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Review Article

Interactions between synchrotron radiation X-ray and biological tissues –– theoretical and clinical significance

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Abstract: Synchrotron radiation (SR) X-ray has great potential for its applications in both diagnosis and treatment of diseases, due to its characteristic properties including coherence, collimation, monochromaticity, and exceptional brightness. Great advances have been made regarding potential medical applications of SR X-ray in recent years, particularly with the development of the third generation of SR light sources. However, multiple studies have also suggested damaging effects of SR X-ray on biological samples ranging from protein crystals to cells and biological tissues. It has become increasingly important to conduct comprehensive studies on two closely related topics regarding SR X-ray in medical applications: The safety issues regarding the medical applications of SR X-ray and the fundamental mechanisms underlying the interactions between SR X-ray and biological tissues. In this article, we attempted to provide an overview of the literatures regarding these two increasingly significant topics. We also proposed our hypothesis that there are significant differences between the biological tissue-damaging mechanisms of SR X-ray and those of normal X-ray, due to the characteristic properties of SR X-ray such as high dose rate. Future studies are warranted to test this hypothesis, which may profoundly improve our understanding regarding the fundamental mechanisms underlying the interactions between light and matter. These studies would also constitute an essential basis for establishing the safety standard for the medical applications of SR X-ray.

Keywords: Synchrotron radiation, X-ray, tissue damage, ROS, radiation safety

Introduction

Synchrotron radiation (SR) X-ray is coherent, collimated, monochromatic and intensely bright. These characteristic properties of SR enable the light to have rapidly increasing applications for basic biomedical research, and to have great potential for medical applications [1, 2]. A number of studies have indicated that SR-based medical imaging could produce images with significantly greater resolutions compared to traditional medical imaging approaches [1, 3]. SR computed tomography (SRCT) has shown superior performance that is close to the theoretical limits of precision and accuracy [4, 5]. SR X-ray-based fluorescence imaging has also shown exceptional capacity in studying the metal distributions in biological tissues [6, 7, 8]. There have been a number of studies suggesting that SR-based microbeam radiation therapy (MRT) may become a novel approach for treating such cancers as gliomas [9-11].

With the development of the third generation SR light sources, such as European SR Facility (ESRF), Shanghai SR Facility (SSRF), and the National Synchrotron Light Source (NSLS), the potential of SR in biomedical applications has been greatly enhanced. For examples, for protein crystallography studies, the intensely bright X-ray from these light sources would enable the use of extremely small crystals, thus resolving the problems in studying the macromolecules that are difficult to obtain in quantity and to crystallize; and based on the exceptional spatial resolution of the third generation SR and the high sensitivity to the iron element of SR X-ray fluorescence, a molecular probe for imaging tumor angiogenesis has been developed [12].

While SR X-ray holds great potential for its applications in medicine and biology, multiple ques-

tions underlying the interactions between SR X-ray and biological tissues remain unanswered. The pivotal question in this topic is: Is there any major differences between the SR X-ray-biological tissue interactions and the conventional X-ray-biological tissue interactions? It is of importance to search for the answers to this question due to several reasons: First, since SR X-ray is the light of multiple characteristic properties and biological tissues belong to matter in a most complex form, novel information resulting from these studies would improve our understanding regarding the interactions between light and matter; second, it is of crucial importance to solidly define the safety doses of SR X-ray for potential medical applications; and third, studies on the interactions between SR X-ray and biological tissues may elucidate the mechanisms underlying the effects of conventional X-ray on biological tissues. For example, because SR X-ray and conventional X-ray are monochromatic and polychromatic, respectively, we may understand the contributions of X-ray at each individual energy level to tissue damage for the first time by applying the monochromatic SR X-ray.

In this article, we attempted to provide an overview of the literatures regarding the studies on the interactions between SR X-ray and biological tissues as well as the safety of SR X-ray in potential medical applications. Based on this overview, valuable directions for future studies might be identified to systemically elucidate the interactions between SR X-ray and biological tissues.

Studies on the interactions between SR X-ray and biological tissues and on biological safety of SR X-ray: Current status

There are four major types of the interactions between X-ray and matter: Absorption, transmission, coherent scattering and Compton scattering. Photoelectric absorption is the process in which all of the X-ray energy is taken up by the atom, which is then transferred to an electron leading to ejection of the electron from the atom. In scattering, the X-ray interacts with the atom, which then continues with an altered direction. There are two types of scattering, i.e., coherent scattering and Compton scattering. In coherent scattering, the X-ray energy is rapidly re-radiated in an arbitrary direction without energy transfer to the atom. In contrast, X-ray continues in altered directions with a reduced energy in Compton scattering, with a portion of the X-ray's energy consumed to free an outer shell electron.

X-ray is one of the major forms of ionizing radiation. Ionizing radiation impairs biological tissues through direct interactions or indirect interactions [13]: In direct interactions, macromolecules are directly attacked by ionizing irradiation [14]; and in indirect interactions, tissues are impaired indirectly by ionization though radiation-induced generation of ROS and reactive nitrogen species (RNS) [15]. It was reported that clinically relevant doses of ionizing radiation can produce significant nuclear DNA damage in cells: Each Gray (Gy) of radiation can cause approximately 3000 damaged bases, 1000 single-strand breaks, and 40 double-strand breaks [14]. X-ray can generate ROS, mainly hydroxyl radicals, by directly inducing radiolysis of water [16]. ROS can produce tissue damage by multiple mechanisms, including disrupting calcium homeostasis, inducing cell apoptosis and necrosis pathways, activating poly(ADP-ribose) polymerase-1, and impairing mitochondria [17, 18].

The radiation from medical imaging can lead to significant health concerns. It has been suggested that a lifetime attributable excess cancer risk of approximately 0.08% can be caused by a single whole-body CT examination on a healthy 45-year-old adult; while this CT examination taken by a person at age 75 would produce an excess risk of cancer mortality of 1.9% [19]. Therefore, in light of the great potential of SR X-ray-based medical imaging, it is required to conduct systemic studies on the safety of this promising technology, so as to enable SR X-ray to be used in medical imaging under clinical settings. However, there have been only few studies regarding the safety of SR X-ray for medical applications, nearly all of which have studied the safety of MRT for the brain [20]. Because it has been established that such organs as gonads and bone marrow, instead of the brain, are the organs that are most vulnerable to radiation, it is essential to study the effects of SR X-ray on these radiosensitive organs.

Multiple studies have determined the effects of SR X-ray on biological macromolecules: In protein crystallography studies, radiation damage to protein crystals is a key problem for obtaining

accurate structures [21]. Studies that apply SR X-ray to study protein structures have shown that SR X-ray can produce specific structural and chemical damage to crystalline proteins [21]. It was also found that the SR X-ray-induced damage on protein crystals are energy-independent [22].

There are several studies that determine the biological safety of MRT. MRT uses arrays of X-ray microbeams of 50-600 keV, which has the beam width of approximately 25-100 microm separated by 100-400 microm microplanar spaces [20]. MRT has been shown to increase the survival of glioma-bearing rats when applied separately or jointly with drugs [23]. In addition to treatment of traditional radiosurgery targets such as cancer, microbeams may allow non-invasive treatment of such diseases as epilepsy and mental illnesses [24]. It was reported recently that, for a unidirectional irradiation and a centrally located tumor, the largest peak and valley doses achievable in the tumor are 55 Gy and 2.6 Gy, respectively. The corresponding maximum valley doses received by healthy brain, the skin, and bone are 7 Gy, 4 Gy, and 14 Gy, respectively, which are within the doses of tolerance (5% probability of complication within 5 years) [25]. It was also found that there was edema in the normal brain exposed to both crossfired arrays about 6 weeks after irradiation, which was associated with changes in blood vessel morphology and an overexpression of vascular endothelial growth factor [2]. In contrast, there was no loss of vascular endothelia or alterations of vascular parameters and vessel morphology in brain regions exposed to only one of the two arrays [2]. A recent study has indicated that on cellular levels, normal and tumor tissues show differential responses after MRT: MRT-treated normal skin appeared to undergo a coordinated repair process, while the tumor zones that were irradiated by peak or valley doses of MRT beams were indistinguishable due to extensive cell migration between the zones [26].

A recent study determined the effects of microbeams on the artery wall. It was found that the artery remained patent [27]. However, microplanar beams appear to dose-dependently induced atrophic and fibrotic changes of the narrow arterial smooth muscle cell layer segments [27]. A latest study also determined the bystander effects of irradiation of SR microbeams on cells. The cells were irradiated either by targeting the nuclei with 10 microm x 10 microm 5.35 keV X-ray beams or by irradiating the whole cells with 50 microm x 50 microm 5.35 keV X-ray beams [28]. When only the nuclei were irradiated, a parabolic enhancement of bystander cell death was observed in a dose-dependent manner in the low-dose region around 1 Gy. In contrast, the surviving fraction of bystander cells decreased monotonically when whole cells were irradiated [28]. Their study has also suggested that nitric oxide - a free radical, mediates the bystander effect [28]. In summary, while the normal brain tissues appear to be resistant to microbeam-induced toxicity, a number of microbeam-induced biological damage on molecular, cellular and tissue levels have been found. It is warranted to further improve the current MRT technology so as to minimize the pathological side effects of the microbeams.

It is noteworthy that the results from the safety studies on MRT can not be extrapolated to answer the questions regarding the safety of SR X-ray-based medical imaging, due to the major differences between MRT and SR X-ray-based medical imaging. These major differences include: First, the microbeams have beam width in the scale of micrometer. In contrast, the beam width of regular SR X-ray beams is in the scales of millimeter or centimeter. Therefore, the affected regions in regular SR X-rays are much greater than that of microbeams, which may significantly affect the extent of tissue damage; and second, the energy of the photons used in MRT is in the range of hundreds of keV to MeV. Thus, the tissues have very low absorbance of the X-ray, and the high-energy photons mainly damage the tissues by such mechanisms as Compton effects. In contrast, the energy levels of the photons used in SR X-ray-based imaging are significantly lower than that of MRT, leading to photoelectric absorption of a significant portion of light by targeted tissues.

SR X-ray and normal X-ray may impair biological tissues by different mechanisms

A study reported that the dose for a mammography using conventional X-ray was 1.28 mGy, while it was 0.86 mGy for phase contrast SR mammography to have an image of higher quality [29]. However, there have been no sufficient studies to support the proposition that com-

pared to conventional X-ray, smaller doses of SR X-ray are needed to produce a medical image with similar or higher image quality. Future studies that test the validity of this proposition are critically needed.

It is noteworthy that safety studies on the medical applications of SR X-ray could still be of great significance due to the following hypothesis: There may be significant differences between the mechanisms of the biological tissue-damaging mechanisms of SR X-ray and those of normal X-ray. Due to these potential differences, SR X-ray at same doses might produce significantly different levels of tissue damage compared with conventional X-ray. This hypothesis is raised on the basis of one of the characteristic properties of SR X-ray – the high dose rate of SR X-ray. There is evidence indicating that at the same doses, the X-ray at high dose rate can induce differential biological changes on molecular levels compared to X-ray at low dose rate. For example, it has been reported that the X-ray of low dose-rate selectively increases the expression of Mmp2 and Mmp15, while the X-ray of high dose-rate selectively increases the expression of Mmp9 and Mmp11 [30]. Two lines of evidence have suggested that the high dose rate of SR X-ray might produce differences between the biological tissue-damaging mechanisms of SR X-ray and that of normal X-ray:

1) High dose rate X-ray may lead to greater tissue damage: Several studies using either cell cultures or protein crystals have found that higher dose rate of X-ray irradiation led to significantly greater cell death or protein damage [31, 32]. For example, Metting et al found that, for the same total dose of 9 Gy, a dose rate of 0.0031 Gy/min resulted in 14% cell survival of CHO-K1 cell line, while a dose rate of 1 Gy/min resulted in only 1.2% survival [31]. It was also found that the lowest dose-rate burns led to less specific radiation damage in the samples. In contrast, higher dose rates of X-ray led to significant signs of structural alterations of protein crystals [33]. The study by Ishizaki [34] suggested important mechanisms underlying the differential effects of high dose-rate X-ray and low dose-rate X-ray on cells: Their study found that high dose rate of X-ray led to significant increases in the levels of phosphorylated histone H2AX –– an index of double-strand DNA damage, while there was virtually no increase in phosphorylated H2AX in low dose rate. A plausible explanation to this observation is that cells are capable of repairing the DNA damage caused by low dose rate of X-ray irradiation, while the rapid accumulation of DNA damage caused by increasing dose rate may overwhelm the DNA repair capacity of the cells, leading to significant accumulation of DNA damage.

2) It has been indicated that high dose-rate X-ray could lead to decreased chain reactions of lipid peroxidation due to the following mechanisms [35]: The probability of recombination of lipid peroxides increases with the square of the concentrations of lipid peroxides. The X-ray of high dose rate can induce generation of significantly higher concentrations of lipid peroxides, which would produce significantly higher levels of recombination of lipid peroxides thus leading to decreased chain reactions of lipid peroxidation.

In addition, it remains possible that the characteristic properties of SR X-ray such as coherence, collimation, and monochromaticity might also lead to differences between the mechanisms of the biological tissue-damaging mechanisms of SR X-ray and those of normal X-ray. Future studies are warranted to investigate these possibilities, which may provide novel information that could greatly improve our understanding regarding the interactions between SR X-ray and biological tissues.

Summary and future perspectives

Increasing evidence has suggested that SR X-ray-based imaging could become an exceptionally powerful medical imaging approach [4]. One of the key prerequisites for SR X-ray to be used in medicine is our comprehensive understanding on the biological effects, particularly damaging effects, of SR X-ray. While there have been multiple studies on the safety of MRT in treating brain tumors, the results from these studies can not be extrapolated to assess the safe doses of SR X-ray-based medical imaging. Therefore, systemic studies on the safety of SR X-ray in medical imaging should be one of the critical research directions for applications of SR-based medical imaging in clinical settings. The following two scientific questions on this topic may be of particular significance: 1) Are there any major differences between the SR X-ray-biological tissue interactions and the con-

ventional X-ray-biological tissue interactions? 2) What are the key mechanisms that determine the vulnerability of biological tissues to SR X-ray? With the increases in both the number of researchers who study applications of SR X-ray in biomedicine and the number of the third generation SR light sources, it is expected that many new pieces of information on these questions would be generated. These studies may profoundly improve our understanding regarding the interactions between light and matter, which would lead to establishment of the safety standard for medical applications of SR X-ray.

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