कैंसर कीमो-रेडियोथेरेपी

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कैंसर कीमो-रेडियोथेरेपी की चुंबकीय अतिताप मध्यस्थता में वृद्धि हेतु लक्षित नैनोकण

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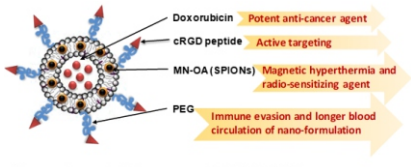

Tumor targeted liposomes (cRGD-LMD)
Indian patent No. 441803

मल्टी-मॉडल नैनो-फॉर्मूलेशन लक्षित ट्यूमर सी. आर. जी. डी.-एल. एम. डी. के डिजाइन के लिए योजना

सारांश

कीमो-रेडियोथेरेपी (CRT) गैर-ट्यूमर विशिष्टता की सीमा में होती है, जो इन विधियों की चिकित्सीय प्रभावकारिता को महत्वपूर्ण रूप से बाधित करती है और अक्सर सामान्य ऊतक की विषाक्तता और रोगी के जीवन की गुणवत्ता से संबद्ध होती है। अतिताप थेरेपी (HT) ट्यूमर के ऑक्सीकरण को बढ़ाकर, ट्यूमर ग्रस्त अंग पर औषधि की सांद्रता बढ़ाने और सेलुलर प्रोटीन के विकृतीकरण को प्रेरित करके DNA की प्रतिकृति, सुधार, कोशिका प्रसार और उत्तरजीविता जैसी महत्वपूर्ण सेलुलर प्रक्रियाओं को विकृत कर CRT की प्रभावकारिता में सुधार करने के लिए जानी जाती है। हालांकि, पारंपरिक HT की चिकित्सीय प्रभावकारिता ट्यूमर कोर के अकुशल तापन और ताप-सह्यता के विकास तक सीमित है। पारंपरिक HT के विपरीत, सुपर-पैरामैग्नेटिक आयरन ऑक्साइड नैनोपार्टिकल्स (SPIONs) मध्यस्थता वाली चुंबकीय अतिताप थेरेपी (MHT) ट्यूमर कोर के ४२-४३ डिग्री सेल्सियस के अतितापन के तापमान तक पर्याप्त और समान तापन को प्रेरित करती है और ताप-सह्यता के विकास को बाधित करती है। वर्तमान शोध में MHT के लिए एक तर्कसंगत रूप से अभिकल्पित ट्यूमर लक्षित चक्रीय RGD (cRGD) पेप्टाइड क्रियाशील लिपोसोमल नैनो-वाहक (cRGD-LMD) के विकास और म्यूरिन फाइब्रोसारकोमा कैंसर कोशिकाओं और इसके ट्यूमर मॉडल में CRT प्रभावकारिता में सुधार का वर्णन किया गया है। लिपोसोम की झिल्ली में SPIONs अनुकूल प्रतिस्थापन ताप के प्रति संवेदनशील कैंसर कोशिका झिल्ली तक इनकी आपूर्ति की सुविधा प्रदान करती है, जिससे MHT की बेहतर प्रभावकारिता सुनिश्चित होती है। इसके अलावा, cRGD लेबलिंग ν ३ इंटीग्रिन रिसेप्टर ओवर-एक्सप्रेसिंग अन्य ऑफ-टारगेट अंगों (जैसे, यकृत, तिल्ली, गुर्दे, हृदय और फेफड़े) की तुलना में माइस के ट्यूमर कोशिकाओं और इसके नव-संवहनी तंत्र में cRGD-LMD के ~ २-९ गुना अधिक संचय को सक्षम बनाता है। उल्लेखनीय रूप से, सीरम CK-MB स्तरों और कार्डियक फाइब्रोसिस के अध्ययन के अनुसार स्वास्थ्य के संदर्भ में हृदय-संवहनी cRGD-LMD को सुरक्षित पाया गया है। cRGD-LMD को भारतीय पेटेंट (सं. ४४१८०३) प्राप्त हुआ है और हमारे परिणाम कैंसर CRT में सुधार के लिए लक्षित नैनो-फॉर्मूलेशन के रूप में इसकी नैदानिक ट्रांसलेशन क्षमता को दर्शाता है।

Cancer Chemo-Radiotherapy

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Targeted Nanoparticles for Magnetic Hyperthermia Mediated Enhancement of Cancer Chemo-Radiotherapy

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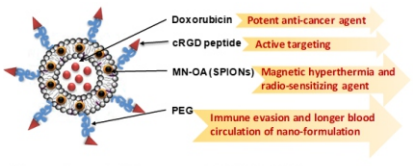

Tumor targeted liposomes (cRGD-LMD)
Indian patent No. 441803

Scheme for design of cRGD-LMD, a tumor targeted multi-modal nano-formulation

ABSTRACT

Chemo-radiotherapy (CRT) suffers the limitation of non-tumor specificity, which significantly hampers the therapeutic efficacy of these modalities and is often associated with normal tissue toxicities and patient's quality of life. Hyperthermia therapy (HT) has been known to improve the efficacy of CRT by increasing tumor oxygenation, increasing drug concentration at tumor site and inhibiting crucial cellular processes such as DNA replication, repair, cell proliferation and survival via inducing denaturation of cellular proteins. However, therapeutic efficacy of conventional HT is limited by the in-efficient heating of tumor core and development of thermo-tolerance. Contrary to conventional HT, super-paramagnetic iron oxide nanoparticles (SPIONs) mediated magnetic hyperthermia therapy (MHT) can induce sufficient and uniform heating of tumor core to hyperthermic temperatures of 42-43°C and prevent development of thermo-tolerance. Present work describes the development of a rationally designed tumor targeted cyclic RGD (cRGD) peptide functionalized liposomal nano-carrier (cRGD-LMD) for MHT and improvement of CRT efficacy in murine fibrosarcoma cancer cells and its tumor model. The strategic placement of SPIONs in the membrane of the liposomes facilitates their delivery to the heat sensitive cancer cell membrane, ensuring improved MHT efficacy. Furthermore, cRGD labelling enables ~ 2-9 fold higher accumulation of cRGD-LMD in αvβ3 integrin receptor over-expressing mice tumor cells and its neo-vasculature compared to other off-target organs (viz., liver, spleen, kidney, heart and lungs). Notably, cRGD-LMD was found to be safe in terms of cardio-vascular health as studied by serum CK-MB levels and cardiac fibrosis. cRGD-LMD has received an Indian patent (No. 441803) and our results demonstrate its clinical translational potential as a targeted nano-formulation for improvement of cancer CRT.

KEYWORDS: *Magnetic hyperthermia therapy, Magneto-liposomes, Radio-sensitization, Cardio-toxicity; Heat Shock Proteins*

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Introduction

Approximately 60 % of solid tumors are treated with Chemo-Radio Therapy (CRT) [1-2]. However, the efficacy of CRT is severely hampered by its non tumor-specificity leading to dose-limiting toxicities and subsequent development of resistance to CRT [2]. Thus, development of alternate and more efficient strategies for cancer treatment has become indispensable.

Nanoparticles mediated Magnetic Hyperthermia Therapy (MHT): Heating Tumors to Death

Hyperthermia therapy (HT) involves heating the tumors in the range of 40 - 43 °C which can either kill the cells directly or sensitize them for subsequent CRT. In clinics, conventional HT is applied using infrared, microwaves, high intensity focused ultrasound, sauna bath or water bath [3]. However, conventional HT suffers the limitations of in-sufficient and non-homogenous heating of tumors often leading to development of thermo-tolerance during subsequent HT sessions [3-4]. Thus, development of alternate and more efficient hyperthermia modalities with ability to induce nano-heating effects at intra-cellular level becomes crucial. In this direction, photothermal therapy (PTT) and magnetic hyperthermia therapy (MHT) are two such modalities with superior hyperthermia efficacies and better therapeutic abilities as compared to conventional HT [5-6]. In the present work we have developed super-paramagnetic iron oxide nanoparticles (SPIONs) for MHT applications. As against conventional HT, SPIONs mediated MHT can induce more efficient and homogenous heating of tumor cells and significantly improve the therapeutic index of CRT. MHT utilizes SPIONs to generate heat under the influence of an alternating current (*AC*) magnetic field (AMH) predominantly by Néel or Brownian relaxation [6]. Unlike conventional HT, in MHT, SPIONs can be functionalized using tumor targeting surface ligands to specifically internalize in the tumor cells and distribute to cellular compartments such as plasma membrane which is known to be a more sensitive target of heat as compared to cytosolic compartment. Thus, in present work we have designed cell membrane targeting SPIONs functionalized with hydrophobic, oleic acid termed as 'MN-OA' and have demonstrated its membrane localization and significant anti-cancer efficacy in combination with MHT in cancer cell cells and its tumor model (Fig. 1A-E) [7-8].

cRGD-LMD : A Targeted and Multi-modal Anti-tumor Nano-formulation

For further improving the tumor cell targeting of MN-OA and to impart multi-modal cancer therapy ability, we have designed a liposomal nano-formulation termed as 'cRGD-LMD'. These liposomes are co-encapsulated with MN-OA and doxorubicin (Dox) in the bilayer and core of the liposomes, respectively and to impart tumor-specificity, they have been functionalized with cyclic RGD (cRGD) peptide. cRGD functionalization enables the targeting of tumor cells and its neo-vasculature over-expressing the αvβ3 integrin receptors [9]. cRGD-LMD has spherical shape and is predominantly unilamellar with MN-OA and Dox encapsulation visible mainly in the bilayer and core of liposomes, respectively as suggested by cryo-TEM (transmission electron microscopy) (Fig. 2A-B). *In vitro* evaluation showed significantly higher cyto-toxicity of cRGD-LMD as compared to commercial nano-formulation of DOX (Lippod™), in cancer cells of skin, breast, lung and brain

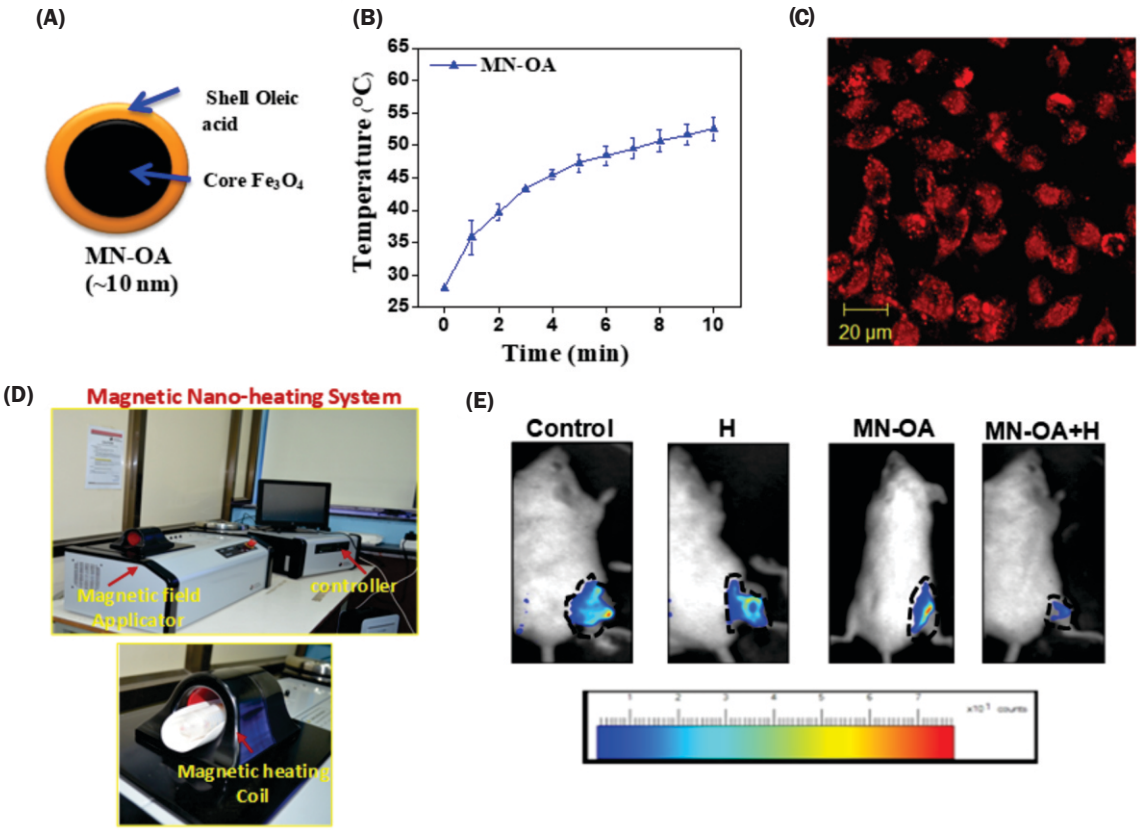

Fig.1: (A) Scheme for SPIONs coated with oleic acid termed as 'MN-OA', (B) graph for temperature achieved by MN-OA (10 mg/mL) under alternating current magnetic field conditions (335Oe, 265 kHz for 10 min) (C) representative confocal microscopy image of WEHI-164 cells treated with MN-OA and stained with nile Blue A. Red foci depict membrane localization of MN-OA, (D) digital photographs of magnetic nano-heating system. Lower image shows the placement of restrained mice inside the copper coils of the instrument during magnetic hyperthermia therapy, (E) representative real time in vivo bio-luminescence imaging of tumor cell growth in fibrosarcoma tumor bearing mice after indicated treatments. (H: magnetic hyperthermia therapy). Reproduced with permission from Elsevier, source: Colloids and Surfaces B: Biointerfaces 108 (2013) 158–168.

origin. Importantly, the cyto-toxicity of cRGD-LMD was significantly lower in normal lung epithelial cells as compared to cancer cells [9]. Moreover, cRGD-LMD showed significant radio-sensitization of murine fibrosarcoma cells predominantly via activation of JNK mediated pro-apoptotic pathway. In addition, cRGD-LMD also showed significant heating of tumors after MHT as determined by thermal imaging using IR camera. (Fig. 2C-E).

Tumor Targeting and Combinatorial Therapy Efficacy of cRGD-LMD: *In vivo* Studies

In vitro studies suggested the superior anti-cancer efficacy of cRGD-LMD in multiple cancer cells. Further, to determine their *in vivo* tumor targeting ability, cRGD-LMD was labelled with a near infrared dye, indocyanine green (ICG). The ICG labelled cRGD-LMD showed ~2-9 folds higher accumulation in fibrosarcoma tumors as compared to other off-target organs (liver, spleen, kidney, heart and lungs) [9]. Anti-tumor efficacy of cRGD-LMD was evaluated in fibrosarcoma tumor model either alone or in combination with radiation (R) or MHT (H) or both. Lippod™ was used as a comparative clinical formulation control. A TGD of ~ 7 days was obtained for cRGD-LMD + H + R as compared to ~ 5 days for cRGD-LMD + R, 4 days for cRGD-LMD + H, 3 days for cRGD-LMD, 0.2 days for Lippod™ and ~ 2 days for Lippod™ + R treatments (Fig. 3A-C).

Toxicological Evaluation of cRGD-LMD in Healthy Animals

To study the toxicological parameters, healthy BALB/c mice were treated with cRGD-LMD or Lippod™ or Dox at 3 mg/Kg dose for 4 days, followed by analysis of whole blood and serum parameters and histopathology of major organs. Dox at 20 mg/Kg of was used as positive control. Serum analysis showed increased expression of early cardiac damage marker, CK-MB in Lippod™ and Dox (20 mg/kg) group but not in cRGD-LMD as compared to control [9]. Furthermore, cRGD-LMD showed in-significant changes in DOX-induced cardiac fibrosis as suggested by trichrome staining and immuno-fluorescence detection of phospho-Smad3, which is one of the mediators of TGFβ induced fibrosis in heart tissue (Fig. 3D-E). These results suggest in-significant toxicity of cRGD-LMD in healthy mice re-emphasizing its plausible clinical potential as a targeted and multi-modal anti-tumor agent.

Conclusion

Present study demonstrates the superior anti-cancer efficacy of cRGD-LMD as compared to Lippod™. The optimized phospholipid composition, size and charge of the liposomes and cRGD functionalization resulted in a significantly (6-18 folds) higher targeting of cRGD-LMD to tumor site as compared to other off-target organs, such as liver, spleen, kidney, lungs, heart and intestine. Thus, these pre-clinical findings support the targeted and combinatorial anti-tumor capabilities of cRGD-LMD involving ferroptosis mediated immunogenic mode of tumor control (10). Currently, toxicological studies in higher rodents and pharmaco-kinetic studies are being carried out to further facilitate the clinical translation of cRGD-LMD.

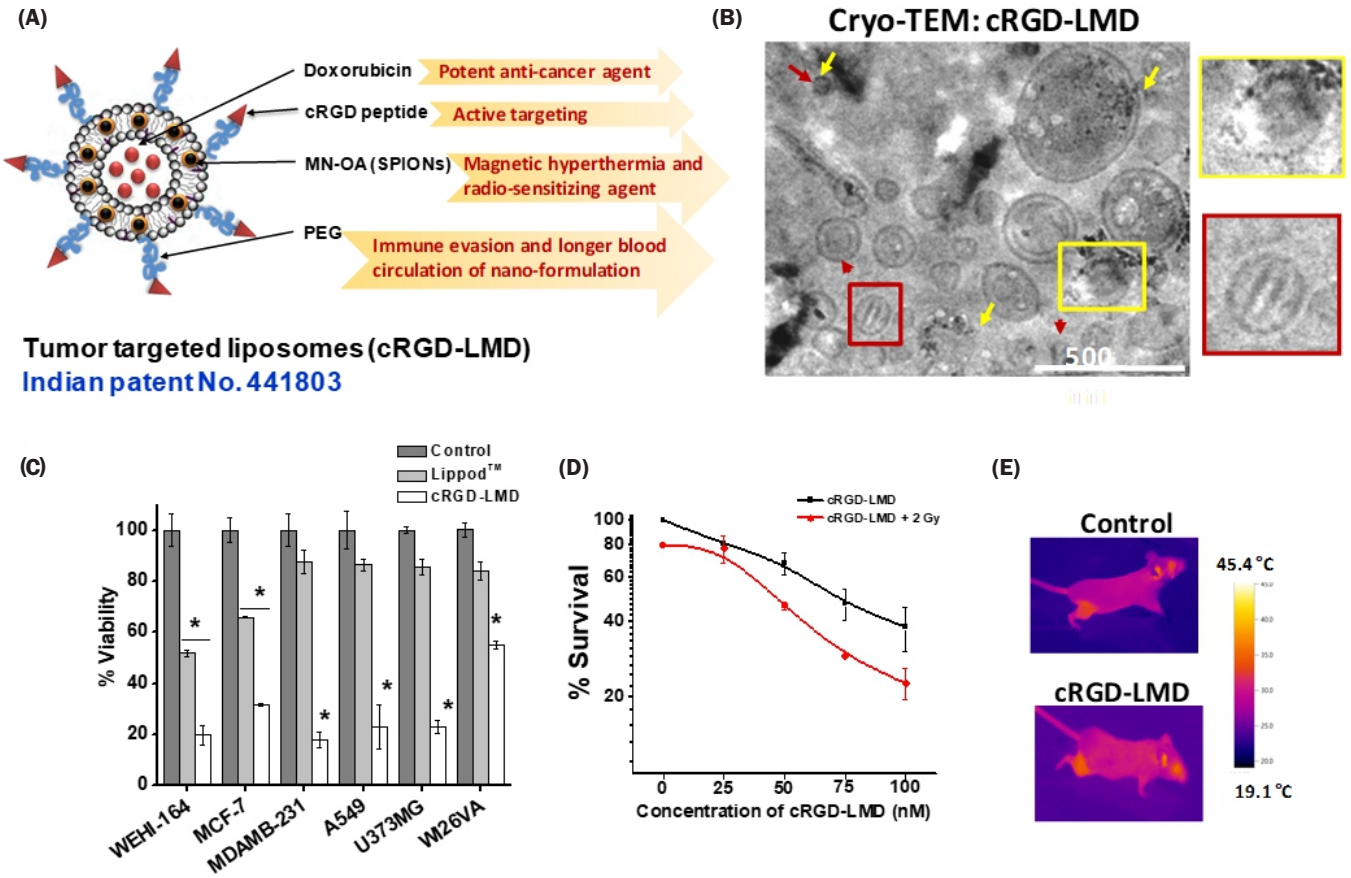

Fig.2: (A) Scheme for design of cRGD-LMD, a tumor targeted multi-modal nano-formulation, (B) representative cryo-TEM image of cRGD-LMD. yellow and red arrows depict MN-OA and doxorubicin (DOX) deposition in predominantly in the bilayer and core of liposomes, respectively. Zoomed inset shows the deposition of MN-OA (yellow box) and DOX (red box). (C) Graph for percentage viability of cancer cells of skin (WEHI-164), breast (MCF-7 and MDAMB-231), lung (A549) and brain (U373MG) and normal lung epithelial cells (WI26VA4) as determined by MTT assay after indicated treatments at 48 h, (D) graph for % survival as determined by clonogenic cell survival assay after indicated treatments. Gamma radiation dose of 2 Gy was used. (E) Representative thermal image captured using IR camera for mice injected with cRGD-LMD intra-venously and subjected to MHT. Reproduced with permission from Elsevier, source: Biomaterials Advances, 142 (2022) 213147.

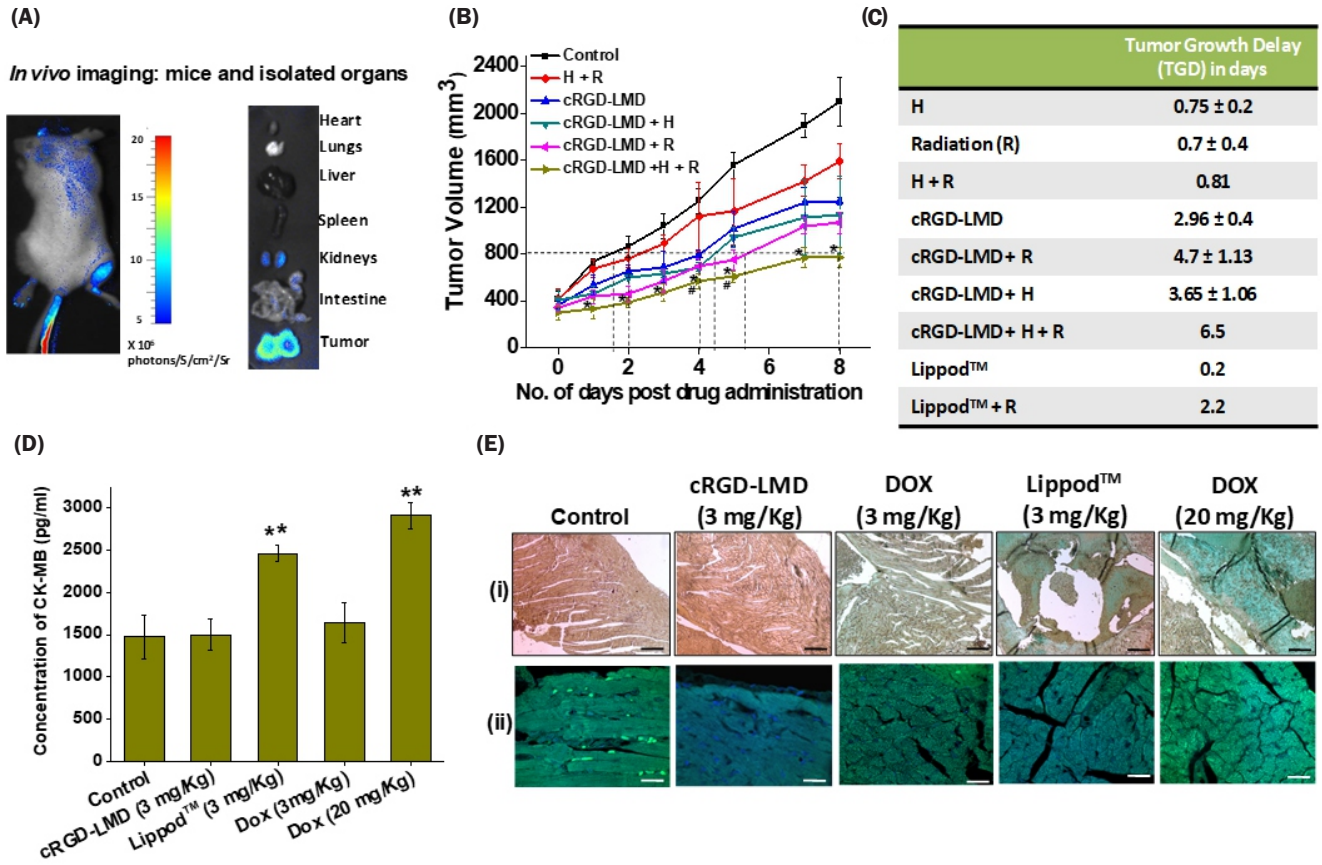

Fig.3: (A) representative real time in vivo fluorescence image of fibrosarcoma tumor bearing in mice on day 7 post intra-venous injection with cRGD-LMD labelled with near infrared dye (indocyanine green:ICG). Right side of the image shows the distribution of ICG fluorescence signal in isolated organs on day 8 after sacrifice of mice,(B) graph for tumor growth kinetics of fibrosarcoma tumors after indicated treatments, (C) table for tumor growth delay obtained after indicated treatments in fibrosarcoma tumor bearing mice, H: MHT and R: Gamma radiation (2 Gy X 3) (D)serum levels of CK-MB, an early cardiac damage marker after indicated treatments in fibrosarcoma tumor bearing mice and (E) representative image of tumor tissue sections after (i) trichrome staining and (ii) immuno-fluorescence staining of p-smad3 expression after indicated treatments. blue color in panel (i) indicates collagen deposition suggesting cardiac fibrosis and green fluorescence in panel (ii) suggests increased expression of p-smad3 suggesting activation of TGF-β mediated cardiac fibrosis pathway. Scale bar : 20 µm. Dox (20 mg/Kg) was used as a positive control. Reproduced with permission from Elsevier, source: Biomaterials Advances 142 (2022) 213147.

Acknowledgment

The authors gratefully acknowledge support and guidance of Head, Radiation Biology & Health Sciences Division and Associate Director, Bioscience Group.

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