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EFFECT OF RADIOTHERAPY-INDUCED ALTERATION OF INDIVIDUAL RADIOSENSITIVITY ON DEVELOPMENT OF SIDE EFFECT IN CANCER PATIENTS

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Keywords: individual radiosensitivity, G2 radiosensitivity assay, radiotherapy.

Summary

Ionizing radiation is commonly used for cancer treatment. Human response to the same dose of ionizing radiation can vary among individuals, therefore individual radiosensitivity (IRS) was proposed to be an important factor for development of radiotherapy (RT) related side effects. Ionizing radiation especially at low doses can modify organism sensitivity causing its sensitization or adaptation to further exposure, thus IRS of cancer patient can change during RT and so effect the development of normal tissue toxicity as well. Therefore, objective of our study was to determine the correlation between IRS of prostate cancer patients during RT and outcome of treatment adverse reactions. This pilot study included six prostate cancer patients without previous exposure to ionizing radiation treated with salvage RT. IRS was assessed using G2 chromosomal radiosensitivity assay with G2-checkpoint abrogation by caffeine three times for each patient: prior RT, after first fraction, and after completing treatment and acute genitourinary (GU) and gastrointestinal (GI) toxicity were reported. It was found that three of selected patients experienced grade 1-2 RT acute GU/GI toxicity. According to IRS tests, before RT two patients were classified as normal, two – as radiosensitive, and two - as highly radiosensitive. After the first fraction there were three individuals classified as normal, one patient remained radiosensitive and two others felt to the highly radiosensitive group. After completion of treatment, the distribution of IRS in selected patients recovered to that observed before the treatment. Despite that pattern of IRS changes during RT varied in every patient, the common tendencies and their correlation with the development of toxicity was observed. It was found that, IRS of patient experienced adverse reaction riced during RT, meanwhile in patients without side effects it decreased. So, it could be concluded that difference in radiation-induced IRS alteration tendency could be reflected in pattern of adverse reaction development. This phenomenon could be associated with attribute of pre-exposure to initiate individually either an adaptive response increasing resistance to further irradiation or sensitization. Therefore, further investigations of more RT patients employing G2 assay are foreseen to reveal the possible correlation between IRS and adverse clinical outcome of RT.

Introduction

Ionizing radiation is commonly used for cancer treatment. Along with healing of tumor RT can cause toxicity in non target tissues including radiation induced carcinogenesis [1, 2]. Assimilation of modernization of RT techniques and control of biological mechanisms involved in adverse reactions would enable to optimize treatment and reduce severity of RT toxicity. Since human can differently respond to the same dose of ionizing radiation, IRS was proposed to be an important factor for development of RT related side effects [3]. Induction of chromosomal aberration and efficiency of DNA reparation system relates to the patient's susceptibility to RT-induced toxicity in normal tissues [4, 5]. Patients with the Ataxia-Telangiectasia (A-T) syndrome has been found to be at higher breast cancer risk due to the suppression of protein kinase ataxia-telangiectasia mutated (ATM), which acts in cellular response to DNA double-strand breaks [6]. So, the frequency of radiation-induced chromosomal aberration can serve as a marker of reparation system status and as a result of IRS [7]. Estimation of IRS based on comparing of exciting cells ability to repair radiation induced

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DNA damage at G2 cell cycle stage to the lowest reparation efficiency similar to found in A-T patients (when G2 checkpoint is abrogated with caffeine) [8] could be employed in RT planning to mitigate toxicity in highly radiosensitive patients and justify dose escalation for resistant one. Since ionizing radiation especially at low doses can modify organism sensitivity causing its sensitization or adaptation to further exposure [9], IRS of cancer patient can change during RT due to fractionated exposure and so influence the development of adverse reactions as well.

Objective of the study - to assess possible correlation between alteration in IRS and patterns of GU and GI toxicity rates during or after salvage RT in prostate cancer patients.

Materials and methods

Selection of patients. Six 47-69 years old prostate cancer patients (C61 according 2021 ICD-10-CM Codes) after prostatectomy, without previous exposure to ionizing radiation or genotoxic medicaments were selected. Patients received RT for either prostate-specific antigen (PSA) rising, or PSA persistence after RP. Within RT plan, prostate/seminal vesicle bed and pelvic lymphnodes were set as a target volume, while bladder, small bowel, rectum and femur heads were defined as organs at risk. Treatment planning employed 3D conformal external beam radiotherapy (3D-CRT) or volumetric modulated arc therapy (VMAT) techniques using 18-MV and 6-MV photon beams respectively with a maximum variable dose rate of 600 MU/min. All patients received total dose of 64-66 Gy (2 Gy/day five times a week) delivered in 44-48 Gy to pelvic lymphnodes followed by boost to the prostate/seminal vesicle bed of 16 – 22 Gy.

Blood sampling, cell culture and irradiation conditions. Peripheral blood sampled to the Li-heparin vacutainers. Culturing of lymphocytes set up by adding 0.5 ml of heparinized whole blood to 4.5 ml of F-10 medium enriched with 13% fetal bovine serum, 2% L-glutamine, antibiotics (penicillin: 100 U/ml, streptomycin: 100 µg/ml) and 2% phytohaemagglutinin (PHA) and incubating for 72 hours in a humidified air atmosphere of 37°C in 5% CO_2 [10]. Lymphocytes cultures were *in vitro* irradiated to 1 Gy in T-105 X-ray therapy unit (Wolf Medizintechnik GmbH, Germany) at room temperature (23 ± 2°C) at a dose rate of 2.3 Gy/min (70 keV, 15 mA). One-half of irradiated lymphocytes culture was supplemented with caffeine solution (4 mM) and incubated together with caffeine-free part for 20 min to initiate cell division and followed by arrest at metaphase through colcemid-block for 1 h. Peripheral lymphocytes were harvested and collected by centrifugation, resuspended in 75 mM KCl solution for 15 min at 37 °C, rinsed three times in fixative (methanol - acetic acid, 3:1), spread on microscope slides over a humidity chamber, air dried and stained with 5 % Giemsa [11].

Experimental procedure and data analysis. Standardized protocol for G2 assay proposed by *Pantelias at al.* was employed to assess possible RT-induced changes in IRS: caffeine - free G2 yield (hereinafter - G2) and caffeine- containing G2 yield (hereinafter - G2 caffeine) simulating high radiosensitivity due to caffeine-induced G2-M checkpoint arrest were set for each patient. IRS was calculated as a percentage of the high radiosensitivity level of AT patients using formula IRS= (G2/G2caffeine) x 100%. Individuals were classified according to IRS cut-off value as radioresistant (IRS < 30%), normal (30% ≤IRS ≤ 50%), radiosensitive (IRS > 50%) and highly radiosensitive (IRS > 70 %) [8]. At least 100 metaphase spreads from each individual were examined (50 cell/per yield) at selected phase of treatment: before RT, after exposure to first 2 Gy, and after treatment was completed. Metaphase spreads were stained with Giemsa and observed at × 600 magnification with oil immersion employing Zeiss Axio Imager Z2 microscope equipped with the high-resolution camera and analysed using Metafer 4 software (Metasystems, Germany).

Within medical supervision and follow-up acute GU and GI toxicity in prostate cancer patients were reported and graded according to the Toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC) [12].

This study was implemented in accordance with bioethi-

Table 1. Individual radiosensitivity (IRS) at different phase of radiotherapy (RT): (before RT, after first fraction and after the completion of RT) and reported acute genitourinary (GU) and gastrointestinal (GI) toxicity graded according EORTC/RTOG in six prostate cancer patients

Patient No.	**1**	**2**	**3**	**4**	**5**	**6**
IRS Before RT	53.2%	52.3%	**74.3%**	31.3%	31.5%	**78.2%**
IRS After the first fraction of RT	59.3%	44.4%	**70.3%**	38.37%	33.9%	**87.6%**
IRS After the completion of RT	63.9%	44.8%	55.7%	48.0%	**76.9%**	**78.5%**
Acute GU/GI toxicity	**2/1**	0/0	0/0	0/0	**0/1**	**1/2**
Total dose (dose to the pelvis lymphnodes + boost to the prostate/seminal vesicle bed), Gy	64 (48+16)	66 (44+22)	66 (46+20)	66 (46+20)	66 (46+20)	66 (46+20)

cal approval to conduct a biomedical research on evaluation of relationship between the occurrence of side effects using ionizing radiation therapy and chromosomal damage in lymphocytes No. L-14-07/1 issued by the Lithuanian Bioethics Committee.

Results and discussion

IRS values and GU/GI toxicity rates of six prostate cancer patients at different phase of RT are shown in table 1. Three of selected patient experienced acute grade 1-2 GU and/or GI toxicity: patient No. 1 demonstrated grade 2 GU and grade 1 GI toxicity, patient No. 5 showed grade 1 GI and patient No. 6 had grade 1 GU and grade 2 GI, while rest three patients did not develop RT side outcomes.

According IRS estimated before RT, two patients were classified as normal (IRS=31.3% - 31.5%), two – as radiosensitive (IRS=53.2% - 52.3%), and two - as highly radiosensitive (IRS=74.3% - 78.2%). After the first RT fraction the number of individuals classified as normal increased to three (IRS=33.9% - 44.4 %), one patient remained as radiosensitive (IRS=59.3%) and two others belonged to the highly radiosensitive group (IRS=70.3% - 87.6%). After the end of treatment, the distribution of IRS in selected patients shifted to the tendency observed before the treatment: two normal (IRS=44.8% - 48.0%), two radiosensitive (IRS=55.7% - 63.9%) and two highly radiosensitive (IRS=76.9 % - 78.5%) patients (Table 1).

However, pattern of IRS changes during RT differs in every single patient. Patient No. 5 experienced escalation in IRS from 31.5% estimated before RT to 76.9% reported after completion of RT and consequently was reclassified from normal to highly radiosensitive during the observation period. The same tendency was observed in patients No. 1 and No. 4, who showed slight increase in IRS (from 53.2% before RT to 63.9% at the end of RT for patient No. 1 and from 31.3% to 48.0% for patient No. 4) remaining classified as radiosensitive and normal respectively during all observation period. Despite alteration in IRS which raised from 78.2% to 87.6% after first fraction of RT and reversion to 78.5% after treatment was completed, patient No. 6 belonged to the highly radiosensitive group in all phases of treatment. Contrariwise, patients No. 2 and No. 3 demonstrated decrease in IRS while undergoing RT (from 52.3% before RT to 44.8% at the end of RT for patient No. 2 and from 74.3% to 55.7% for patient No. 3) and were felt in classification from radiosensitive to normal and from highly radiosensitive to radiosensitive respectively. Figure 1 depicts the distribution of IRS in accordance with phase of RT in different patients.

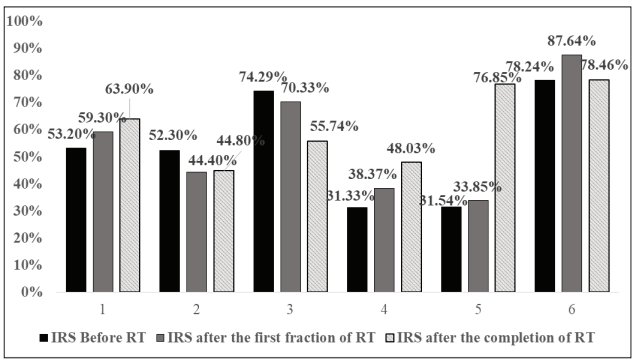

Figure 1. Distribution of IRS in cancer patient in different phase of radiotherapy: IRS – Individual radiosensitivity (IRS) calculated as a percentage of the high radiosensitivity level of A-T patients using formula IRS=(G2/G2 caffeine) x 100%

It is remarkably, that patients who experienced an increase in IRS during RT (patients No. 1, 5, 6) and by the end of RT belonged to radiosensitive or highly radiosensitive also developed RT-induced 1-2 grade acute GU/GI toxicity. Meanwhile, an increase of IRS took place within an interval of normal sensitivity to ionizing radiation, observed in patient No. 4 was not accompanied with side effects due to RT. Unlike increasing tendency of IRS during RT, RT toxicity was not reported in cases of decrease in IRS during RT observed in patient No. 2 and 3. This is especially notable for patient No. 3, for whom RT-induced toxicity was expectable due to the classification as highly radiosensitive before RT (IRS=74.3%).

Our pilot study revealed positive correlation between tendencies in changes of IRS during RT and development of acute toxicity among 6 prostate cancer patients: increase in IRS associated with development of acute normal tissue toxicity (except if increase in IRS is with normal sensitivity interval), meanwhile in case of decrease in IRS any toxicity was not reported. Observed results are in line with previous findings regarding possibilities of an organism to modify its sensitivity to ionizing radiation. It is well known that living cells, particularly human lymphocytes, in order to retain their viability can modify reparation rate in response to low-dose ionizing radiation initiating either adaptation or hypersensitivity. *Joiner et al.* previously discussed phenomenon of potential interest for developing improved RT comprising hypersensitivity of cells to acute low doses and at the same time increased resistance to high doses of ionizing radiation [13]. *Lambin et al.* also reported changes of sensitivity to ionizing radiation in human tumor cell lines expressed as hypersensitivity at low doses followed by an induced radioresistance at higher doses [4]. Adaptive response cause higher resistance of an organism to irradiation and can be reflected as a decrease in IRS, otherwise sensitization leads to increase in IRS. Therefore, risk associated with low doses per fraction

outside the target volume, especially in case when subsequent RT of pre-exposed regions is required, should be of clinical concern [14]. Especially it is true taking into account the fact that individual organism can develop opposite outcome in response to low dose radiation such as higher resistance or sensitivity for further irradiation. According these findings, further investigation of mechanisms of correlation between IRS and acute normal tissue toxicity are required.

Conclusions

Observed phenomenon of alterations of IRS during RT may have clinical implication and play an important role in prediction of development of acute toxicity due to RT. Changes in IRS during RT can be triggered individually by adaption or hypersensitivity of an organism to ionizing radiation initiated by protracted exposure to low-doses. Mechanisms laying down behind the alterations of IRS due to fractionated exposure may determine the rate of the development of RT induced toxicity. Therefore, further multidisciplinary research on individual susceptibility to initiate adaption or sensitization of an organism to ionizing radiation in response to low-dose pre-exposure would provide data enabling to improve and optimize clinical outcome of RT.

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References

1. Kron T. Reduction of margins in external beam radiotherapy. JMed Phys 2008; 33(2):41-42.
https://doi.org/10.4103/0971-6203.41190
2. Gamulin M, Kopjar N, GrgiM, Rami S, Viculin T, Petkovi, Garaj-Vrhovac V. Cytogenetic follow-up in testicular seminoma patients exposed to adjuvant radiotherapy. Collegium antropologicum 2010; 34(2):455-465.
3. Auer J, Keller U, Schmidt M, Ott O, Fietkau R, Distel LV. Individual radiosensitivity in a breast cancer collective is changed with the patients' age. Radiol and Oncol 2014;48(1):80-86.
https://doi.org/10.2478/raon-2013-0061
4. Lambin P, Coco-Martin J, Legal JD, Begg AC, Parmentier C, Joiner MC, Malaise EP. Intrinsic radiosensitivity and chromosome aberration analysis using fluorescence in situ hybridization in cells of two human tumor cell lines. Radiat Res 1994;138(1):S40-3.
https://doi.org/10.2307/3578758
5. Popanda O, Ebbeler R, Twardella D, Helmbold I, Gotzes F, Schmezer P,Thielmann HW, von Fournier D, Haase W, Sautter-Bihl ML, Wenz F, Bartsch H, Chang-Claude J. Radiation-induced DNA damage and repair in lymphocytes from breast cancer patients and their correlation with acute skin reactions to radiotherapy. Int J Radiation Oncology Biol Phys 2003; 55 (5):1216-1225.
https://doi.org/10.1016/S0360-3016(02)04415-2
6. Bogdanova N, Helbig S, and Dörkcorresponding T. Hereditary breast cancer: ever more pieces to the polygenic puzzle. Hered Cancer Clin Pract 2013;11(1):12.
https://doi.org/10.1186/1897-4287-11-12
7. Greve B, Bölling T, Amler S, Rössler U, Gomolka M, Mayer C,Popanda O, Dreffke K, Rickinger A, Fritz E, Eckardt-Schupp F, Sauerland C, Braselmann H, Sauter W, Illig T, Riesenbeck D, Könemann S, Willich N, Mörtl S, Eich HT, Schmezer P. Evaluation of different biomarkers to predict individual radiosensitivity in an inter-laboratory comparison-lessons for future studies. PLoS One 2012;7(10):e47185.
https://doi.org/10.1371/journal.pone.0047185
8. Pantelias GE, Terzoudi GI. A standardized G2-assay for the prediction of individual radiosensitivity. Radiother Oncol 2011;101(1):28-34.
https://doi.org/10.1016/j.radonc.2011.09.021
9. Pollycove M, Feinendegen LE. Biologic responses to low doses of ionizing radiation: detriment versus hormesis part 2. Dose responses of organisms. J Nucl Med 2001;42(9):26-32.
10. IAEA. Cytogenetic dosimetry: applications in preparedness for and response to radiation emergencies. IAEA EPR-biodosimetry series. International Atomic Energy Agency, Vienna 2011.
11. Guogytė K, Plieskienė A, Sevriukova O, Ladygienė R, Žiliukas J, Janušonis V. Micronuclei and G2 assays for assessment of chromosomal radiosensitivity as assistant tool in radiotherapy: method-comparison study. Sveikatos Mokslai/Health Sciences In Eastern Europe 2016;26(5):63-68.
https://doi.org/10.5200/sm-hs.2016.073
12. Cox JD, Stetz J, Pajak TF. Toxicity criteria of the radiation therapy oncology group (RTOG) and the European organization for research and treatment of cancer (EORTC). Int J Radiat Oncol Biol Phys 1995;30;31(5):1341-6.
https://doi.org/10.1016/0360-3016(95)00060-C
13. Joiner MC, Marples B, Lambin P, Short SC, Turesson I. Low-dose hypersensitivity: current status and possible mechanisms. ICTR 2001;49 (2):379-389.
https://doi.org/10.1016/S0360-3016(00)01471-1
14. Malaise EP, Lambin P, Joiner MC. Radiosensitivity of human cell lines to small doses. Are there some clinical implications? Radiat Res 1994;138(1):S25-7.
https://doi.org/10.2307/3578754

INDIVIDUALAUS RADIOJAUTRUMO KITIMO GELBSTINČIOS PROSTATOS VĖŽIO RADIOTERAPIJOS METU POVEIKIS NEPAGEIDAUJAMŲ ŠVITINIMO REAKCIJŲ IŠSIVYSTYMUI

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Raktažodžiai: individualus radiojautrumas, chromosomų radiojautrumo G2 stadijoje analizė, radioterapija.

Santrauka

Jonizuojančioji spinduliuotė plačiai taikoma vėžiui gydyti. Žmonių atsakas į tą pačią jonizuojančiosios spinduliuotės dozę gali skirtis, todėl individualus radiojautrumas (IRJ) buvo pasiūlytas kaip svarbus veiksnys, lemiantis radioterapijos (RT) metu atsirandančių šalutinių reiškinių sveikuose audiniuose raidą. Mažos jonizuojančiosios spinduliuotės dozės gali keisti organizmo jautrumą tiek sukeldamos adaptacinį atsaką, tiek ir didinančios atsparumą tolesniam jonizuojančiosios spinduliuotės poveikiui. Vėžiu sergančio paciento IRJ, dėl frakcionuoto švitinimo, gali pasikeisti gydymo metu ir taip paveikti aplinkinių sveikų audinių reakciją į RT. Tyrimo tikslas – nustatyti koreliaciją tarp prostatos vėžiu sergančių pacientų IRJ ir RT sukeliamų nepageidaujamų reakcijų sveikuose audiniuose pasireiškimo.Tyrime dalyvavo šeši vyrai, sergantys prostatos vėžiu, anksčiau nepatyrę apšvitos jonizuojančiąja spinduliuote, kuriems dėl ligos progresavimo po prostatektomijos buvo skirta gelbstinti RT į prostatos ir sėklinių pūslelių ložę bei sritinius limfmazgius. IRJ buvo vertinamas taikant modifikuotą chromatidžių trūkių G2 ląstelės ciklo fazėje tyrimą su G2 patikros taško blokavimu kofeinu kiekvienam pacientui tris kartus: prieš RT, po pirmosios frakcijos ir baigus gydymą. Nepageidaujami RT sukelti reiškiniai – spindulinės reakcijos buvo vertinamos stebint toksinį poveikį urogenitalinei (GU) ir virškinimo (GI) sistemai. Tyrimo metu nustatyta, kad trims atrinktiems pacientams pasireiškė 1-2 laipsnio ūmios spindulinės reakcijos (GU/GI toksiškumas). Pagal IRJ vertinimo rezultatus, prieš RT dviejų pacientų radiojautrumas buvo normalus, du – radiojautrūs ir du – labai radiojautrūs. Po pirmosios RT frakcijos trijų pacientų radiojautrumas buvo normalus, vienas pacientas išliko radiojautrus, o likusieji du buvo priskirti labai radiojautrių asmenų grupei. Baigus RT IRJ pasiskirstymas buvo toks pat, kaip ir prieš gydymą. Nors IRJ pokyčiai RT metu kiekvieno paciento buvo skirtingi, pastebėtos bendros tendencijos ir jų teigiama koreliacija su toksiškumo raida. Nustatyta, kad pacientams, patyrusiems nepageidaujamų reakcijų, IRJ padidėjo RT metu, o tiems, kuriems spindulinės reakcijos nepasireiškė, IRJ sumažėjo. Galima daryti prielaidą, kad RT sukeltų IRJ pokyčių tendencijos skirtumas gali atsispindėti nepageidaujamų reakcijų vystymosi modelyje. Šis reiškinys gali būti siejamas su apšvitai mažomis jonizuojančiosios spinduliuotės dozėmis būdinga savybe individualiai sužadinti organizmo atsparumo ar jautrumo tolesniam švitinimui padidėjimą. Kol mechanizmai, lemiantys individualų organizmo atsaką, nėra iki galo išaiškinti, siekiant atskleisti galimą koreliaciją tarp IRJ kaitos ir nepageidaujamų RT reiškinių (spindulinių reakcijų) vystymosi eigos, numatoma tęsti tyrimus taikant modifikuotą G2 metodą IRJ nustatyti, įtraukiant daugiau vėžiu sergančių pacientų.

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