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Meso Porous Silica Nanospheres For Delivery of Multikinase Inhibitor Sorafenib in Chemotherapy- an Invitro Study

KEYWORDS: Sorafenib, Multikinase inhibitors, Colon cancer.

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ABSTRACT *The major draw backs of chemotherapy includes mainly the poor solubility, specficity, stability of drugs and assisted delivery systems are found promising in overcoming this problem. Sorafenib, a class of novel multikinase inhibitors is being restircted to fewer pharmacological effects due to its poor solubility and pharmacokinetics. In the present study sorfenib was successfully loaded on mesoporous silica nanospheres with a loading efficiency of 93% and size of 396 nm. Cytotoxicity checking with normal fibroblast cells shown increased toxicity of sorafenib alone over loaded form which can be attributed to specificity of drug release. The particles were found to induce cell death in HT 29 colorectal cancer cell lines in a dose dependent manner with IC 50 values of 50µg/ml. The results draw new insights in use of silica nanospheres as potent delivery systems for multikinase inhibitors*

Introduction
The multikinase inhibitors sorafenib and sunitinib is clinically proved to be effective in patients with colorectal cancer, renal cell cancer and hepatocellular cancer (liu l et al, 2006) Sorafenib is a multikinase inhibitor of Raf, and it inhibits tumor cell proliferation and vascularisation by the activation of the receptor for tyrosine kinase signaling in the Ras/Raf/Mek/Erk cascade pathway. It has also been shown to block tumor cell proliferation and angiogenesis by inhibiting serine/threonine kinases (c-RAF, and mutant and wild-type BRAF) as well as the receptor tyrosine kinases vascular endothelial growth factor receptor 2 (VEGFR2), VEGFR3, platelet-derived growth factor receptor (PDGFR), FLT3, Ret, and c-KIT. The limitations of sorafenib including lesser solubility pose concerns over pharmacokinetics of the drug. The recent findings reported regarding adverse drug reactions including Hand-foot disease(Wood et al,2010), hypertension, nausea, muscle pain is quite alarming. In this aspect assisted delivery systems can be quite worthy and silica mesospheres offer a promising candidate in this context.

Silicon is the second most abundant element in the earth and it is readly excreted into urine as orthosilicic acid (reffitt et al,1999) generating minimum toxicity. Silica in its Nano form has good properties like good mechanical and thermal stability, it is chemically inert and has low density. According to WCRF, colorectal cancer is the third most common cancer in both men and women. The recent reports of National cancer institute estimates, that around 136,830 new cases of colorectal cancer will be reporting and death due to colon cancer is about 96,830 in 2014. It is a cancer that forms in the tissues of colon. Most colon cancers are adenocarcinomas (cancers that begin in cells that make and release mucus or other fluids) and inefficacy of existing therapeutic regimens necessitates the need for novel delivery systems.

Materials and Methods
Tetraethyl ortho silicate (TEOS) 99%, Ethanol, Ammonia solution 25% were obtained from Merck specialities, India. Dulbecco,s Modified eagles media(DMEM), Trypsin, Fetal bovine serum(FBS), 3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) were obtained from Invitrogen. All the other chemicals used were of superior analytical grade.

Cell lines L929 (Murine fibroblast cells), HT 29 (colorectal carcinoma) were obtained from NCCS, pune and maintained in Dulbecco,s Modified eagles media supplemented with 10% FBS

Synthesis of Mesoporous silica Nanospheres
Mesoporous silica nanospheres was prepared by sol-gel method (Stober et al,1968) . 1.5ml of tetraethyl orthosilicate (TEOS-99%) and 50 ml of ethanol was heated at 64°C for 1-2 hours. 2.5ml ammonia was added drop wise to the resulting solution and stirred continuously for 18 hours. Nanospheres were extracted by centrifugation at 10,000g at 4°C for 10 minutes. The pellets were washed with ethanol 3times and particles (MSN) were air dried.

Preparation of drug loaded Nanospheres
Drug loaded Nanospheres was prepared by dissolving 200mg sorafenib in 1ml ethanol. 200mg of synthesized particles were added to the above mixture and stirred for 48hours at 500rpm. The particles were recovered by centrifugation at 10,000g at 4°C for 15 minutes. The pellet was washed for 3 times with acetone and particles (SMSN) were air dried.

Zeta Sizer Analysis
Particle size and size distribution were determined using Zetasizer (brookhaven Instruments cooperation) . Measurements were recorded at 25°C suspended in Hepes buffer (ionic strength 40mM, pH 7.4) with a Ag electrode using Phase Analysis light scattering mode. The zeta (ζ)potential was automatically calculated from electrophoretic mobility based on the smoluchowski equation, $v=(\varepsilon.E/\eta)\xi$. Where, v= electrophoretic velocity

η= Viscosity

E= electric field

ε= electrical permittivity

Determination of Loading capacity and Entrapment efficiency
Loading capacity and entrapment efficiency was calculated by measuring the free drug content in the supernatant by UV-visible spectrophotometer at 265nm.

Loading efficiency = (Amount of drug added-amount of free drug) ×100

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Amount of drug added

Determination of Cytotoxicity
L929 cells were grown to confluency at 37°C in 5% CO2 incubator (NBS, Eppendorf-Germany) in a humidified atmosphere. The cells were trypsinized and 100µl of medium containing cells was added to a 96 well plate. After 24 hours incubation, the cells were treated to measure the in-vitro cytotoxicity. Phase contrast microscope was used to examine the cell morphology after 24 hours treatment. Cyto toxicity was measured by MTT Assay.

MTT Assay (Arung et al., 2000)
The cell culture suspension was washed with 1x PBS and then added 30 µl of MTT solution to the culture (MTT -5mg/ml dissolved in PBS) and plates were incubated for 3 hours followed by 200 µlof DMSO addition and further incubated at room temperature. Optical density was read at 540 nm using DMSO as blank.

Anti-Cancer Activity of SMSN on HT29
HT 29 cells were exposed with different concentrations of SMSN such as (6.25, 12.5, 25, 50,100µg) and incubated for 24 hours. The resultant cell death was determined by MTT Assay.

Results
Zeta Sizer Analysis
Zeta potential was performed to validate the size of mesoporous silica nanospheres was 396nm which range in nano scale.

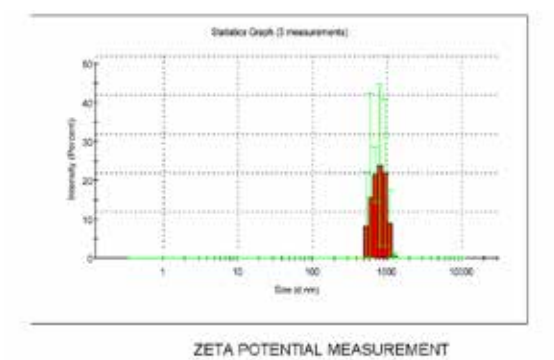

Fig 1: Size measurement of sorafenib loaded silica nanospheres

Determination of loading efficiency
Loading efficiency of SMSN was determined using the equation and showed 93%drug was efficiently entrapped in the silica nanospheres

Determination of Cytotoxicity

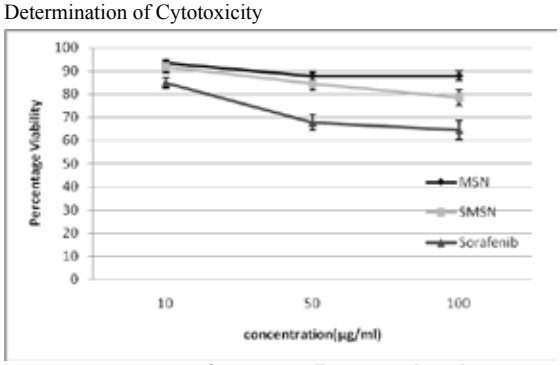

Fig 2: MTT assay of L929 cells treated with MSN and SMSN. Along X axis concentration in µg/ml and Y axiz % viability

Cytotoxicity accounts to the response of sorafenib loaded silica nanoparticles to normal cells. From the results it is clear that mesoporous silica nanospheres is non toxic to normal fibroblast cells .

Determination of Anticancer Activity
MTT Assay

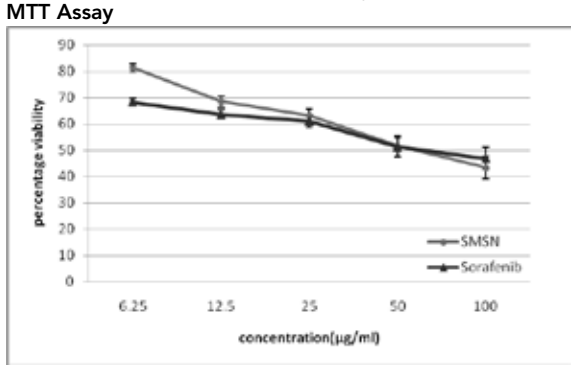

Fig 3: MTT assay of HT 29 cells treated with MSN and SMSN. Along X axis concentration in µg/ml and Y axiz % viability

MTT assay measures the mitochondrial metabolic stability after exposure with drug. From the results it can be observed that our loaded silica nanospheres is exhibiting an IC50 values of µg/ml which is comparable with standard sorafenib.

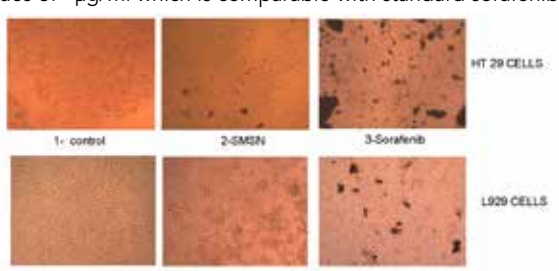

Fig 4: Phase contrast analysis of cell treated with Mesoporous silica nanospheres (SMSN) and Sorafenib. Sorafenib and SMSN showing increased cell death in HT 29 cells.

DISCUSSION
Multikinase inhibitors is gaining increased acceptance since a decade owing to their increased therapeutic potential over other drugs. Sorafenib is approved by European medicines agency for treatment of patients with HCC (Hepatocellular Carcinoma) and RCC (renal cell carcinoma) (Moore et al , 2005) and is found effective against metastatic colorectal cancer and KRAS mutated tumors (Samalin et al, 2014; Josep et al, 2013). We hypotheise targetting of sorafenib can overcome the exisiting limitations such as solubility, stability and increase the specificity and silica was selected as the carrier of interest in this aspect.

The synthesized silica nanospheres was found to have average particle size of approximately 396nm and loading efficiency was relative high. The inherent high loading capacity of silica nanospheres is previously reported by many workers and we got a loading capacity of 93% which is quite acceptable in this range. The anticancer activity of loaded sorafenib in colon cancer cell lines give an IC 50 value of 50µg which is in accordance with previous findings of Jin et al, 2011. Morphological analysis of cell lines shows increased membrane blebbing and cytological changes associated with silica nanospheres loaded cells rather than that of sorafenib alone with confirms the anticancer potential. The results first time reports sucessfully incorporation of sorafenib in silica nanospheres and anticancer activity in colon cancer. This study can offer valuable data regarding incorporation of sorafenib in delivery systems.

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