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THERAPEUTIC APPLICATION OF GOLD NANOPARTICLES OVER TUMOR BEARING MICE

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ABSTRACT

Nanotechnology is enabling technology that deals with nano-meter sized objects.Nanobiotechnology is a branch of nanotechnology that deals with the biological aspects of this science. Now a days nanobiotechnology has emerged as a new field for many therapeutic applications. Among them gold nanoparticles are of great interest. Gold nanoparticles can be used as the drug carrier for tumor directed drugs very well due to their size and many unique properties.

Keywords: Drug Delivery, Gold Nanoparticles, PG, TNF, Tumor

I. INTRODUCTION

Generally, gold nanoparticles are produced in a liquid by reduction of hydrogen tetra chloro aurate. After dissolving $H[AuCl_4]$, the solution is rapidly stirred while a reducing agent is added. Citrate initially acts as the reducing agent and finally as the capping agent.Constructive pathways for clinical treatment of cancer are confined. Surgical restriction, chemotherapy and irradiation are some common tactics used for cancer therapy but these approaches are non specific.Cancer patients undergoing radio and chemotherapy face drug resistance such as cisplatin, cancer related fatigue and several cardio vascular effects such as cardiomyopathy, ischemia, arrhythmias, hyper tension, thromboembolism, pericardial diseases or heart attack as they damage the cancer cells along with the destruction of healthy cells.Besides of some conventional approaches, chemotherapy remains the primary treatment for cancer but it is not much restorative because of various side effects caused by unspecified drug distribution in the body due to several chemotherapeutic agents such as cytotoxic drugs.Paclitaxel (PTX) shows cytotoxicity against different types of cancer so it is considered to be an essential chemotherapeutic drug with limited therapeutic effects due to toxicity caused by poor water solubility and selectivity. Because of these shortcomings, effective approaches should be considered. Chemotherapeutic agents cause various side effects in cancer patients such as nephrotoxicity, vomiting, myelosuppression, severe nausea, ototoxicity and neurotoxicity due to CDDP (cisplatin) administration whereas gastro intestinal disturbances, acute nausea, vomiting, stomatitis, alopecia baldness, neurologic disturbances, bone marrow, aplasia, cumulative cardio toxicity and bone marrow depressant effects due to doxorubicin administration. Due to fluoropyrimidines (5FU), methotrexate, irinotecan and cisplatin patient may suffer from adverse diarrhea and constipation. When treated with temsirolimus as a single drug anemia, hyperglycemia, stomatitis, hypophosphatemia, interstitial lung disease and pneumonia were reported in patients suffering from advanced

renal cell carcinoma.Eachyear, large numbers of deaths are caused by cancer because lack of selectivity, drug targeting ability, inefficient metastatic tumor therapy and drug resistant tumor cells.Therefore advanced chemotherapic treatments have been needed to kill cancerous cells.Today in order to avoid side effects fuscous of scientists are shifted towards natural products which still needs to prove their effectiveness.

II. TNF

The protein (TNF) is the prototypical member of a pro-apoptotic ligand family and have significance for cancer therapy. Studies show that after binding with cytokine receptor on the tumor TNF is able to induce necrosis of several types of tumors.TNF is also a known pro-inflammatory cytokine that regulates immunity.Potential side effects when introducing TNF are expected, that raises many concerns about its safety and restricting its applications in cancer therapies.Targeted drug delivery, use the nanotechnological platforms to reduce interaction with healthy tissue to improve therapeutic efficacy.

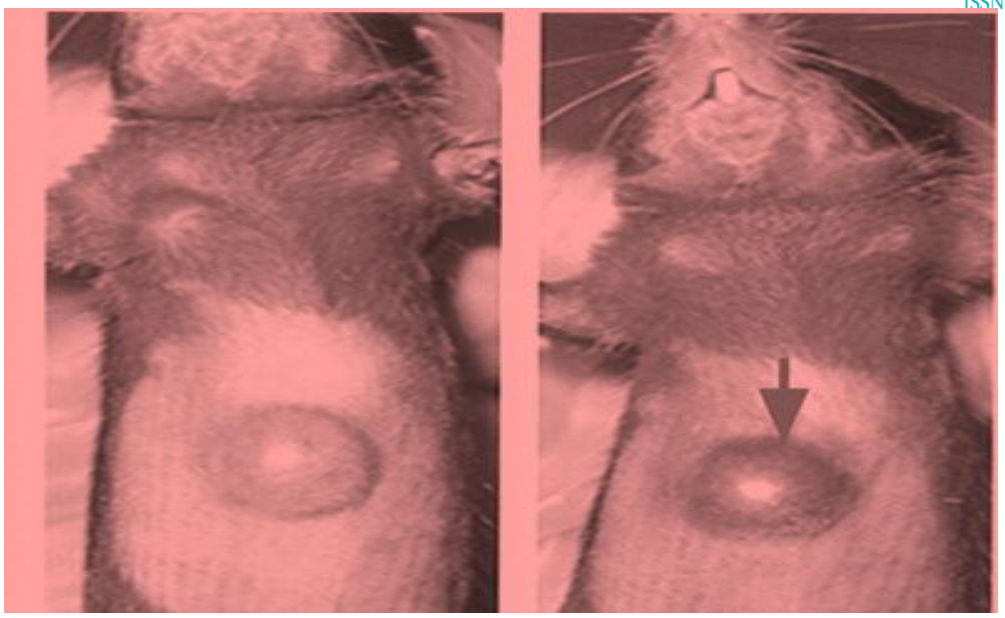
III. EXPERIMENTAL

3.1 Preparation of cAu-TNF-PG Vector

cAu-TNF-PG vector was designed by binding molecules of TNF and PG(Propylene glycol) on the same particle of colloidal gold. The binding of proteins to colloidal gold nanoparticles is dependent on the pH of the colloidal gold and the protein solutions. To know the optimal pH for binding of the particles, the pH of of 32 nm colloidal gold sol was adjusted, as measured on pH strips, from pH 6 to 10 using 1N NaOH. TNF was reconstituted in DI water to a concentration of 1.5 mg/ml in TRIS base at pH 9. 10 microlitre TNF stock was added to the 2 ml pH adjusted colloidal gold in 12 separate tubes and incubated for 30 min. Following this incubation, NaCl solution was added to the aliquots to induce particle precipitation. The optimum pH was defined as the pH that prevent their precipitation by salt. The optimal pH for binding TNF to colloidal gold was around 9.At lower pH values, the addition of the TNF caused the particles to concentrate as bunches, while adjusting the pH of the sol above 9 resulted in the generation of unstable vector preparations.The mixture of cAu-TNF was allowed to stir for 30 min followed by the addition of the 10 microlitre PG (20 μg/ml added in DIH2O) in each tube. For Binding studies 1 ml of the various samples was collected and centrifuged at 8,500 rpms for 10 min. Sample of the supernatant was added to EIA assay and analyzed for TNF concentration by EIA.

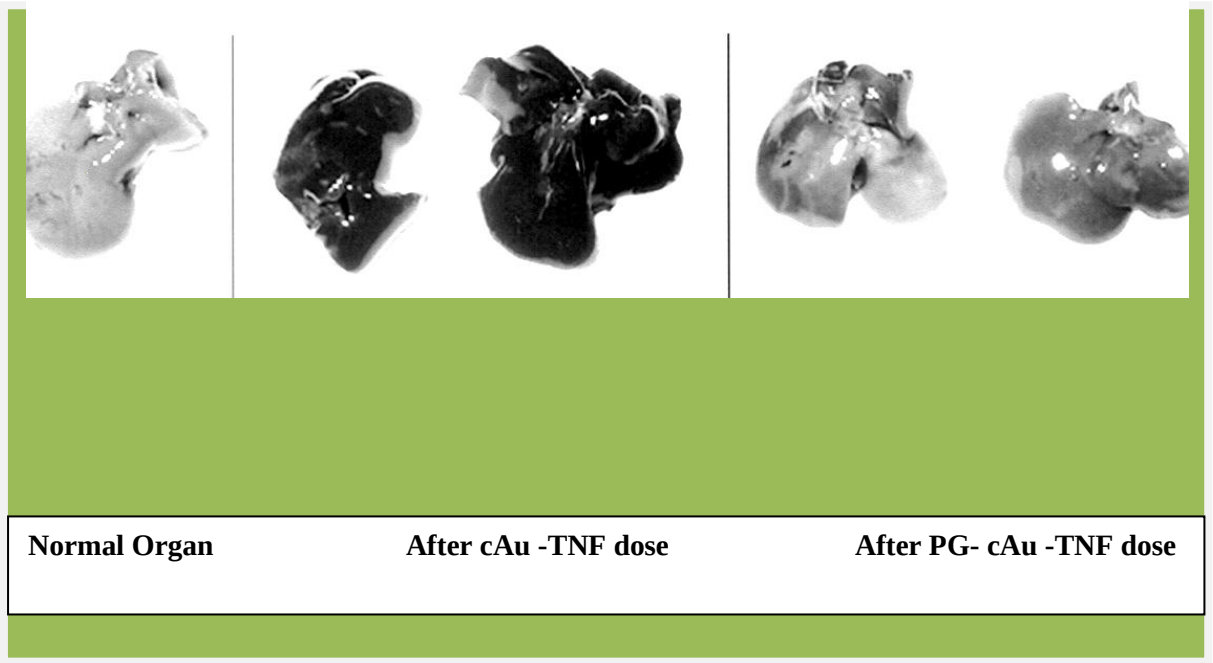
IV. TUMOR IMPLANTATION

C57 BL/6 mice implanted with the colon carcinoma tumor cells was used for tumor implantation. Here The K12/TR cell line derived from a transplantable colon carcinoma induced by dimethylhydrazine are used in the mice. K12/TR cells produced tumors following s.c. injection of 2 mu L,(100ng/mu L) into mice. The cells were allowed to grow and they formed a tumor measuring 0.6 cm3, as determined by measuring the tumor in three dimensions using a digital caliper.



V. INJECTION OF THE VECTORS

Native TNF, cAu-TNF, or PG-cAu-TNF formulations were generated as described above. Depending on the study, 5–24 μg of native TNF, cAu-TNF, or PG-cAu-TNF formulations were intravenously injected, through the tail vein, of tumor burdened mice. One group of mice treated with either the cAu-TNF or PG-cAu-TNF vector was sacrificed after the injection. The organs from these mice were collected and photographed to document the presence of the colloidal gold vector. Further studies have also been done by taking BSA (Bovine serum albumin) PG-cAu-BSA as control vector.



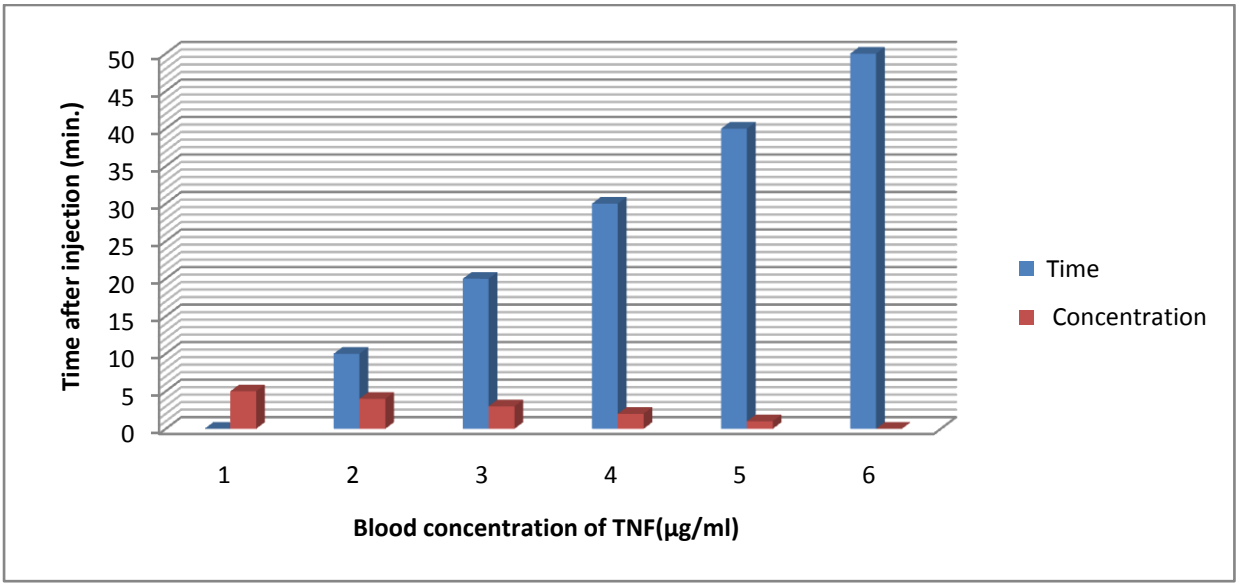
Color characteristics were used to track the location of both the cAu-TNF and the PG-cAu-TNF vectors after their injection into tumor-burdened mice. As shown in Figure the cAu-TNF vector is sequestered within the livers and spleens as evidenced by change in the color This uptake was rapid. Finally, these organs remained

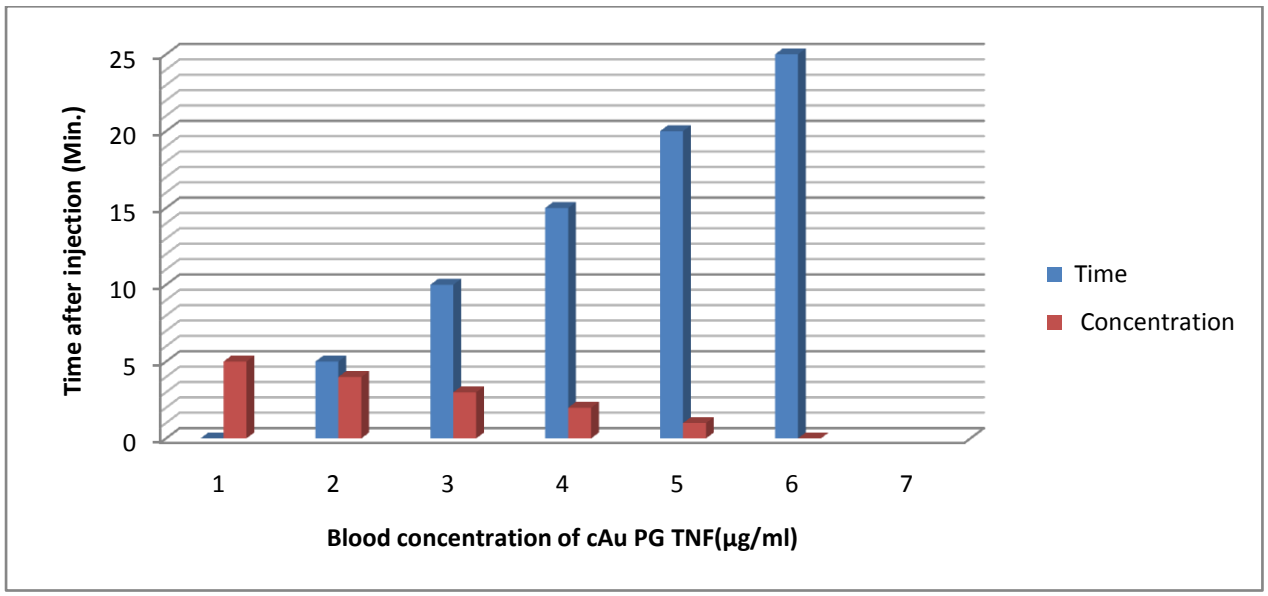
black for a month after treatment . The visual data obtained with the PG-cAu- TNF vector clearly show less gold uptake by the livers and spleens.Unlike the color of the gold that accumulated in the liver and spleen by cAu-TNF treatment, the color of the gold seen in the tumor following the administration of the PG-cAu-TNF was reddish-purple, suggesting that the gold particles remained in a colloidal state in the circulation and their accumulation in the tumor. Interestingly, the pattern of PG-cAu-TNF accumulation in and around the tumor site changed with time. The PG-cAu-TNF was initially sequestered solely in the tumor.

VI. COMPARISON OF THE ANTITUMOR EFFICACY OF NATIVE TNF, cAu-TNF, and PG-cAu-TNF: Biodistribution & Pharmacokinetic studies –

The toxicity of native TNF was dose-dependent. Around Five to six micrograms of native TNF caused piloerection within 1.5 hr of injection.While increasing the doses of TNF more toxicities were observed. At a dose of 10 μg of TNF per mouse 50% of the animals were cold and without response, and ultimately died within 24 hr. However, 24 μg of cAu-TNF did induce significant tumor reduction. This vector was also safer than native TNF since 24 μg of native TNF caused 100% of the animals to die.These safety and efficacy data are consistent.Yet the vectors' ability to deliver the remainder of the TNF in the tumor may account for its antitumor efficacy. The ability of the PG-cAu-TNF vector to actively sequester TNF within tumors supported its ability to improve safety and efficacy.

The pharmacokinetic profiles of native TNF and the cAu-TNF vector in tumor-burdened mice is as below in the figure. Tumor-burdened mice were intravenously injected with 10 μg of either native TNF or the PG-cAu-TNF vector.





VI. CONCLUSION

Gold nanoparticles mediated drug delivery systems have many advantages over other nanocarriers as well as to conventional drugs. Gold nanoparticles have been widely used as a cancer antigen and in tumor therapies.Some advantages are listed here; (i) Gol nanoparticles have unique properties due to their size and shap (ii) Gold nanoparticles have high surface area which provide dense drug loading; (iii) These particles are biocompatible and are readily available for conjugation with small biomolecules such as proteins, enzymes, carboxylic acid, DNA, and amino acids (iv) Gold nanoparticles have controlled dispersity(v) Due to small size and uniform dispersion they can easily reach to the targeted site with blood flow (vi) They are non-cytotoxic to the normal cells and (vii) Gold nanoparticles are easily synthesized by various methods.So this study shows that gold nanoparticls loaded with TNF and PG are very helpful in reducing the tumor burden in mice. The methodology developed in this study can be applied to investigate other types of antibody–antigen interactions, as well as the formulation of nanoparticle-based therapeutic platforms.

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