Ultra-high dose rate (FLASH) treatment: A novel radiotherapy modality (Review)

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Abstract. Ultra-high dose rate radiotherapy defined as FLASH radiotherapy is a potential technology to improve local tumor therapeutic gain ratio. It relies on linear accelerator capable of delivering large doses in a single microsecond pulse (>40 Gy/sec). This therapy would lead to sparing of normal tissue which has been termed the FLASH effect. As significant reduction of radiation-induced toxicity, a greater dose of FLASH radiotherapy could be administered in tumor region. Some evidences prove the relation between FLASH effect and oxygen. Yet, the underlying physicochemical and biological mechanism remain to be fully demonstrated. The current hypotheses that may explain the normal and tumor tissue different response were We summarized and the future direction of study and clinic implementation was proposed.

Contents

1. Introduction

Conventional radiation is an essential treatment prescribed to >50% of patients in developed countries (1). However, numerous tumors are insensitive to radiotherapy with limited doses. A novel delivery methodology is the ultra-high dose rate (FLASH), that refers to differential radiobiological responses compared with conventional dose rates (2). FLASH irradiation was established using 4-6 MeV electrons with an intra-pulse dose rate in the range of 10^6-10^7 Gy/sec-a time-averaged dose rate >40 Gy/sec (3). Radiation exposures at ultrahigh dose rates have been shown to control tumor tissues while sparing normal tissues (4). Proton, X-ray and ion irradiations maintain similar efficiency in this regard (5). Preclinical studies have shown that the lungs, skin, brain and abdomen suffer less damage after FLASH radiation (6,7). This phenomenon was treated as the FLASH effect. Oxygen measurement experiments indicated a decrease in oxygen during radiation (8). Several groups have reported radiation-induced transient oxygen depletion (9). This is a vital factor of how hypoxic tumors exhibit radio-resistance. Some researchers in the past have proposed the effect of radical-radical reactions (10). DNA destruction is caused by interactions with free radicals (11). However, the current hypothetical mechanisms extracted from indirect evidence are neither clear nor comprehensive. In the present review, the reported theories of the effect of FLASH are summarized and the effect of different levels of oxygen and the mechanisms of oxygen conditions are discussed.

2. Advantages of FLASH radiotherapy

Radiation-induced side effects that affect the tumor control remain an important dose-limiting factor. Irradiation-induced damage ranges from ulceration to lymphedema, fibrosis and necrosis (12,13). Previous studies have pointed towards the role of elevated expression of TGF-β levels on irradiated regions in triggering the process of fibrosis (14). Other studies have implicated waves of inflammation following persistent radiation-induced normal tissue toxicity (15). The secretion of inflammatory cytokines such as TNF-α and IL-6 ιs activated as a radio-response (16). These therapeutic disadvantages, including acute and chronic toxicities, restrict the radiation dose. The aim of innovations in radiation therapy is to optimize the biological efficacy and tolerance. FLASH-radiotherapy (FLASH-RT) is defined as ultra-high dose rate (≥40 Gy/sec) technology that spares normal tissue while retaining tumor therapeutic responses (17). This reduction in normal tissue toxicity has also been observed in pre-clinical studies.

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Buonanno *et al* (18) observed that the DNA damage foci were significantly reduced when protons were delivered at the highest dose rate (1,000 Gy/sec). The survival curves of both the normal and affected cells were slightly influenced by the FLASH dose rates.

A prominent difference between FLASH-RT and conventional radiation is the exposure time. FLASH-RT can deliver electrons in only some microsec, whereas conventional radiation requires a few min for completion. The original study rediscovered a linear accelerator, that could emit gamma radiation at certain intervals and generate sub-millisecond pulses to elicit less genomic instability (19). Normal smooth muscle and epithelial cells were protected from acute radiation-induced apoptosis. Later, these effects were observed for a variety of beam currents, including those of electrons, photons, protons and carbon ions.

Some experiments have used 4-20 MeV electron beams in high-energy electron FLASH-RT (20). To penetrate into deep anatomical locations, a very high-energy electron radiotherapy of 50-250 MeV has been proposed to deliver required doses (21). Its accuracy could be only minimally affected by tissue heterogeneities (22). Further studies were implemented using simple 3D-conformal electron beams portals to enable a quasi-instantaneous fraction delivery, while achieving dosimetric conformity (23).

The photon beam is converted from the energy fluence of the electrons. A recent study compared the toxicity of microbeam radiation therapy and high-dose-rate synchrotron broad-beam radiation (24). The dose of valley microbeam radiation therapy dose was correlated with acute toxicity. Cerebral and brainstem histology did not change, and neurological toxicities, such as loss of balance and ataxia were unremarkable. To date, conventional radiation uses only 15 MeV photon beams, whereas ultra-high dose rates of photons are severely limited by low transformation efficiency and various other negative phenomena (25). The capability of photon linear accelerators to produce a sufficient number of electrons for energy transfer must be improved.

The purpose of proton beam treatment is to pass radiation through deep-seated tumors (26). It has been previously shown that the pencil-beam scanning technique can produce instantaneous high-dose-rate proton beam (27). The local dose was accumulated by 3-dimensional position matrix beam spots. A maximum dose rate of 1,000 Gy/sec was investigated in lung fibroblast cells (18). When compared with the ow dose rate, the overall cell survival did not differ at high dose rates. DNA damage was significantly lower at 1,000 Gy/sec. To obtain continuous current output, an isochronous cyclotron was employed at hundreds of nano-amperes (28). Fixed cyclotron energy output, facility-specific parameters, and beam delivery hardware are crucial and limiting factors in fulfilling the establishment of FLASH dose rate.

Carbon ion-beam therapy based on synchrotrons, is an increasing modality (29). The Heidelberg Ion-Beam Therapy Central examined the FLASH effect of carbon ions with a dose of 7.5 Gy for a 280 MeV/u beam (30). Technologically, it is difficult to convert carbon-ions into ultra-high dose rates. The synchrotrons for the carbon-ions were obtained using a slow-beam extraction method. The FLASH conditions (>40 Gy) which required <1 sec were hard to achieve. Hence, the optimum extraction methods such as quadruple-resonance extraction and betatron core extraction should be further improved.

3. The effect of FLASH radiation

The FLASH effect exploited the biological differences between tumors and healthy tissues. The term, first explained by the Favaudon and Vozenin groups denotes the phenotype of sparing normal tissue along with tumor cytotoxicity (31). The advantages of FLASH radiation over conventional therapeutic modalities, such as conventional dose rate irradiation, standard-proton radiotherapy, have been extended in different experiments. The evidences of the FLASH effect from various regions are listed in Table I.

Modern FLASH radiation combined with three-dimensional planning has resulted in accurate treatment. The feasibility focus, dose and dose rate distribution are recognized as crucial factors (23). Desirable multi-portal very high energy electron therapy could maximize the FLASH effect. To optimize the tissue-receiving dose rate distribution in depth, the Gao *et al* (38) developed a simultaneous dose and dose rate optimization method. This helped provide a useful paradigm for understanding the availability and quantification of treatment plans.

4. Oxygen condition influences the FLASH effect

A study by Chaudhary *et al* (39) using a portable chamber revealed enhanced DNA damage under hypoxic conditions. In a normoxic environment, hypoxia-inducible factor (HIF)-1α was stained in the cytoplasm while upon oxygen-deprived conditions, it was intensely observed in the nucleus. HIF-1 expression is related with maintaining tissue oxygenation within the normal scope. This phenomenon was extended to overcome hypoxic radioresistance. Najaf *et al* (40) elucidated that increased cell elimination under hypoxia resulted in solid tumor control because of tumor hypoxia and local oxygen depletion at high dose-rate. To test the effect of oxygen concentration, the cancer cells were exposed to normoxic, hypoxic (oxygen pressure of 1.6%) and anoxic conditions (oxygen pressure of 0%) (41). The surviving fraction of cells showed an apparent difference between the FLASH and conventional irradiation groups under hypoxic conditions. McKeown *et al* (42) found that tumor cells with the same intracellular oxygen concentrations as normal tissues have an increased tolerance to FLASH compared with conventional therapy. When oxygen level ranged from 0-20%, HIF-1α and HIF-1β expression was active in returning the tissue to its preferred oxygen metabolism.

5. Mechanism of oxygen reaction

The correlation between the FLASH effect and oxygen consumption was confirmed. Oxygen is a critical material that affects FLASH effect. Normal tissues under hypoxic conditions are resistant to ultrahigh dose-rate radiation (14). Tumors surrounded by oxidized normal tissue have minimal effects. However, the different biological mechanisms of FLASH have not been elucidated.

Table I. Responses of normal tissue (organized in order of targeted organs).

First author/s, year	Model (site)	Endpoint	Dose (Gy)	Dose rate (Gy/sec)	Radiation source	Conclusions	(Refs.)
Velalopoulou *et al*, 2021	Mice (leg)	Bone resorption, myofiber atrophy	30	69-124	Proton	Fewer severe toxicities in skin and mesenchymal tissue	(32)
Levy *et al*, 2020	Mice (abdomen)	Gastrointestinal function	14	216	Electron	Single high dose radiation spred gut function and epithelial integrity	(33)
Alaghband *et al*, 2020	Mice (brain)	The objects in updated location	8	$4.4x10^6$	Electron	FLASH irradiation preserves neurons, minimize microgliosis	(34)
Wright *et al*, 2021	Mice (lung)	Fibrosis, bronchial damage	30	$1.4x10^4$	Proton	The grade and extent of fibrotic lesions, inflammation, bronchial and vascular damage were remarkably low in FLASH therapy	(35)
Konradsson *et al*, 2021	Canine (limb)	Skin adverse event	15-35	400-500	Electron	Adverse events were mild in electron FLASH radiotherapy	(36)
Zhu *et al*, 2022	Mice (abdomen)	Immune cells	10	125	X-rays	FLASH radiotherapy reduces radiation-induced damage in the spleen and intestine	(37)

Conventional irradiation technology destroys microvessel structure and contributes to tissue fibrosis. In addition, if the vessels are closed, tumor cells are protected from radiation therapy or chemotherapy (43). At the same dose, using FLASH therapy can overcome both radioresistance and control hypoxic tumors. Irradiation disrupts the metabolism of agents such as superoxide, hydrogen peroxide and organic hydroperoxides (44). When compared with normal tissues, tumor tissues have higher levels of peroxidized compounds. These compounds would destroy the metabolic processes in cells.

On the one hand, experiments verified that water in cells breaks down and produces reactive oxygen species (ROS). The tissue response was related to the level of ROS which attack DNA with hydroxyl radicals. A coupled ROS was constructed (45). The simulation revealed oxygen depletion and a high ROS concentration in FLASH. ROS develop into more stable chemical complexes and cause less DNA damage in normal tissues. Tumors in hypoxic regions were less productive and the radiation eliminated more resistant cells.

On the other hand, the ionizing current causes radiolysis of the water molecules. Electrons and H^+ are reactive for a short duration of radiation and interact with O_2 (8). The reaction products remained stable after 10^{-6} sec of abrupt and transient increase in H^+-mediated pH values in the cellular environment. This response remained constant for the acid spike constantly (46). The biochemistry of O_2 in biological systems is highly sensitive to the pH. O_2 was simultaneously ionized with electrons' loss. Normal tissue had lower peroxidized compounds and labile iron content than the tumor tissue. Highly reacting atomic oxygen combines with H from the DNA to generate •OH radical (45). These •OH radicals capture ROS and reduce their population under ultra-high dose rate radiotherapy conditions. Although no evidence has confirmed the relationship between the decrease degree in ROS and the radiation dose rate, the reduction in ROS was more significant when the dose rate was <100 Gy/sec (47).

Molecular oxygen is also used as a radiation sensitizer. Oxidative metabolism contributes to the effects of ionizing radiation during exposure (48). The radiolytic formation of O_2 in the tumor region converts hypoxic conditions into oxygenated conditions. This effect leads to an improvement in cytotoxicity and causes tumor cell death (49). In normal tissues, oxygen generation at this dose level not occur owing to the plateau region of the depth-dose distribution of ions.

6. Conclusions

FLASH therapy is a novel and potential technology. The use of ultra-high dose rate overcomes the limitation of radiation-induced toxicity because of normal tissue sparing. FLASH therapy might refine the radiation dose of cancer. In FLASH effect process, oxygen level plays a key role in ultra-high dose rate radiation. The ultra-high dose rate radiation could deplete oxygen and induce protective hypoxic environment in the normal tissue. These mechanism theories suggested sparing of normal tissue and remaining tumor control following FLASH radiotherapy. The biological changes resulting in the FLASH effect are significantly correlated with oxygen concentration and ROS.

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Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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