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Nitric Oxide Releasing Photoresponsive Nanohybrids As Excellent Therapeutic Agent for Cervical Cancer Cell Lines

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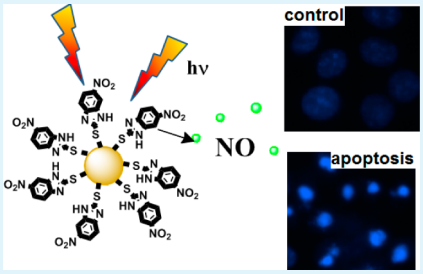
Supporting Information

ABSTRACT: Gold nanoparticles (GNPs) that can release nitric oxide (NO) on visible-light irradiation were prepared using 2-mercapto-5-nitro benzimidazole (MNBI) as stabilizer. These nanoparticles meet overall prerequisites for biomedical applications like small sizes, water solubility, and stability. It was found that even a very low dosage of MNBI-stabilized GNPs exhibit appreciable tumor cell mortality against cervical cancer cell lines, demonstrating the role of NO in killing cancer cells.



KEYWORDS: *photoresponsive, nanohybrids, 2-mercapto-5-nitrobenzimidazole (MNBI), gold nanoparticles (GNP), nitric oxide (NO), apoptosis*

INTRODUCTION

Nitric oxide (NO) is a fascinating molecule, because of its key role in several physiological processes.[1] The exciting discoveries of multiple roles of NO in physiological processes such as neurotransmission, vasodilation and hormone secretion, has led to a prolific growth in the area of synthesis of NO delivering materials.[2−5] Also NO proved to be an excellent antioxidant in free radical induced lipid peroxidation and an efficient anticancer agent.[6−8] High level expression of NO produced by activated macrophages may be cytostatic or cytotoxic for tumor cells but low level expression can have opposite effect and can promote tumor proliferation.[7] So the investigation of the role of NO in cancer at the molecular level can have profound effect on its treatment and therapeutic application. Many classes of NO donors are known viz., nitrosothiols (RSNOs),[9] diazeniumdiolates (NONOates),[10−12] 4-alkyl-2-hydroxyimino-5-nitro-3-hexenes (NORs),[13−15] etc. These compounds release NO by autolysis. Substances that release NO by light stimuli were found to be more attractive than those based on autolysis. The easy manipulation of light along with the fast response of NO release by photochemical reactions allows one to have temporal and targeted NO delivery.

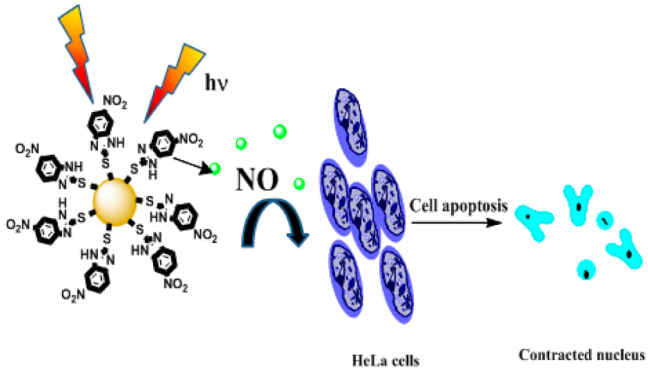
Benzimidazole nucleus acts as the key building block for developing molecules of pharmaceutical and biological interest.[16−19] Nitro benzimidazole derivatives have been reported as efficient antitumor compounds by Ramla et.al and the studies show that the cytotoxic effect is mainly due to the presence of nitro goup.[18] Herein, we report on the synthesis of 2-mercapto-5-nitro benzimidazole (MNBI)-capped GNPs in aqueous conditions which are intended to release NO under visible-light irradiation in controlled fashion. In the present case, benzimidazole acts as bridging ligand between chromophore (NO_2) and gold core. The synergistic effect of high surface to volume ratio of GNPs along with its water solubility and light-induced NO release are taken as advantages in designing a potent therapeutic agent against cervical cancer cell lines.

RESULTS AND DISCUSSION

The aqueous soluble 2-mercapto-5-nitro benzimidazole stabilized gold nanoparticles (MNBI-GNPs), prepared in our lab, showed enhanced antitumor efficacy against cervical cancer cell lines (HeLa) (Scheme 1).

The synthesis of water-soluble MNBI stabilized GNPs was achieved by borohydride reduction method. This method involves a simple two step procedure (Supporting Information) in which tetrachloroauric acid was mixed with MNBI in alkaline

Scheme 1. Schematic Representation Showing HeLa Cell Apoptosis in the Presence of MNBI-Stabilized GNPs



Received: May 31, 2013
Accepted: August 16, 2013
Published: August 16, 2013

condition. This solution was allowed to stir at room temperature (RT) under N_2 atmosphere. To the above solution ice cold $NaBH_4$ was added after an hour. Color of the solution changes from yellow orange to dark brown confirming nanoparticle formation. Nanoparticles were separated from excess reactants via dialysis for 24 h and maintained in neutral pH thereafter. Figure 1 shows the photographs of GNPs

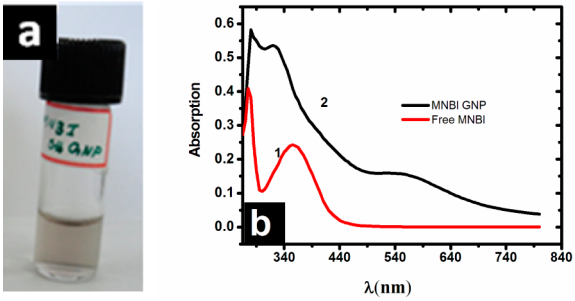


Figure 1. (a) Photograph of prepared MNBI-GNPs; (b) UV−vis spectra of (1) free MNBI and (2) prepared GNPs.

prepared by us and their corresponding UV−vis spectra. Curve 1 represents UV−vis spectrum of free MNBI and curve 2 corresponds to MNBI-GNP. Small shift toward shorter wavelength side is observed for nitro group of MNBI after binding with gold. This is due to the influence of gold core on chromophores.[20−23] UV−vis spectra of these nanoparticles show two absorption bands one at 540 nm corresponding to the surface plasmon band of GNPs and other one at 350 nm corresponding to nitro group of MNBI.

A significant dampening and broadening of plasmon band is observed after MNBI capping, which is characteristic of thiolate-capped GNPs.[20−23] Absorption of chromophores is influenced by length of bridging units. Long units can block electronic interaction between gold core and chromophores while short units can facilitate interactions. Such interactions can cause dampening or shifting of absorption of chromophore. Here the short bridging unit mercapto benzimidazole facilitates chromophore - gold core interaction. The blue shift of NO_2 group might be due to increased interaction of emanating electrostatic field of gold core with chromophore.[24]

The difference between free MNBI and MNBI-stabilized GNPs was studied through IR analysis (see the Supporting Information, Figure S1). Loss of the stretching frequency of MNBI (ν S−H) at 2548 cm^{-1} in MNBI-GNPs confirms thiol binding on gold surface. Also the shifting of $-NO_2$ stretching frequency from 1340 cm^{-1} to 1387 cm^{-1} confirms MNBI grafted on the gold surface. Although the above analysis confirms stabilization of GNPs by MNBI, the core size and size distribution of MNBI-GNPs was studied by transmission electron microscope (TEM) as shown in Figure 2. The MNBI-GNPs show relatively narrow size distribution with average core size of 7 ± 1 nm. TEM micrographs show that the particles are nearly monodisperse in nature.

The concentration of MNBI-GNPs was found to be 2 nM based on the average core diameter of 7 nm (see the Supporting Information). From the weight loss observed in thermogravimetric analysis (TGA) the number of ligands per GNP was found to be 2331 (see the Supporting Information, Figure.S2 and calculations).The MNBI-GNPs were found to be highly air stable and they remain dispersed in water without aggregation for one month. We have conducted NO release

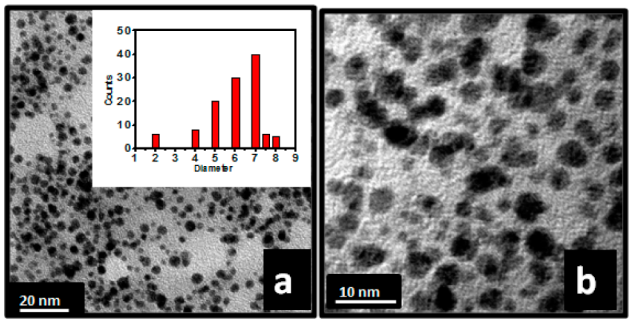


Figure 2. TEM images of aqueous Au colloidal suspension after dropcasting on carbon coated copper grids. (a) Scale bar = 20 nm, inset shows the histogram showing the size distribution of MNBI GNP; and (b) scale bar = 10 nm.

studies on bare MNBI and MNBI GNP using Griess assay (Supporting Information). On the basis of the studies, it can be concluded that only MNBI-stabilized GNP shows enhanced NO release and the NO release from the free ligand is insignificant. Studies of Sortino et al. showed that NO release from Pt nanoparticles can be increased by improving the intensity of absorption of nitro group and by shifting the absorption toward longer wavelength side.[25−29] But, in our case, we found that MNBI stabilized gold nanoparticles showed improved NO release, due to inter chromophore interaction of densely packed photoresponsive capping agent and chromophore-plasmon electron interaction. These interactions lead to intermolecular nitro-nitrite rearrangement because of non-planar torsional conformation of nitro group in relation to aromatic ring. This is confirmed by the photolysis profile of MNBI-GNPs upon visible light irradiation as shown in Figure 3. On visible-light irradiation, the initial dark brown color of

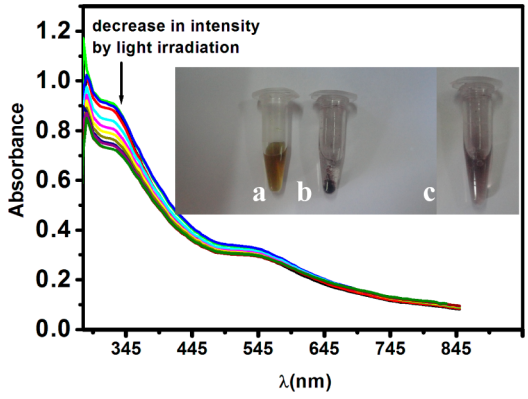


Figure 3. Photolysis profile of MNBI GNP in aqueous condition at neutral pH. Inset shows the color change observed for GNP before and after NO release. (a) GNP before NO release , (b) GNP after NO release, and (c) aggregated GNP after sonication shows pink color.

MNBI-GNPs changes to pink, confirming the nitro group degradation. The absorption spectra also showed decrease in intensity at 350 nm on continuous illumination of visible light indicating the possible degradation of NO_2 group (see the Supporting Information, Figures S3 and S4), which in turn demonstrates the suitability of MNBI-GNPs as an appropriate NO releasing material. NO release takes place through nitro-to-nitrite photo rearrangement followed by O-NO bond rupturing resulting in NO release and phenoxy radical formation (Scheme in the Supporting Information).[25−29]

dx.doi.org/10.1021/am402086m | ACS Appl. Mater. Interfaces 2013, 5, 8263−8266

To evaluate the potential biomedical application of these MNBI-GNPs, we carried out in vitro experiments with different cancer cell lines (cervical cancer cell lines, breast cancer cell lines, and lung cancer cell lines). The effectiveness of MNBI-GNPs to induce antitumor effect was studied both in dark and in the presence of light using MTT {(3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide)} assay against HeLa cells (figure. 4a). A tubular fluorescent lamp of 40W is used as light

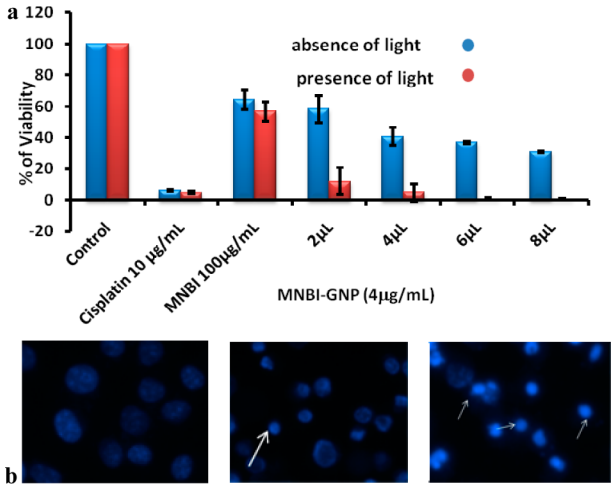


Figure 4. (a) Cytotoxicity effect of MNBI stabilized GNP of various concentration compared with Cisplatin (10 μg/mL), free MNBI (100 μg/mL) and control sample in the presence and in the absence of visible light. (b) Hoechst 33342 staining of HeLa nucleus, white arrows indicates the apoptotic cells.

source (see the assay protocol in the Supporting Information). For comparison, the effect of free ligand and concentration effect of GNP was evaluated. We noticed high mortality rate in the presence of light for MNBI-GNPs. Effect of GNP is more pronounced compared to free ligand and was found that by increasing the concentratation, cell mortality rate is also increased. The mortality rate of free ligand in the absence and presence of light was found to be approximately the same and indicates the need of GNP for enhanced NO release. The anti cancer activity was compared by doing a control experiment with the widely used drug Cisplatin. Figure 4a shows the cytotoxicity effects expressed as % of cell viability for cisplatin, free MNBI and MNBI-GNPs. From the figure, it is clear that antitumor effect of MNBI-GNP system is comparable with that of cisplatin. Here cytotoxic effect of 10 μL (10 μg/mL) Cisplatin and 4 μL (4 μg/mL) MNBI-GNP are found to be almost equal. This shows that GNPs with a 80% lower dosage is equally effective to 10 μg/mL Cisplatin. Also our method of preparation can introduce a large number of NO relesaing ligands (2331) across each gold core. Hence even small concentrations are sufficient to bring out anti cancer activity. Sortino et.al have prepared Pt nanopartcles by introducing NO releasing ligands by place exchange reaction, which can bring in only around six ligands across each gold core.[26] Cell apoptosis of HeLa cells were monitored using Hochest 33342 staining.[30] After treatment with MNBI GNP, HeLa cells show the morphological characteristics of apoptosis such as contracted nucleus and shrinking of cytoplasm. Figure. 4b shows Hoechest stained HeLa nucleus. When treated with MNBI-GNP more number of HeLa cells were found to be brighter compared to normal and MNBI treated case. This is due to condensed chromatin which would be stained brighter than that of normal cells. Apoptosis studies were conducted in the presence of light among HeLa (cervical cancer cell lines, see Figure S5 in the Supporting Information), Siha (cervical cancer cell lines, see Figure S6 in the Supporting Information), MCF-7 (breast cancer cell lines, see Figure S7 in the Supporting Information), and A549 (lung cancer cell lines, see Figure S8 in the Supporting Information) in a dose- and time-dependent manner. The inhibition proliferation was found to be more significant among cervical cancer cell lines, HeLa and Siha, than that in lung and breast cancer cells. MTT assay studies and Hochest staining shows good apoptotic rate among these cells within 24 h after treatment with MNBI-GNP. Thus the prepared nanohybrid was found to be an efficient and effective anticancer agent aganist cervical cancer cell line. For practical utilities, we propose to focus on shifting the absorption of GNPs to IR region and on the targeted delivery of NO to cancer cells.

CONCLUSIONS

In summary, we have reported the photoinduced release of NO from GNPS stabilized with MNBI, a pharmaceutically important molecule. Here the densely packed photoresponsive shell encapsulating gold core helps in the controlled NO delivery of the nanocomposite system under visible-light irradiation. This nanohybrid system exhibits enhanced anticancer activity due to the concomitant cytotoxic effect of NO.

ASSOCIATED CONTENT

Supporting Information

Preparation of MNBI-GNP, IR, UV, TGA data of MNBI-GNP, concentration and number of ligands calculations, anticancer studies and their corresponding histograms, confocal images. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The author S. Priya acknowledges CSIR, New Delhi for granting the financial support for the above work in the form of Junior Research Fellowship (JRF) and the funding received from CSIR network project (NWP0035 and M2D). The authors acknowledge Dr. K. Pandima devi and R.Sakthivel of Alagappa University for carrying out cytotoxicity study. Confocal images of anticancer studies were carried out in Rajiv Gandhi Centre for Biotechnology,Trivandrum, with the help of Dr. T.R. Santhosh Kumar and R. Prakash.

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