Bone 81 (2015) 513–523

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Bone metastasis treatment using magnetic resonance-guided high intensity focused ultrasound

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ARTICLE INFO

Article history:
Received 23 June 2015
Revised 16 August 2015
Accepted 26 August 2015
Available online 29 August 2015

Keywords:
Focused ultrasound
Bone metastases
Palliative treatment
HIFU

ABSTRACT

Objectives: Bone pain resulting from cancer metastases reduces a patient's quality of life. Magnetic Resonance-guided High Intensity Focused Ultrasound (MR-HIFU) is a promising alternative palliative thermal treatment technique for bone metastases that has been tested in a few clinical studies. Here, we describe a comprehensive pre-clinical study to investigate the effects, and efficacy of MR-HIFU ablation for the palliative treatment of osteoblastic bone metastases in rats.

Materials and methods: Prostate cancer cells (MATLyLu) were injected intra-osseously in Copenhagen rats. Upon detection of pain, as determined with a dynamic weight bearing (DWB) system, a MR-HIFU system was used to thermally ablate the bone region with tumor. Treatment effect and efficacy were assessed using magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT) with technetium-99m medronate (^{99m}Tc-MDP), micro-computed tomography (μCT) and histology.

Results: DWB analysis demonstrated that MR-HIFU-treated animals retained 58.6 ± 20.4% of limb usage as compared to 2.6 ± 6.3% in untreated animals ($P = 0.003$). MR-HIFU delayed tumor specific growth rates (SGR) from 29 ± 6 to 13 ± 5%/day ($P < 0.001$). Untreated animals (316.5 ± 78.9 mm^3) had a greater accumulation of ^{99m}Tc-MDP than HIFU-treated animals (127.0 ± 42.7 mm^3, $P = 0.004$). The total bone volume increase for untreated and HIFU-treated animals was 15.6 ± 9.6% and 3.0 ± 4.1% ($P = 0.004$), respectively. Histological analysis showed ablation of nerve fibers, tumor, inflammatory and bone cells.

Conclusions: Our study provides a detailed characterization of the effects of MR-HIFU treatment on bone metastases, and provides fundamental data, which may motivate and advance its use in the clinical treatment of painful bone metastases with MR-HIFU.

1. Introduction

Cancer-induced bone pain is a devastating symptom affecting 70% of advanced cancer patients [1]. Though there is an increasing body of evidence that elucidates the pathophysiology of bone cancer pain, pain management remains a challenge. A repertoire of treatment strategies is available and yet, pain relief efficacy is suboptimal. Radiation therapy, the standard palliative treatment for bone metastases, is ineffective in 20–30% of the patients and 23–25% of the initially effective patients experience pain recurrence [2]. While re-irradiation could be given, patients who have exceeded the allowed cumulative radiation doses do not qualify for re-treatment. Moreover, previously published meta-analysis showed that 40% patients who underwent re-irradiation did not benefit from it [3]. Besides that, radiation therapy comes with adverse side effects, such as mucositis, fatigue and nausea [4]. Other treatment options, such as opioids, non-steroidal anti-inflammatory drugs, systemic radioisotopes and bisphosphonates, suffer from complications and side effects [4]. Therefore, new treatment strategies should be implemented to manage the pain and improve patients' quality of life.

Magnetic Resonance-guided High Intensity Focused Ultrasound (MR-HIFU) has been proposed as an alternative thermal treatment strategy for the palliative treatment for bone metastases. MR-HIFU is a non-invasive technique that focuses acoustic energy on metastatic lesions to raise the temperatures of the intact cortical bones locally to cause irreversible periosteum damage [5]. As the periosteum is richly innervated with nerve fibers and perturbation of this tissue layer has been shown to result in pain, ablation of the periosteum should logically alleviate pain [6]. Unlike other thermal ablation techniques, such as radiofrequency ablation and cryoablation, HIFU ablation is usually

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http://dx.doi.org/10.1016/j.bone.2015.08.025
8756-3282/

performed under MR guidance, which enables three-dimensional visualization of the lesions for accurate treatment planning, real-time monitoring of the temperature increase in the adjacent soft tissue, and of the corresponding thermal damage, thus allowing immediate alteration and optimization of treatment delivery, and post treatment assessments for follow-up therapies. Clinical data have shown that a significant number of radiation refractory patients receiving MR-HIFU ablation reported pain relief after treatments for up to 6 months [7–11]. Catane et al. were the first to demonstrate that MR-HIFU ablation provided pain relief 3 months after treatments [7]. In the latest phase 3 randomized trial where 112 patients were treated, Hurwitz et al. showed that pain relief was evident at 3 days after the treatments, and that the response rate in MR-HIFU treated patients was 64.3% compared to 20% in the placebo group [10]. Of the responding patients in the MR-HIFU treated group, 27% of the patients reported discontinuation of pain medication, whereas an additional 17% of them required less medication compared to baseline. MR-HIFU has also been used as first line treatment in 18 patients with bone metastases [12]. In this study, 72.7% patients experienced complete pain relief in the absence of pain medication, and 16.7% patients obtained partial pain relief with no increase in analgesia intake. Also, based on the MD Anderson criteria, local tumor control was observed in 33.3% patients. Interestingly, skeletal remodeling, in the form of de novo mineralization, cortical thickening and morphology rearrangement, was observed to restore the integrity of the bone [12]. Based on above studies, MR-HIFU is promising pain palliation technique, which provides not only immediate pain relief, but also long lasting effects after treatment. Hence, to ensure and improve treatment efficacy for widespread application of this technique in treatment of bone metastases, an in-depth understanding of the effects MR-HIFU ablation on bone is warranted.

Pre-clinical studies have been conducted in rabbits [13] and swine [14–17] to address the safety and effects of this technique on biomechanical properties of bones. However, in these preclinical studies, healthy bones were used and thus effects of MR-HIFU ablation on bone metastases and the pain relief mechanisms were not assessed. Furthermore, denervation of the periosteum has been suggested as the mechanism for pain palliation in clinical studies [7–10,12], but preclinical evidence and data are yet missing to substantiate this claim. The aim of this research was therefore to perform a comprehensive investigation of the effects and efficacy of MR-HIFU ablation for palliative treatment of osteoblastic bone metastasis in rats. For this purpose, multi-modality imaging techniques, detailed histological and behavioral analyses were employed (Fig. 1).

2. Materials and methods

2.1. Experimental groups

A total of 5 experimental groups were included in this study: (i) Tumor bearing animals euthanized 3 weeks after tumor inoculation, for treatment efficacy comparison ("tumor/no HIFU"); (ii) HIFU ablated tumor bearing animals ("tumor/HIFU"); (iii) HIFU ablated healthy animals ("no tumor/HIFU"), (iv) Sham-operated animals injected with Hank's Buffered Salt Solution (HBSS, "sham"), and (v) tumor bearing animals euthanized 2 weeks after tumor inoculation, corresponding to the MR-HIFU treatment time point. This group was specifically included for histological analysis of bone morphology on the day of treatment. For each experimental group, n = 6.

2.2. Cell culture

MATLyLu prostate cancer cells were purchased from the European Collection of Cell Cultures. Cells were cultured in monolayer in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 IU Penicillin, 100 μg/mL streptomycin and 250 nM dexamethasone and maintained at a maximum of 70–80% confluency. Prior to intra-osseous injection, cells were trypsinized with 0.25% trypsin and pelleted by centrifugation for 5 min at 1000 rpm. The pellet was washed twice with HBSS and re-suspended in HBSS at 1×10^6 cells/mL.

2.3. Animal model

All animal experiments were approved by the local animal welfare committee (Maastricht University, The Netherlands) and conformed to the ethical guidelines set by the institutional animal care committee. Male Copenhagen rats with a minimum age of 12 weeks were used (Jackson Laboratory, USA). The surgical procedures for intra-osseous injection of tumor cells were modified and optimized from a pre-existing osteoblastic rat model [18]. Prior to tumor induction, buprenorphine was administered subcutaneously at 0.05 mg per kg body weight to relieve pain resulting from surgery. Animals were anesthetized by inhalation of 0.6 L/min filtered compressed air with 3% isoflurane and maintained at 1–2% thereafter. Animals were laid in a supine position on a heated plate to maintain their body temperature at approximately 37 °C using a rectal temperature probe feedback loop. The left limb was shaved and disinfected. Under a dissecting microscope, a 1 cm incision was made in the skin to expose the *musculus biceps femoris* and *musculus quadriceps femoris*. A further incision was made over the intermuscular septus and the muscle strands were separated using a pair of spreading scissors to expose the femoral shaft. A cavity was made on the femoral shaft using a 0.99 mm (diameter) ball mill, carbide steel connected to a surgical drill (Microtorque II, Harvard Apparatus, USA). A bent 30-G needle was inserted at a 45° angle to reach the intramedullary cavity of the femur and a 1 mL syringe containing cell suspension was connected to the needle. A 50 μL cell suspension (50,000 cells) was injected through a drilled cavity. The cavity was sealed with synthetic bone graft paste (Osiq®, Kyeron, Enschede, The Netherlands). The surgical site was flushed with 5 mL sterile saline. A hole was made using a 30-G needle in the bone paste. This step was necessary to allow outgrowth of tumor cells from the cavity and interaction of tumor cells with the periosteum for development of pain. The fascia was closed with a surgical knot using an absorbable 4-0 suture (Polysorb™, Covidien, Dublin, Ireland). The skin was sealed using a continuous absorbable 4-0 suture. Finally, the wound was washed with sterile saline. For the animals in the sham-operated group, 50 μL HBSS was injected instead of tumor cells.

2.4. MR-HIFU ablation

MR-HIFU ablations were performed when the ipsilateral paw weight, determined as described in Section 2.5, showed a decrease of >5% as compared to pre tumor injection, corresponding to tumor volumes of 0.39 ± 0.22 cm^3. This occurred in the second week after tumor inoculation. Following anesthesia induction, animals were given carprofen (Rimadyl®, Pfizer Inc., New York, USA) once, at 4 mg/kg body weight, to relieve pain caused by HIFU-induced damage to healthy tissue. The limb to be treated was shaved and covered with degassed ultrasound gel (Aquasonic 100, Parker Laboratories, Fairfield, USA). The limb was then submerged in degassed water and positioned in a multichannel small animal MR receiver coil to enable usage with a clinical 3 T MR-HIFU platform [19] (Philips Sonalleve®, Vantaa, Finland). T_2-weighted MR planning images were acquired using a turbo spin echo (TSE) sequence (repetition time (TR) = 20,752 ms, echo time (TE) = 43 ms, field of view (FOV) = $100 \times 70 \times 71$ mm^3, voxel size = $0.5 \times 0.5 \times 1.0$ mm^3, number of signal averages (NSA) = 2). 4 treatment cells ($2 \times 2 \times 7$ mm^3) were positioned behind the bone and along the femoral shaft, avoiding the distal and proximal joints (Fig. 2a). Several sub-therapeutic sonications (acoustic frequency = 1.44 MHz, acoustic power = 5 W, duration = 20 s per sonication, continuous wave ultrasound) were performed to ensure temperature increase in the planned treatment sites. HIFU ablation was performed using 10–15 W acoustic power. During treatment, MR-thermometry sequences (RF-spoiled gradient with echo planar imaging