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High-energy photons *vs* protons in their action on vascular function in rats

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The goal of this work was to compare the effects of a photon (PTI) and proton/hadron (HTI) irradiation on rat's cardiovascular system. Cardiovascular functions were studied in rats after PTI and HTI impact in the equivalent total absorbed dose of 6 Gy. Photons were delivered using ^{60}Co gamma-rays (0.8 Gy min^{-1}). The particle irradiation was done by using a $9{,}6\times10^{-12}$ J proton beam accelerated in the U-240 isochronous cyclotron. Both PTI and HTI decreased the acetylcholine-induced relaxation in rat's aorta smooth muscle (SM) and outward potassium currents in aortic SM cells on the 9th day post-irradiation but HTI appeared to produce a more profound effect. HTI had no significant effect on systolic blood pressure (SBP) in rats while PTI produced clearly defined systemic hypertension. HTI, unlike PTI, significantly increased the left ventricle pressure in Langendorff - perfused rat's heart. Thus, the biological effects of PTI and HTI on rat's aorta endothelium-dependent relaxation and net potassium currents in the SM cells appear to be similar, although the effects of HTI are more pronounced. However, PTI, unlike HTI, produced significant systemic hypertension.

Keywords: photon and proton irradiation; cardiovascular function; potassium currents; endothelium-dependent relaxation.

INTRODUCTION

It is well known that exposure to high levels of radiation can cause acute, short-term and long-term health deteriorative effects causing various cancers and cardiovascular diseases. At the same time, external beam radiation therapy is commonly used as primary and adjuvant therapy in patients with neoplasm, and despite many advances in modern technology, such therapy often affected normal tissues, including the vasculature and heart. These unwanted radiation-induced cardiovascular effects seriously complicate the course and the treatment of the main disease [1].

Experiments on irradiated (6 Gy) rats showed that blood pressure became significantly increased on the 9th day of post-irradiation, and such hypertension persisted over six months [2]. The same clinical observations of elevated blood pressure were reported in the group of survivors after the Chornobyl accident [3] and it was confirmed by experimental data on vascular reactivity [4].

Cell damages caused by radiation are the subject of intensive research. However, many of the mechanisms behind the side effects caused by ionizing radiation and their short-term and long-term effects remain obscure. The majority of the literature is concentrated on investigating conditions leading to cell death or to the prevention of cell division at relatively high-absorbed doses. There is a very limited number of studies addressing changes not leading to cell death [5].

It is clear that the physical properties of high-energy photons and high-energy protons

are distinct but their radiobiological properties and biological effects appear to be comparable. For instance, γ-irradiation (PTI) demonstrates an exponentially decreasing energy deposition with increasing depth of penetration in tissues. In contrast, charged particles (HTI) show an increasing level of energy deposition with penetration distance leading to a maximum near the end of the particle beam range, i.e. moving through tissues, protons to a much lesser extent lose their energy on atomic and nuclear events compared to photons [6]. The mechanism of charged particle energy loss by electronic and nuclear stopping yields the maximal energy loss at the end of the particle's path, denoted as the Bragg peak, and hence delivers the maximal amount of energy in a well-defined depth in contrast to photons [6, 7].

Thus, HTI has an advantage from the clinical point of view due to the spatial distribution of dose delivered to the target since the heavier ions demonstrate an increasing energy loss with the depth of penetration, producing maximal energy deposition in a given place. Protons are appearing slightly more biologically effective than photons [8]; and in combination with their physical properties, it makes them highly useful for precise targeted clinical proton beam therapy.

The recent and ongoing developments in the field of accelerator technology make proton therapy a realistic form of radiation treatment for an increasing amount of patients. Today, facility-related investment costs are constantly being reduced and this trend is expected to continue making this kind of treatment more accessible. Additionally, there is evidence that the use of proton therapy in the treatment of some tumors (*medulloblastoma*) appears to be more economically preferable than the use of photon therapy [9].

The patients with neoplasm who receive radiotherapy, specifically photon or hadrons (proton) therapy (PT/HT), may suffer from unwanted cardiovascular effects in the areas adjacent to the targets (tumors). Up to date, there have been no convincing investigations that directly compare the acute effects of PTI and HTI on cardiac and vascular function in case the tumor is located close to the heart. The problem is in the limited data on the mechanisms of side effects on healthy tissues when influencing protons or other charged particles.

The main goal of this work was a comparative study of the effects of proton and photon irradiation on the functional state of the rat's cardiovascular system at the same absorbed dose.

METHODS

Whole-body animal photon and proton irradiation. During the PTI and HTI impacts, the animals were restrained in respectively plastic and metal containers specifically designed for the experimental needs. There were no changes in housing, standard food, or drink before and following irradiation as well as in the post-irradiation period. The protocols were approved by the Institute of Pharmacology and Toxicology and Liverpool University Institutional Animal Care and Use Committees and followed all recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

Experiments were performed on adult male Wistar-Kyoto rats (250-300 g) aged 6-8 weeks. Animals were divided into three groups: a control group, rats after PTI and rats after HTI impact (7 rats in each group). Single whole-body γ-irradiation (PTI) was performed with gamma rays delivered at a rate of 0.9 Gy/min from a cobalt^{60} source (TGT ROCUS M, USSR) positioned 50 cm from the animal. During irradiation, the animals were fixed inside the plastic box specifically designed for this study, which was placed in such a way that the axis of the radiation beam with a square of 20 × 20 cm was centered on the animal's chest.

Particle beam parameters (HTI) were established based on the data simulated with the semiempirical model [10]. We took into account the average values of the tissue density and ionic

composition in the irradiated area. In accordance with Paganetti (2014), relative biological effectiveness was chosen equal to 1.1 for the proton beam with the Bragg peak within the rat's thorax. The camera with the experimental animal was placed at a distance of 10 cm from the proton beam exit point (transverse beam size of 20 mm) so that the Bragg peak was localized in the projection of the middle of the animal's chest.

Rats were exposed to an equal total absorbed dose of 6 Gy in both casess of irradiation impact and then were euthanized on the 9th day following the irradiation.

Contractile recording experiments (ACh – test). Experiments were performed on the thoracic aorta tissues obtained from healthy and irradiated adult rats as previously described [2]. Briefly, the vascular rings were removed after thoracotomy under anesthesia (alpha chloralose, 40 mg/kg plus urethane, 400 mg/kg b/w, i.p.). To evaluate endothelium function, isolated thoracic aorta rings were preconstricted with 10 μM arterenol and then, when the contraction reached a plateau level, acetylcholine was added to the bath solution in a dose-dependent manner. The relaxation responses of the rings to Ach were expressed as a percentage of the contractile response of the aorta to arterenol.

Outward net potassium current recordings. The whole-cell perforated patch-clamp technique in the whole-cell configuration was used to study whole-cell potassium currents (voltage-clamp mode) as previously described [11].

Non-invasive measurement of blood pressure. Systolic blood pressure values in rats (three consecutive readings) were obtained using a tail-cuff sphygmomanometer (S-2 Hugo-Sachs Elektronik, Germany) connected to analog-to-digital converter PowerLab 4/30 (ADInstruments, Australia).

Langendorff preparation of isolated heart perfused at a constant flow. The procedure of isolation and connection of the heart to the perfusion apparatus for the measurements of left ventricular pressure, coronary perfusion pressure and electrocardiogram has been previously described by Dhein et al. [12]. The left ventricular end-diastolic pressure was initially set to 5 mm Hg by balloon inflation. Oxygenation of the perfusion solution was maintained with a gas mixture containing 95% oxygen and 5% carbon dioxide.

The pressure transducers and EG electrodes were connected to a converter PowerLab 4/30 (ADInstruments, Australia).

Statistical analysis. The data are shown as means ± SEM; *n* indicates the number of vascular preparations tested. Curves were fitted to the Hill equation. Half-maximally effective concentration (EC_{50}) values were expressed as pD_2 (-Log EC_{50}). A comparison of variables obtained by various treatments with basal values was made by one-way analysis of variance with a repeated measurement design, and if any significant difference was found, the Scheffe's multiple comparison test was applied. Differences were considered to be statistically significant when $P < 0.05$.

RESULTS

It has been determined that both PTI and HTI led to a decrease in the amplitude of the Ach-induced relaxation on the 9th day of post-irradiation. Dose-responses curves showed that the maximal amplitude of endothelium-dependent SM relaxation (Rmax) had decreased from 91 ± 2% in control to 80 ± 3%, and 53 ± 3% in PTI and HTI aortic tissues, respectively ($n = 14$, $P < 0.001$). Furthermore, the sensitivity of the aortic tissues to acetylcholine after both PTI and HTI had also significantly decreased (mean values of pD_2 (-log EC_{50}) were 7.8 ± 0.2 in control, 7.0 ± 0.02 and 6.9 ± 0.04 under PTI and HTI, respectively, $n = 14$, $P < 0.001$) (Fig 1). Thus, HTI had a stronger effect on the aorta endothelial function as compared to PTI: These groups differed significantly in the values of both Rmax and pD_2.

On the 9th day of post-irradiation, outward net potassium currents (IKo) in aortic SM

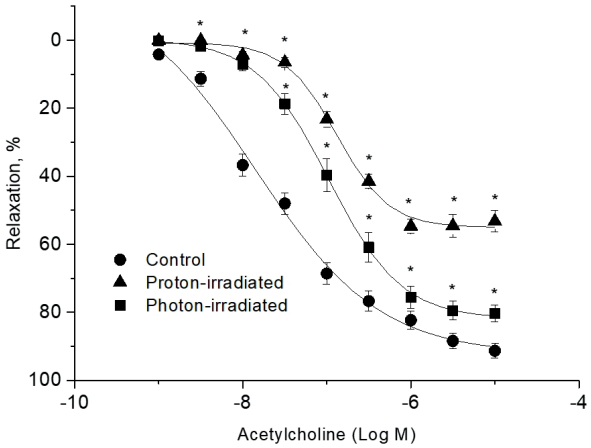

Fig. 1. Endothelium-dependent Ach-induced relaxation in rat aorta rings obtained from control, proton-irradiated and photon-irradiated rats and preconstricted with 10 μM arterenol. Data shown are mean ± SEM (n = 14). *P < 0.05 versus control

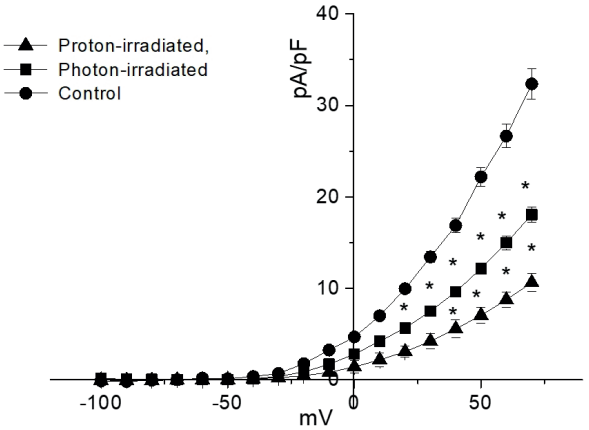

Fig. 2. I-V relationship of outward currents recorded in isolated rat thoracic aorta smooth muscle cells (SMC) obtained from control, proton-irradiated and photon-irradiated rats. Currents were elicited by 10 mV step pulses with 300 msec duration between -100 and +70 mV from a holding potential of -60 mV. Data shown are mean ± SEM (n = 7). *P < 0.05 versus control

cells, which is mainly due to the current carried through Maxi-K^+ channels, have been reduced from control 32 ± 2 to 18 ± 1 pA/pF, and to 10±1 pA/pF at +70 mV, following PTI and HTI, respectively, i.e. the effect of HTI appeared to be more profound than that seen after PTI (n = 7, P < 0.001) (Fig 2).

The HTI had no significant effect on rat's systolic BP (128 ± 8 *vs* 126 ± 8 mm Hg in control) while PTI produced sustained and significant hypertension development on the 9th day of post-irradiation (158 ± 6 vs 126 ± 8 mm Hg in control, n = 7, P < 0.05 (Fig 3).

As for the functional state of the myocardium, it should be noted a more significant increase of left ventricle pressure after HTI (53.6 ± 5.1)% vs control) as compared with PTI (29.6 ± 4.6)% vs control) (Fig. 4). Both HTI and PTI had no effects on the index of myocardial contractility in Langendorff-perfused rat hearts.

DISCUSSION

The data obtained suggest that HTI causes endothelial dysfunction and ion channelopathy, like exposure to PTI. It is important to note that these effects were more clearly expressed after HTI. At the same time, the results indicate that HTI did not affect such a system parameter as

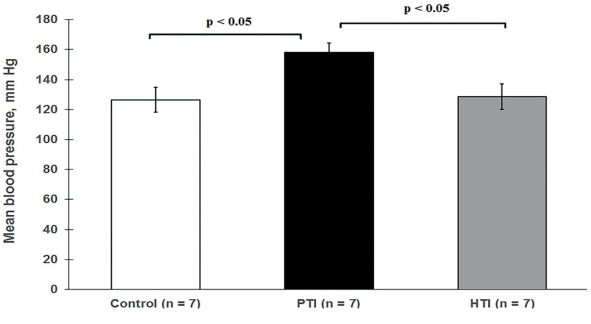

Fig. 3. The effect of photon and proton irradiation on systolic arterial blood pressure in rats (in percents of the initial value). Data shown are mean ± SEM (n = 7)

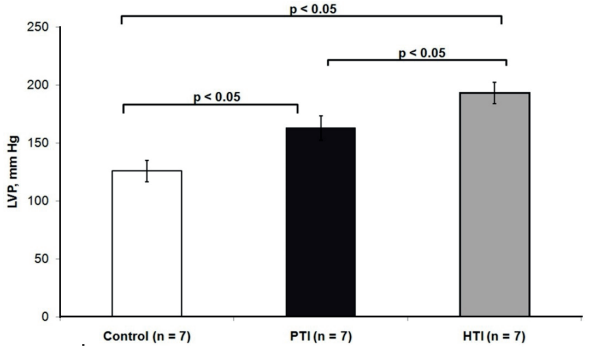

Fig. 4. The effect of PTI and HTI on left ventriculi pressure (LVP) changes in Langendorff perfused rat hearts. Data shown are mean ± SEM (n = 7)

arterial blood pressure. PTI, on the contrary, led to the persistent increase in blood pressure. So, one of the main findings of this study was the absence of radiation-induced arterial hypertension after HTI.

The systemic blood pressure is primarily stipulated by the total resistance of all peripheral vessels and the heart work. Due to the specifics of energy distribution in the animal's body, photon irradiation leads to dysfunction of the endothelium in a larger volume of the vascular bed and, accordingly, to a greater decrease in the production of the endothelial relaxing factor. When irradiated with protons, the area of endothelial damage is limited to the area of the Bragg peak localization, i.e. zone of the heart and thoracic aorta.

It is difficult to say how rat's blood pressure will change as a delayed effect of proton irradiation. In any case, it is unlikely that this will be hypertension since the endothelial damage zone is localized in the heart and aortic arch projection. It might be explained by the absence of massive damage to the peripheral endothelium as in the case of photons. If changes in pressure take place in the long perspective, then more likely it will be a development of hypotension due to violation of the contractile function of the heart.

We studied intensively the effect of PTI on blood vessels in the different post-radiation periods. We firmly established that PTI damages endothelial function, suppresses BK_{Ca} channels in both endothelial and SM cells, and leads to a pronounced increase of systolic blood pressure [13-15]. These dates clearly demonstrated that the effects of PTI on endothelium function and potassium conductivity depend on the dose of radiation and appear to be even increased in the post-radiation period for at least 30 days.

It is interesting to note that a comparative analysis of the data obtained in this study with our previous observations indicates that the changes in endothelium-dependent relaxation and outward potassium currents caused by HTI on the 9th day of post-irradiation were similar to those after exposure PTI but on the 30th day after irradiation. The HTI effect is maximally realized in the zone of formation of Bragg peak and to a lesser extent affects the surrounding tissues. It has been shown that PTI suppresses BK_{Ca} channels, which control the driving force for Ca^{2+} entry and NO synthesis in endothelial cells [13]. This may contribute to radiation-induced endothelium dysfunction and an increase in arterial blood pressure. At the same time, the BK_{Ca} suppression in vascular SM cells following PTI can promote decreasing its vasorelaxant activity [11].

Thus, our previous data allowed us to form the concept of a leading role of the endothelium in the vascular malfunctions developed due to PTI impact. This conception was later confirmed by several other researchers [5, 16-18].

The observed differences in the HTI and PTI effects on BP can be explained by differences in the radiation principles. Protons differ significantly as compared to photons in their physical interaction with the vascular tissue atoms and molecules due to their higher concentration of energy. Protons and photons have also differences in energy absorption and distribution in tissues, and this might explain the differences in their clinical outcomes.

It has long been known that heavy particles move along a relatively straight path and form long tracks with a characteristic concentration of energy. That is why protons are more likely to produce long-range nuclear secondaries, and due to greater relative biological effectiveness, they have a potential therapeutic advantage [8].

Photons, on the other hand, are characterized by a tendency toward scattering and distribution of energy. Uniform energy dissipation across all tissues following PTI leads to the destruction of the most sensitive cells, including endothelial cells. Besides endothelial cells death, PTI can also cause premature senescence, leading to increase in many senescent cells that are unable to perform their functions [19,20].

CONCLUSIONS

Thus, the effects of PTI and HTI on vascular function (endothelium-dependent relaxation and ion potassium conductivity), except arterial blood pressure, appeared to be inhibitory, but their intensity differed significantly. The data obtained provide new relevant information on the higher effectiveness of protons compared to photon exposure on the vascular tissues due to their different physical properties and the ability of protons to release energy in the therapeutic target area without affecting the surrounding tissue. The undoubted advantage of HTI is the absence of such side effects as arterial hypertension.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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ПОРІВНЯННЯ ДІЇ ФОТОННОГО ТА ПРОТОННОГО ОПРОМІНЕННЯ НА СЕРЦЕВО-СУДИННУ СИСТЕМУ ЩУРІВ

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Метою цієї роботи було порівняти дію фотонного (ФО) і протонного (ПО) опромінення на серцево-судинну систему щурів. Функціональний стан серцево-судинної системи тварин вивчали після їх впливу в еквівалентній сумарній дозі 6 Гр. Щурів піддавали ФО через зовнішнє одноразове γ-опромінення джерелом ^{60}Co (0,8 Гр хв$^{-1}$) або ПО – за допомогою пучка протонів з енергією 9,6×10^{-12} Дж, прискореного в ізохронному циклотроні У-240. Встановлено, що дія як ФО, так і ПО зменшувала ендотелійзалежне розслаблення ізольованих сегментів аорти та вихідний калієвий струм у гладеньком'язових клітинах аорти щура на 9-й день після опромінення, проте ПО спричиняло більш глибокий ефект. Його дія не викликала значного збільшення систолічного артеріального тиску у щурів, тоді як ФО спричиняло виражену системну гіпертензію. У разі ПО, на відміну від ФО, значно підвищувався тиск у лівому шлуночку серця щура, перфузованого за Лангендорфом. Таким чином, біологічні ефекти ФО та ПО, що проявлялись у пригніченні ендотелійзалежних судинних реакцій та калієвих струмів у гладеньком'язових клітинах аорти, виявилися подібними, хоча ефекти ПО були більш вираженими. Однак ФО, на відміну від ПО, викликало значну системну гіпертензію.

Ключові слова: фотонне і протонне опромінення; функції серцево-судинної системи; калієві струми; ендотелій-залежна релаксація

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