

Hormone Peptide Conjugated Ironoxide Nanoparticles For Detection and Treatment of Metastases

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BACKGROUND

Despite new discoveries of drugs the mortality rate among cancer patients failed to improve over the last decades. In 2005 over 600,000 new cases of breast cancer were reported in the US [1]; more than 41,000 deaths occurred due to metastatic disease [2]. Breast cancer is the most common cause of cancer death in women; more than 75 % of patients die from skeletal metastases [3]. The accurate diagnosis of metastatic disease is therefore crucial for treatment and survival. Current treatments are highly toxic, have poor specificity for cancer cells and fail to destroy slow growing tumors and metastases.

Consequently, new drugs and contrast agents need to be developed to monitor the efficacy of response to treatments.

It has become evident that most cancers and their metastases overexpress functional receptors for luteinizing hormone releasing hormone (LHRH) [4]; more than 60 % of human breast cancers overexpress functional receptors for luteinizing hormone releasing hormone (LHRH) [4, 5]. We have demonstrated the usefulness of nanoparticles for diagnosis of single cancer cells, by linking covalently the hormone ligand, LHRH, to superparamagnetic iron oxide nanoparticles (SPION) [6]. The treatment of cancers with hormone ligand conjugated membrane disrupting peptides has been very successful in xenograft models with minimal side effects [7, 8]. In nude mouse models, these xenografts and their metastases have been targeted through receptors for LHRH linked to the membrane disrupting peptide, Hecate [7, 9-11].

Based on these observations a new nanoparticle construct has been developed for monitoring and treatment of tumors and metastases: This nanoparticle consists of LHRH as targeting moiety and is also linked to a membrane disrupting peptide drug, Hecate.

We hypothesized that targeted delivery of LHRH-SPION-Hecate facilitates and increases the accumulation of SPIONs in metastatic cancer cells that have spread to peripheral tissues, lymph nodes, brain and bones, thus increasing the sensitivity of MR imaging and specifically destroying cancer cells at the same time. We have developed nanoparticles that target LHRH

receptors for specific delivery, higher efficiency and faster accumulation within tumor cells [6]. The nanoparticles are iron oxide based particles of less than 30 nm diameter, they are monodisperse, neutral of charge and have a high saturation magnetization [6, 11]. Surface modification allows binding of membrane disrupting peptides on these functionalized nanoparticles creating particles that enhance magnetic resonance imaging sensitivity in vivo and can at the same time destroy tumors and metastases.

In this study we tested whether LHRH-SPION-Hecate specifically target and accumulate in and destroy metastatic cells from breast cancer xenografts.

METHODS

SPIONs were fabricated as described in Kumar et al [6, 11] using wet chemical methods and then conjugated to LHRH and Hecate by carbodiimide reactions [11].

Female nude mice bearing human breast cancer xenografts (MDA-MB-435S.luc) [6] were injected into the lateral tail vein with 33 mg Fe/kg LHRH-SPION-Hecate or LHRH-SPION with and without co-injections of LHRH. Mice were treated with a single injection once a week for three weeks and sacrificed in the fourth week. At necropsy, organs and tumors were collected for iron and metastasis analysis. Metastatic cells were determined by luciferase assays from organ homogenates; iron contents were determined spectrophotometrically by a Prussian blue reaction from organ homogenates and from paraffin embedded and sectioned tissues.

RESULTS

1. LHRH-SPIONs were incorporated directly into the cancer cells of primary tumors and metastatic cells from peripheral tissues.
2. The amounts of iron accumulated through LHRH-SPIONs correlated with the number of metastatic cells.

3. Repeated injection of LHRH-SPION increased the iron content in tumors and metastases and was retained over a period of 4 weeks.
4. LHRH-SPIONs did not reduce tumor volume or tumor weight, nor cause destruction of metastatic cells.
5. Magnetic Resonance Imaging of LHRH-SPION injected mice resulted in increased resolution up to 300 micrometer.
6. LHRH-SPION-Hecate accumulated in tumor tissue and metastases.
7. LHRH-SPION-Hecate caused significant reduction in tumor volume and tumor weights
8. The number of metastases in LHRH-SPION-Hecate treated mice was significantly reduced compared to LHRH-SPION or saline treated mice (Fig 1).
9. Co-injection of LHRH prevented destruction of tumor cells and metastases in LHRH-SPION-Hecate treated mice (Fig 1).
10. SPION-Hecate failed to reduce or accumulate tumors and metastases.
11. Ironoxide nanoparticles were retained in diseased tissue.
12. Co-injection of LHRH inhibited the accumulation of LHRH-SPION and LHRH-SPION-Hecate in tumors and metastases, no iron was detected in these groups of mice.

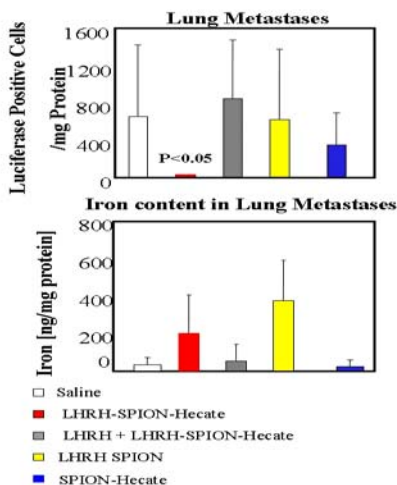


Figure 1: LHRH-SPION-Hecate destroys metastases in lungs in MDA-MB-435S.luc tumor bearing mice; co-injection with LHRH prevents reduction of lung metastases and accumulation of iron in lung tissue. LHRH-SPION fails to destroy metastases in lungs, yet accumulates in metastatic cells. SPION-Hecate fails to destroy or accumulate in lung metastases.

CONCLUSION

LHRH-SPION and LHRH-SPION-Hecate specifically target LHRH receptors on cancer cells and allowed accumulation through receptor mediated endocytosis.

The LHRH-SPION-Hecate can serve as a contrast agent and anticancer drug that specifically increase the sensitivity of detection of metastases and disseminated cells in lymph nodes, bones and peripheral organs in MRI and opens the possibility to monitor a treatment response in a non-invasive manner. This approach may have promising applications for treatment and imaging and facilitate monitoring of treatment response in patients.

Keywords: Metastases, nanoparticles, luteinizing hormone releasing hormone receptors, Magnetic Resonance Imaging, lytic peptides, treatment monitoring

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