Radiotherapy and Oncology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Original article

Effects of local irradiation combined with sunitinib on early remodeling, mitochondria, and oxidative stress in the rat heart

Vijayalakshmi Sridharan [a], Chanice J. Thomas [b], Maohua Cao [a], Stepan B. Melnyk [c], Oleksandra Pavliv [c], Jacob Joseph [d,e], Sharda P. Singh [f], Sunil Sharma [g], Eduardo G. Moros [h], Marjan Boerma [a,*]

[a] University of Arkansas for Medical Sciences, Department of Pharmaceutical Sciences, Division of Radiation Health, Little Rock; [b] Oakwood University, Huntsville; [c] University of Arkansas for Medical Sciences, Department of Pediatrics, Little Rock; [d] Veterans Affairs Boston Healthcare System, Department of Medicine; [e] Brigham and Women's Hospital, Department of Medicine, Harvard Medical School, Boston; [f] University of Arkansas for Medical Sciences, Department of Pharmacology and Toxicology; [g] University of Arkansas for Medical Sciences, Department of Radiation Oncology, Little Rock; and [h] Moffitt Cancer Center and Research Institute, Department of Radiation Oncology, Tampa, United States

ARTICLE INFO

Article history:
Received 13 January 2016
Received in revised form 23 March 2016
Accepted 28 March 2016
Available online xxxx

Keywords:
Heart
Ionizing radiation
Sunitinib
Oxidative stress
Mitochondria

ABSTRACT

Background and purpose: Thoracic (chemo)radiation therapy is increasingly administered with tyrosine kinase inhibitors (TKI). While TKI have adverse effects on the heart, it is unknown whether combination with other cancer therapies causes enhanced toxicity. We used an animal model to investigate whether radiation and sunitinib interact in their effects on the heart.

Material and methods: Male Sprague–Dawley rats received local heart irradiation (9 Gy per day, 5 days). Oral sunitinib (8 or 15 mg/kg bodyweight per day) started on day 1 of irradiation and continued for 2 weeks. Cardiac function was examined with echocardiography. Cardiac remodeling, cell death, left ventricular (LV) oxidative stress markers, mitochondrial morphology and mitochondrial permeability transition pore (mPTP) opening were assessed.

Results: Cardiac diameter, stroke volume, and LV volume, mass and anterior wall thickness increased in time, but only in the vehicle group. Sunitinib reduced LV inner diameter and volume in systole, which were counteracted by radiation. Sunitinib and radiation showed enhanced effects on mitochondrial morphology and mPTP opening, but not on cardiac troponin I, mast cell numbers or markers of oxidative stress.

Conclusions: This study found no early enhanced effects of radiation and sunitinib on cardiac function or structure. Long-term effects remain to be determined.

 Radiotherapy and Oncology xxx (2016) xxx–xxx

Long-term survivors of thoracic cancers whose heart was exposed during radiation therapy, may present with cardiac side effects such as conduction abnormalities, accelerated atherosclerosis, myocardial and pericardial fibrosis and injury to cardiac valves [1–3]. Radiation therapy has undergone many improvements in treatment planning and radiation delivery. Nonetheless, a significant subset of patients with thoracic cancers, including those of the lung, esophagus, and proximal stomach, still receive considerable doses of radiation to the heart [4–6].

Tyrosine kinase inhibitors (TKI) have emerged as a new class of targeted cancer treatments. By inhibiting tyrosine kinases, these agents target pathways involved in tumor angiogenesis, proliferation, and metastasis [7]. Several TKIs have been approved or are in clinical development for the treatment of thoracic cancers (e.g., breast, esophagus, and lung) [8–11] and metastatic cancers [12–15]. TKIs are commonly administered concurrently with (chemo)radiation therapy, and continuous dosing of the TKI follows for several months to years afterward. Unfortunately, TKI have shown cardiac side effects in about 1–30% of patients [16–18]. Since radiation and several common chemotherapeutic agents have their own adverse effects on the heart, there is a concern about potential additive or synergistic cardiac toxicity of these treatments [19,20]. Here, we start to investigate potential interactions by examining the early effects of TKI in combination with radiation in a preclinical animal model.

Sunitinib malate is an orally active inhibitor of multiple receptor tyrosine kinases [21]. Because of its broad targeting, sunitinib is an effective anti-cancer agent, but it also carries a possibility of side effects that may result from inhibition of off-target kinases. Sunitinib is currently in use for the treatment of several solid tumors, including gastrointestinal stromal tumors, and is tested in clinical trials for breast cancer and non–small cell lung cancer [9,10,13]. Since the cardiotoxic effects of sunitinib have been

* Corresponding author at: University of Arkansas for Medical Sciences, Division of Radiation Health, 4301 West Markham, Slot 522-10, Little Rock, AR 72205, United States.

E-mail address: mboerma@uams.edu (M. Boerma).

http://dx.doi.org/10.1016/j.radonc.2016.03.027
0167-8140/

studied in previous animal models [22–24] that may provide results for comparison, we selected sunitinib as our model TKI.

Materials and methods

Animal model and local heart irradiation

All procedures were approved by the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences (UAMS), in accordance with the Guide for the Care and Use of Laboratory Animals (eighth edition) under protocol number 3405. Male Sprague–Dawley rats (Harlan Laboratories) were maintained in our Division of Laboratory Animal Medicine on a 12:12 light-to-dark cycle with free access to food and water.

At a weight of 250–290 g, rats received local heart irradiation with the Small Animal Conformal Radiation Therapy Device developed at UAMS as described before [25] and in Supplementary Materials. The heart was exposed in 19 mm-diameter fields, one anterior-posterior and two lateral, at 3 Gy each (225 kV, 13 mA, 0.5 mm Cu-filtration resulting in 1.92 Gy/min at 1 cm tissue depth) to a total of 9 Gy per day for 5 consecutive days.

Sunitinib treatment

Oral administration of sunitinib (R&D Systems) or vehicle started on the first day of irradiation and continued once a day for 2 weeks. Two separate experiments were performed. Because the cardiac toxicity of radiation in combination with sunitinib was unknown, local heart irradiation was first combined with sunitinib at a relatively low dose of 8 mg/kg bodyweight per day. When no severe signs of toxicity were observed, a second experiment was performed with sunitinib at 15 mg/kg bodyweight per day. An oral dose of 15 mg/kg bodyweight is required for rats to reach sunitinib plasma levels comparable to those in patients at 24 h after clinical doses of sunitinib [26]. Sunitinib was dissolved to 50 mg/ml in DMSO, diluted in saline, and a total volume of 500 µl was administered by gavage. The rats were weighed every 2–3 days, and the ratio of sunitinib in DMSO and saline was adjusted every 6 days to correct for an increase in average rat weight. Each experiment contained 4 experimental groups: sham-irradiation + vehicle, sham-irradiation + sunitinib, 5×9 Gy + vehicle, and 5×9 Gy + sunitinib (5–6 animals per group). Every day, the vehicle group received oral DMSO and saline in the same ratio (total volume 500 µl) as the sunitinib group.

Echocardiography

Echocardiography was performed as described before [27], using a Vevo 2100 imaging system (VisualSonics) with the MS250 transducer (13–24 MHz). Short axis M-mode recordings at the mid left ventricular (LV) level were used to obtain echocardiographic parameters.

Tissue and plasma preparation

At 2 weeks after local heart irradiation, rats were anesthetized with 3% isoflurane and injected i.v. with 100 U/kg heparin. Peripheral blood samples were collected into EDTA coated tubes and spun down to prepare plasma. The hearts were collected and cut longitudinally. One half, containing LV, right ventricle, and the interventricular septum, was placed in 10% formalin (8 mg/kg sunitinib experiment) or 5% formalin (15 mg/kg sunitinib experiment) for histological analysis. The remaining cardiac tissue was dissected to obtain the LV, and a total of 180–200 mg was immediately used to isolate mitochondria. The remainder of the LV was divided and snap-frozen, to ensure that for each animal the glutathione analyses and Western-Blots described below were performed on cardiac tissue of the same anatomical location.

Ex vivo analysis of mitochondria

Isolation of mitochondria and measurements of mitochondrial permeability transition pore (mPTP) opening were performed as described before [28]. Freshly isolated mitochondria were resuspended in a swelling buffer and exposed to vehicle, 250 µM $CaCl_2$, or 250 µM $CaCl_2$ in combination with the mPTP opening inhibitor cyclosporine A (CsA). MPTP opening in response to calcium leads to mitochondrial swelling as detected by a reduction in optical density at 540 nm (OD540). OD540 was measured with a Synergy 4 microplate reader (BioTek), immediately before the assay and every 2 min thereafter for a total of 20 min.

High-performance liquid chromatography quantification of glutathione

Approximately 50 mg of snap-frozen LV tissue was weighed and homogenized to evaluate levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) by high-performance liquid chromatography (HPLC), as described before [29].

Western-Blots

Western-Blots were performed on LV tissue samples as described before [30,31]. Primary and HRP conjugated secondary antibodies are listed in Table 1S in the Supplementary Materials. Antibody binding was visualized with the Immobilon detection system (EMD Millipore) on CL-Xposure Film (Thermo Scientific). Films were scanned using an AlphaImager® gel documentation

Table 1
Effects of local heart irradiation and sunitinib on cardiac apoptosis, cardiac mast cell numbers, and plasma cTnI. Average ± sem (n = 5–6).

Experimental group	Number of apoptotic nuclei	Number of mast cells	Plasma cTnI (ng/ml)
Experiment 1: Sunitinib at 8 mg/kg bodyweight per day			
5×0 Gy + vehicle	ND	210 ± 14	0.08 ± 0.004
5×0 Gy + sunitinib	ND	203 ± 41	0.09 ± 0.003
5×9 Gy + vehicle	ND	22 ± 6*#	0.12 ± 0.001*#
5×9 Gy + sunitinib	ND	11 ± 3*#	0.13 ± 0.004*#
Experiment 2: Sunitinib at 15 mg/kg bodyweight per day			
5×0 Gy + vehicle	827 ± 61	182 ± 13	0.08 ± 0.002
5×0 Gy + sunitinib	904 ± 97	155 ± 20	0.09 ± 0.001
5×9 Gy + vehicle	1631 ± 97*#	20 ± 3*#	0.13 ± 0.002*#
5×9 Gy + sunitinib	1053 ± 70†	4 ± 1*#	0.14 ± 0.007*#

ND: not determined.
* $p < 0.05$ vs. sham + vehicle;
# $p < 0.05$ vs. sham + sunitinib.
† $p < 0.05$ vs. radiation + vehicle.

Please cite this article in press as: Sridharan V et al. Effects of local irradiation combined with sunitinib on early remodeling, mitochondria, and oxidative stress in the rat heart. Radiother Oncol (2016), http://dx.doi.org/10.1016/j.radonc.2016.03.027