CDH, India
GALA were synthesized from: USV Pharma Ltd Mumbai, India.

NOPARTICLES PREPARED IN THREE STEP

Step I: Synthesisi of biodegradable amphiphilic polymer i.e. poly (H_2NPEGCA-co-HDCA) having high encapsulation efficiency Peracchia (1997) and Stella (2000).

Step II: Preparation of doxorubicin and GALA encapsulated nanoparticles double emulsification method reported by Stella (2000)

Step III: Conjugated of nanoparticles with transferrin reported by Minghuang (2009)



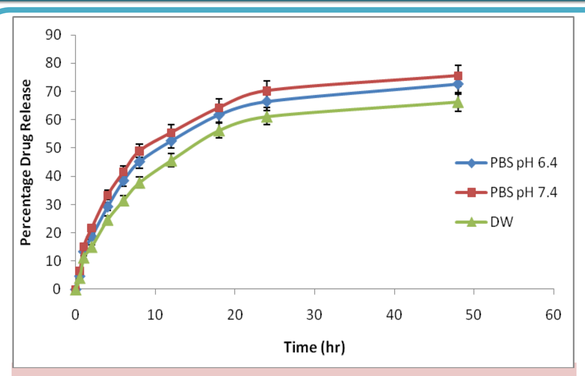


Fig. 2 (a) In vitro drug release of Doxorubicin from NPF-DOX-TF-GALA formulation(Mean±S.D, n=6)

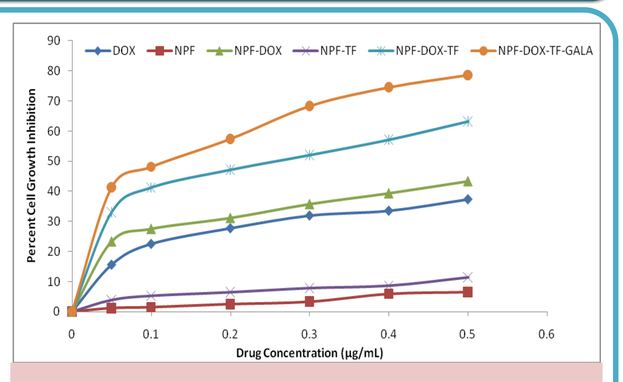


Fig. 2(b) Percent Cell Growth Inhibition Assay on MCF-7 cell line

EX-VIVO STUDIES

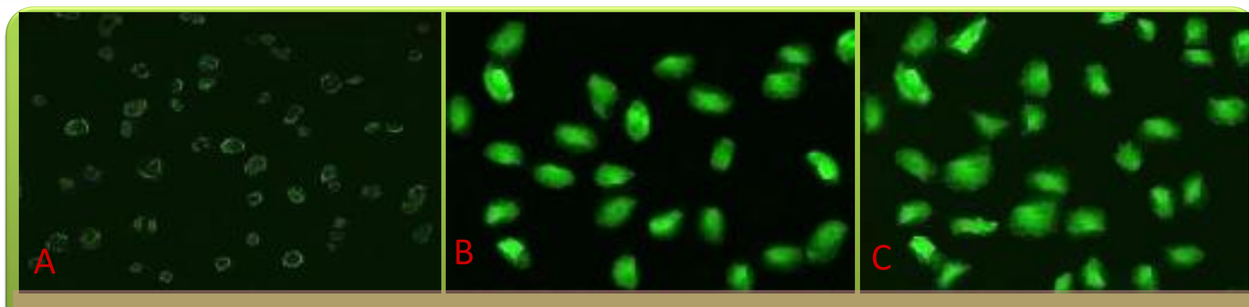


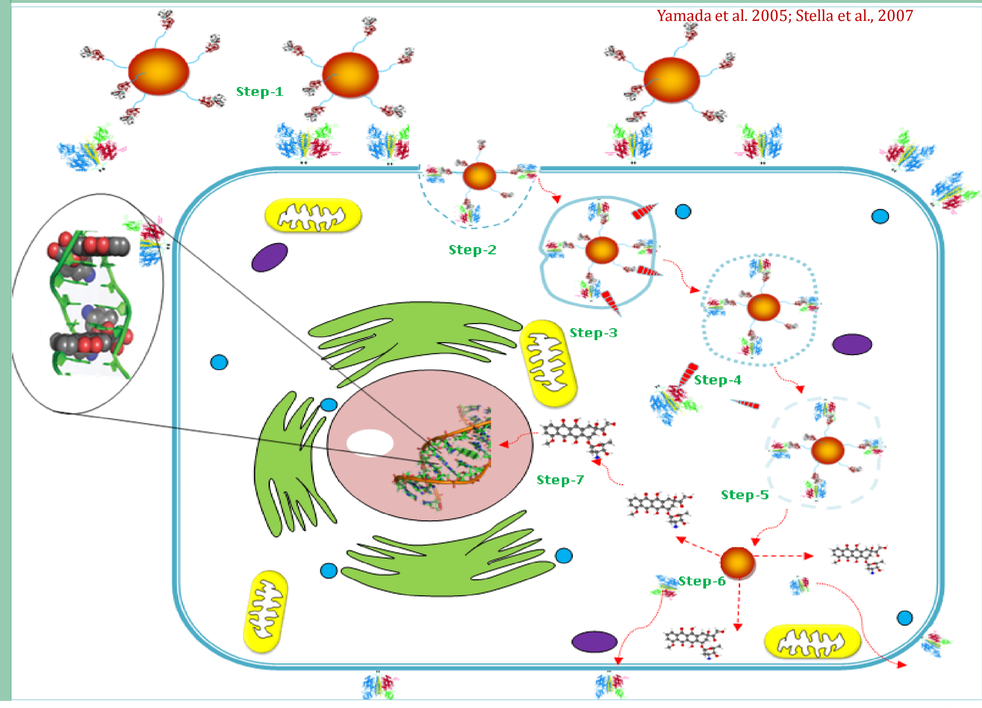
Fig. 3. Fluorescence photomicrograph of cell uptake assay. (A) NPF-DOX, (B) NPF-DOX-TF, (C) NPF-DOX-TF-GALA

IN-VIVO STUDIES

Schemetic Representation of Tumor Targeting of Nanoparticles

Step 1: Administered transferrin conjugated nanoparticles bearing Dox and GALA bind to the transferring receptor which are over expressed on the tumor cell surface.
Step 2: After binding of nanoparticles uptake by tumor cell through endocytosis process.
Step 3 & 4: During endocytosis, nanoparticles will come and face lysosomal (Late endosome) environment i.e. pH (5.5-6.5) and enzymes. In this pH condition GALA release from nanoparticle and fuse to endosomal layer and make pores on it.
Step 5 & 6: In this step endosomal layer breakdown totally and release drug loaded nanoparticles safely in and available for release drug cytosol of tumor cell.
Step 7: Drug is uptake by nucleus where Dox interact from DNA and interrupt their function (protein synthesis, and other function) that by cell death occur.

Yamada et al. 2005; Stella et al., 2007



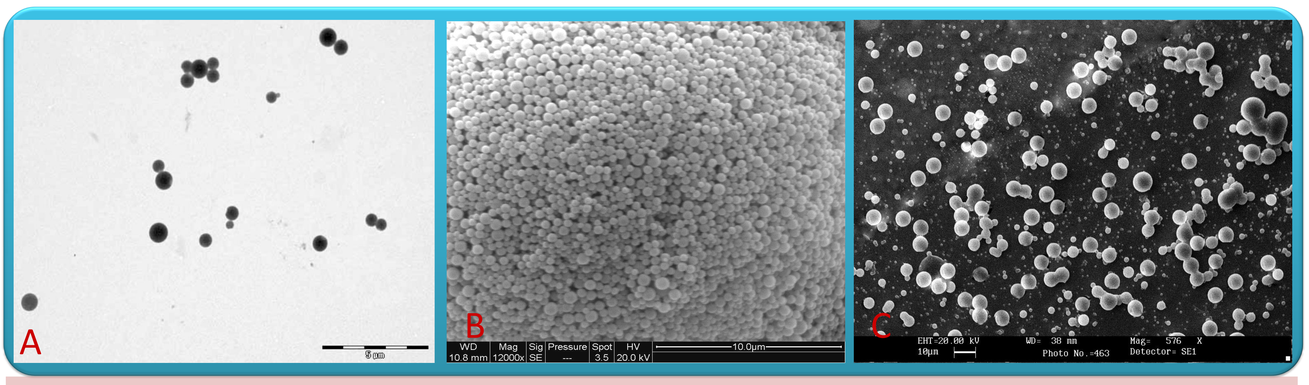


Fig 1. TEM & SEM Photomicrograph of Prepared Nanoparticles (A)TEM Photomicrograph, (B) & (C) SEM Photomicrograph

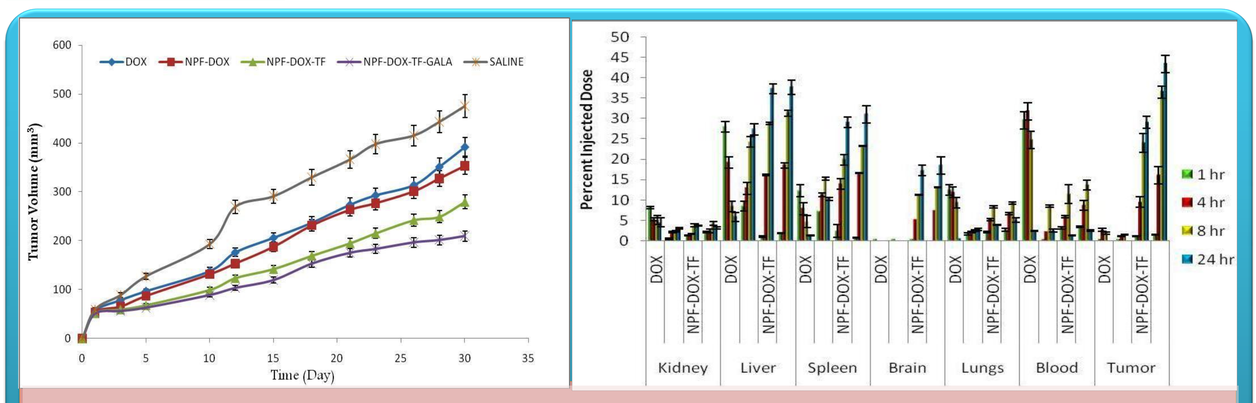


Fig. 4. (A) Tumor growth inhibition assay and (B) drug biodistribution study of DOX and DOX loaded formulations. Values represent mean ±SD (n = 6)

PERFORMANCE OF NANOPARTICLES FORMULATIONS

Stella et al., 2007; Li et al., 2003

- Average Size (Zetasizer, Malvern UK): 192.86±2.03 nm
- Poly-dispersity Index (Zetasizer, Malvern UK): 0.186
- Shape and Surface Morphology (SEM): Spherical in shape and smooth in surface (Fig. 1).
- % Drug Entrapment Efficiency: 67.74±2.14%
- In-Vitro Release Profile: 60-75% release in 24 hr. (Fig. 2 (a))
- Ex-Vivo Cell Growth Inhibition Studies: NPF-TF-DOX-GALA exhibiting highest percent cell growth inhibition (Fig. 2(b)) and higher cell uptake (flouroscence microscopy shown in fig.3, compared to other formulations as well as drug itself.
- In-Vivo Drug Distribution Studies and Tumor Growth Inhibition Studies:

  As expected, DOX loaded, NPF-DOX-TF, NPF-DOX-TF-GALA NPs are showing greater activity in tumor as compared to plain DOX and NPF-DOX (fig. 5A). drug recovered in tumor after 8 hr of administration of various nanoparticulate formulations is, NPF-DOX-TF-GALA>NPF-DOX-TF>NPF-DOX>DOX (Fig. 4). It is only possible due to presence of GALA (endosomolytic agent)

CONCLUSION: The surface modified NPs were found to protect entrapped drug and showed a release profile that was suitable for systemic delivery. *Ex vivo* and In *vivo* results indicate that delivery of DOX loaded NPs shows considerable promise in complimenting the therapy of cancer. This is apparent from the exciting results in timorous rats with significant tumor growth retardation for prolonged period. The anticancer effect of drug loaded formulation further synergism with the help of GALA peptide which help in increasing drug concentration in cytosol by lysing the endosomal layer. Therefore, it is conclude that prepared formulation using can be successfully exploited for the treatment of cancer.

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