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REVIEW

Irradiation affects the structural, cellular and molecular components of jawbones

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ABSTRACT

Purpose: Emerging evidence shows that changes in the bone and its microenvironment following radiotherapy are associated with either an inhibition or a state of low bone formation. Ionizing radiation is damaging to the jawbone as it increases the complication rate due to the development of hypovascular, hypocellular, and hypoxic tissue. This review summarizes and correlates the current knowledge on the effects of irradiation on the bone with an emphasis on jawbone, as these have been a less extensively studied area.

Conclusions: The stringent regulation of bone formation and bone resorption can be influenced by radiation, causing detrimental effects at structural, cellular, vascular, and molecular levels. It is also associated with a high risk of damage to surrounding healthy tissues and an increased risk of fracture. Technological advances and research on animal models as well as a few human bone tissue studies have provided novel insights into the ways in which bone can be affected by high, low and sublethal dose of radiation. The influence of radiation on bone metabolism, cellular properties, vascularity, collagen, and other factors like inflammation, reactive oxygen species are discussed.

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Introduction

Since 1895 and the discovery of X-rays, radiation has had important applications in the treatment and diagnosis of many diseases (Bernier et al. 2004). Radiation therapy (RT) is used in the therapeutic, adjuvant, and palliative therapy for a wide range of malignant conditions. Treatment of jawbone, either maxilla or mandible, and the rehabilitation following resection and reconstruction, is a difficult problem faced by maxillofacial surgeons. RT in the oro-facial region is often accompanied by inevitable damage to the healthy surrounding hard and soft tissues. Thus, the overall morbidity due to head and neck cancer (HNC) therapy is compounded by a failure to reinstate and rebuild the damge which leads to impaired oral functioning and decreased quality of life (Dholam and Gurav 2012).

Bone remodeling is a dynamic process; it occurs throughout the lifetime of an organism in a coordinated and tightly regulated manner in order to maintain a functional skeletal system. The bone remodeling process involves two opposing processes – bone resorption and bone formation (Mello et al. 2018) executed by three distinct cell types present in bone cells; osteoclasts, osteoblasts, and osteocytes. The physiological process of bone remodeling is based on the interactions not only between these cells but also multiple molecular agents including hormones, growth factors, and cytokines (Feng and McDonald 2011). Bone turnover is necessary to allow new bone to replace the existing bone, ensuring the adaptation of the newly formed bone to its microenvironment (Misch et al. 2001). Exposure to radiation causes a deterioration of the quantity and quality of bone by interfering with bone remodeling/turnover activity which ultimately impacts on the bone's microstructure (Costa and Reagan 2019).

It is known that radiotherapy adversely affects the jawbone; this may result in bone loss and osteoradionecrosis (ORN) increasing the risk of fracture (Fornetti et al. 2018; Elliott et al. 2011). Furthermore, radiotherapy can lead to changes in the oral flora (salivary quantity and composition), mucositis, loss of the sense of taste, trismus, fibroatrophy of bone, and surrounding soft tissue. The initial bone changes caused by irradiation alter the bone remodeling process. Later radiation damage to bone and vessels is thought to result in the characteristic signs of hypoxia, hypocellularity, and hypovascular tissue, the persistence of which leads to a chronic non-healing wound, thereby increasing the risk of ORN (Jereczek-Fossa and Orecchia 2002; Tanaka et al. 2013). There is a wide range of incidence reported although it does seem that the incidence of ORN has declined in recent years. Due to the recent

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improvements in radiation techniques, it was reported that the incidences of ORN have decreased from 20% down to 4–8% (Kubota et al. 2021). The microscopic changes evident in bone after irradiation are empty osteocytic lacunae in cortical and trabecular bone, changes in cellularity and vascularity of the periosteum, fibrosis, decreased numbers of vessels in interstitial spaces, obliterative endarteritis, loss of hematopoiesis, narrowing or obliteration of blood vessels, with a decreased proliferation of new bone (Rohrer et al. 1979). Overall, it does seem that exposure to radiation causes a suppression of bone formation and a state of low bone turnover.

Advances in radiation therapy have resulted in a reduction of adverse side effects, but there is still a concern about its systemic and localized effects on the adjacent tissues and cells including vessels and bone (Costa and Reagan 2019). Most of the studies on the irradiation damage of the jawbone, especially the mandible, have been performed in animal models, which are a valuable tool because one can apply standardized protocols to study the effects of radiation and potential treatments. However, studies on human material are less common since the available data has to be derived either from the tumor resection specimens or the excised jawbone from the bony lesions. This review examines how jawbone reacts to irradiation and updates the impact of radiation at structural, cellular, and molecular levels in terms of both the bone and the microenvironment around bone and to discover novel ways enhance bone health during and after irradiation.

Physiological aspects of bone

Bone remodeling or bone turnover is regulated by the coordinated action of the osteocyte, osteoblast, and osteoclast (Kajarabille et al. 2013). The osteoblast, a mononuclear cell, is of mesenchymal origin and its major function is bone matrix formation and mineralization. Osteoblasts are uniformly present on the surface of the bone matrix where they secrete an organic matrix (collagen and proteoglycans) and an inorganic matrix (hydroxyapatite crystals via vesicles) (Donaubauer et al. 2020). The osteoclast, a large multinucleated cell, is formed by the fusion of monocytes. Mature osteoclasts are positioned in the Howship lacunae on the bone's surface where they degrade the inorganic mineralized component of the bone (Donaubauer et al. 2020; De Souza Faloni et al. 2011). For this purpose, they secrete enzymes like acid phosphatase, cathepsins, and matrix metalloproteinases, to carry out the process of bone resorption (Parikka et al. 2001; Henriksen et al. 2011). The major cell type in bone is the osteocyte, which are astrocyte-shaped cells that reside within the bone matrix (Tate et al. 2004). These cells can detect mechanical strain through their extensions that allow cell-cell communication i.e. between osteocyte-osteocyte and osteocyte-osteoblast through the lacuno-canalicular system and stimulate signaling pathways for osteoclastogenesis and osteoblastogenesis (Lanyon 1993; Poulsen et al. 2007).

The RANKL pathway is an important component of bone physiology; this consists of three proteins, RANKL (Receptor-activated nuclear kappa-β ligand), its receptor RANK, and osteoprotegerin (OPG, a decoy receptor). The RANKL pathway controls osteoclast development (Wada et al. 2006; Leibbrandt and Penninger 2008). RANKL and OPG can be produced by a variety of cells including osteoblasts. RANKL and OPG can bind to RANK, which is located on osteoclast progenitors and osteoclasts. RANKL-RANK binding initiates osteoclastogenesis whereas binding of RANKL-OPG prevents the osteoclastogenesis induced by RANKL-RANK. Hence, a balance between this triad of proteins is a major factor controlling osteoclast numbers (Quinn and Gillespie 2005; Poulsen et al. 2007). Osteocytes and osteoblasts can release prostaglandin E2 (PGE2) in response to mechanical signals, and this prostanoid promotes osteoclastogenesis through RANKL-RANK binding and by suppressing OPG expression. The PGE2 released from osteocytes can also activate the Wnt signaling pathway to stimulate osteoblastogenesis. The coordinated action of bone cells, proteins, and lipids modulate bone remodeling through formation and resorption (Figure 1).

Irradiation parameters and biological loss

Radiation is a form of energy that spreads as an electromagnetic wave or particle radiation and can be either non-ionizing or ionizing (Donaubauer et al. 2020). Non-ionizing radiation energy is not sufficient to eject electrons from an atom, whereas ionizing radiation energy can remove electrons from atoms, and consequently, it possesses the potential to break molecular bonds and this can cause biological damage to DNA, RNA, or cellular organelles (Willey et al. 2011). Ionizing radiation can occur in the form of alpha or gamma radiation or X-rays, which have different physical origins, properties, and different biological effects (Donaubauer et al. 2020). Although cells have a remarkable ability to repair radiation damage, the indirect radiation-induced on biological molecules and the irreversible damage to cells lead to the inevitable death of various cell types and thus to further damage. Irradiated tissue, mainly bone, presents a different degree of sensitivity. The mineralized component of bone is not considered to be radiosensitive whereas the non-mineralized component (cells and supporting tissues) is radiosensitive (Dholam and Gurav 2012; Zhai et al. 2019). In contrast, another report claims that the high calcium content in bone, which absorbs 30–40% more irradiation than the surrounding tissues, making it a tissue sensitive to radiation-induced damage (Curi et al. 2016).

The degree of biological damage can vary depending on the type of radiation (alpha, beta, or gamma, neutrons, or heavy ions) and the dose of radiation. One of the important events in radiotherapy is the choice of dosage and the fractionation mode (Wernle et al. 2010; Hui et al. 2013). A fractionated radiation dosage has often been used in different animal models with different doses and fractions, but these tend to be much lower than the therapeutic dose administered to humans (Willey et al. 2008). Some authors also

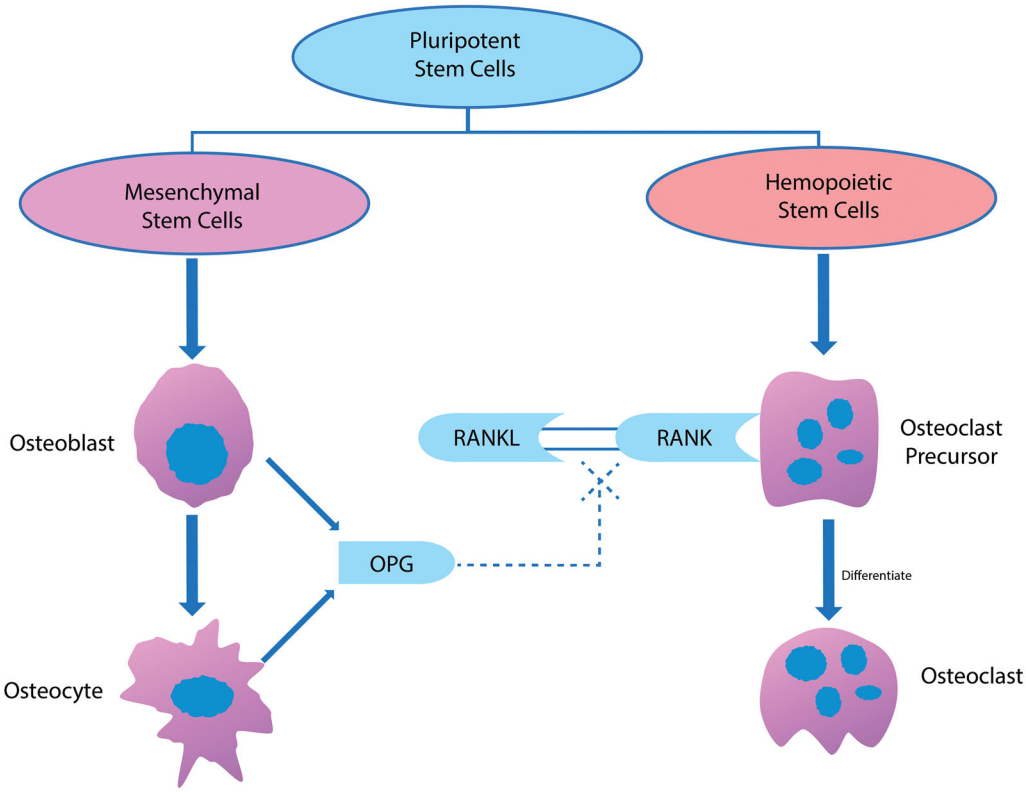

Figure 1. Physiological interplay of RANKL (Receptor-activated nuclear kappa-β ligand) RANK (RANKL receptor) and OPG (Osteoprotegerin) on bone cells.

proposed that it is optimal to use 2 Gy fractionated irradiation several times or a single large (20 Gy) dose administration or 5 Gy large dose fractionated radiation four times, in animal models (Xu et al. 2017; Oest et al. 2018). Therefore, if the fractionation is increased then the total cumulated dose must be increased to obtain the same biological effect. Brasseur et al. (2006) suggested that a fractioned irradiation of 10 daily fractions of 4.3 Gy in the canine jawbone would be equivalent to 60 Gy delivered over 6 weeks with 5 sessions a week in humans. Kim et al (2001) considered a 15 Gy single dose in the animal bone to be biologically equivalent to 25 sessions of 1.8 Gy administered 5 times per week over 5 weeks in humans. The same dose can be obtained with 26 Gy administered in 2 fractions of 6.5 Gy per week over 2 weeks and this is related to the cell repopulation (Weinlaender et al. 2006). Fractionated radiation is used because with low dose radiation, normal tissue repairs sublethal damage better than tumor tissue. According to Asikainen et al. (1998), bone remodeling can occur only when irradiation is fractioned because this leads to a higher tissue tolerance. Concerning the jawbone periapical area, a dosimetric analysis demonstrated that the area of higher radiation dose (>66–70.2 Gy) is more likely to develop inflammation of the jawbone due to bacterial infection from decayed teeth. A shift of the oral microflora or a change in the compositions of root canal flora might result in periapical inflammation after radiotherapy (Hommez et al. 2012). Dosimetric parameters were reviewed and found to represent a significantly increased risk of ORN in patients receiving a high dose (>60–75 Gy) of radiation to the jaw. A dosimetric comparison of the irradiated jaws revealed significant differences between patients with and without ORN of the jaw (Kubota et al. 2021).

The fractionated radiation augments the slight survival advantage that normal tissue has over tumor tissue when irradiated with small exposure. The fractionation radiation effect consists of four independent processes that are thought to occur between fractions and favor the survival of normal tissues over cancers i.e. (1) repair of sublethal cellular damage, (2) reoxygenation of the hypoxic portions of tumors, (3) redistribution of tumor cells from radioresistant (late S phase) into radiosensitive (G2-M phase) portions of cell cycle and, (4) migration of normal cells into irradiated areas to repopulate these normal tissues with healthy cells. However, the risk of complications following fractionated radiation depends on several factors including fraction size, total radiation dose, and the site that is irradiated (Connell and Hellman 2009). The few relevant studies performed by various authors in animal and human jawbone are summarized in Table 1.

Irradiation effect on bone microarchitecture

Irradiation causes deteriorations in bone's properties such as demineralization of bone, thinning of cortical bone, and loss of trabecular connections (Ergun and Howland 1980; Mitchell and Logan 1998; Hopewell 2003). Micro-CT (computed tomography) is a commonly used method to measure the microarchitecture of bone such as bone volume, bone surface, cortical thickness, and trabecular connectivity. In

Table 1. Irradiation induced changes in human and animal jawbone.

Authors	Site	Subjects	Radiation dose	Radiation effects
Human studies				
Kubota et al. (2021)	Mandible	Human	69.96 Gy (50–75)	Development of osteoradionecrosis of jawbone (7.5%) with cortical erosion, loss of spongiosa trabeculation, pathological fracture.
Dekker et al. (2020)	Mandible	Human	66 Gy	Impaired bone turnover and deterioration in bone microarchitecture.
Zhuang and Zhou (2020)	Jawbone – Gingiva	Human cell culture	0–16 Gy	Irradiation activated fibroblasts increased miR-23a expression in human bone mesenchymal stem cells (hBMSCs)
Dekker et al. (2018)	Mandible	Human	54Gy-70 Gy	Effects micro-vascularity of jawbone with decreased percentage of small vessels, vessel density, vascular function.
Curi et al. (2016)	Mandible	Human	5942 cGy	Hypocellularity, hypovascularity, increase of fat in bone marrow, fibrosis in bone tissue.
Singh et al. (2015)	Mandible	Human	60 Gy	Loss of lamellar structure, deformation in collagen structure, decrease in matrix and mineral contents, changes in vasculature and osteocytes.
Hommez et al. (2012)	Maxilla and Mandible	Human	66.0–70.2 Gy	With a high radiation dose, inflammation of the jawbone develops due to periapical bacterial infection
Animal Studies				
Heinonen et al. (2018)	Mandible	Beagle dogs	40 Gy	Changes in the lacuno-canalicular system, decrease in canalicular connections between osteocytes and periosteum.
Poort et al. (2017)	Mandible	Minipigs	6.5, 9.7, 11.8 Gy	Architectural bone changes damage vascularization, decreased bone formation.
Damek-Poprawa et al. (2013)	Mandible, Tibia	Rats	50 Gy	Hypocellularity, hypoxia, and oxidative stress were higher in the irradiated mandible than in tibia but the extent of vascular damage was similar.
Weinlaender et al. (2006)	Mandible	Dogs	5000 cGy	Increased resorption of bone and retarded bone formation
Brasseur et al. (2006)	Mandible	Dogs	43 Gy	Compatible osseointegration with higher porosity and a less homogenous mineral distribution.
Asikainen et al. (1998)	Mandible	Dogs	40, 50, 60 Gy	A low dose causes bone remodeling, a high dose causes bone resorption and rapid bone loss
Aitasalo (1986)	Mandible	Rats	15, 20, 30, 35, 40 Gy	Cell damage, inflammatory change, vascular, enzymatic, and cellular responses to periosteum and bone.
Rohrer et al. (1979)	Mandible	Monkeys	4,500 rads (cobalt 60)	Periosteum exhibited a loss of cellularity, loss of vascularity, loss of osteoid formation. Marrow showed signs of fibrosis, proliferation of new bone, and obliterative endarteritis.

maxilla and mandible bone, micro-CT revealed changes in periodontal ligament thickness, and specific osseous sites in both the cortical and trabecular compartments (Alikhani et al. 2012; Shimizu et al. 2013; Xu et al. 2013; Dai et al. 2014; Chatterjee et al. 2017). A recent histomorphometry and micro-CT study on irradiated human mandibular bone detected a dramatic impairment in bone turnover and a deterioration in bone microarchitecture (Dekker et al. 2020). Tooth loss and progressive periodontal attachment loss of teeth within the area of high-radiation dose have been observed in some studies (Epstein et al. 1998; Marques and Dib 2004).

It was found that the anatomical areas of jawbone surrounding molars are difficult to quantify, as their trabecular bone is less homogenous in comparison to long bones (Faot et al. 2015). Hence, to address such a challenge, it is useful to assess the size of the volume of interest (VOI), as the trabecular structure and number are site-dependent. A recent study (Kubota et al. 2021) showed that the predominant location of ORN was in the body of the mandible followed by the angle or ramus of the mandible and the least in the symphyseal or parasymphyseal areas. The condylar and coronoid processes were not involved in ORN. In the posterior part of the mandible, it was thought that radiation will affect the endosteal blood flow due to an intimal proliferation of the inferior alveolar artery and soft tissue fibrosis that may diminish periosteal blood flow and result in decreased vascularity to the cortical and medullary bone. After irradiation, the anterior part of the mandible seems to have a better remodeling potential, possibly due to the supplemental vascular supply from the facial artery (de Oliveira et al. 2012; Nishimura et al. 1998). Bone loss and structural damage (a rapid decline in bone mineral density, a decrease in the bone volume fraction, a reduction in several bone trabeculae, a decrease in the trabecular junction points, and an increase in the degree of trabecular separation) have been identified in the directly irradiated and the adjacent unexposed areas, as early signs of radiation-induced bone damage (Zhai et al. 2019). The remodeling of cortical and trabecular bone is decreased by radiation; these changes

appear to be a reversible phenomenon secondary to the quantitative and qualitative alterations occurring in the bony cellular microenvironment (Jegoux et al. 2010).

Cellular changes in irradiated bone

Irradiation with high precision to the tumor tissue leads to death or senescence of rapidly dividing malignant cells but it inevitably also damages the adjacent healthy cells. In bone, irradiation causes the deleterious effect on osteoblasts, osteoclast, and osteocytes within the bone microenvironment (Ergun and Howland 1980; Mitchell and Logan 1998; Hopewell 2003). The in vitro and in vivo data indicate that high-dose irradiation can diminish bone formation by reducing the proliferation and differentiation of osteoblasts along with cell cycle arrest, a decrease in collagen production, and enhanced sensitivity to apoptotic agents (Dudziak et al. 2000; Gal et al. 2000; Szymczyk et al. 2004; Sakurai et al. 2007). Kondo et al. (2009) demonstrated that irradiation damaged osteoblast precursors and the oxidative stress triggered by irradiation contributed to early damage to osteoprogenitors. Osteoblasts are essential for the proper differentiation of osteoclast and the maintenance of functional bone hemostasis, damage to these processes due to irradiation will indirectly affect the biology of the osteoclast (Cao et al. 2011). Low dosage exposure can have a stimulatory effect on osteoblasts. Studies conducted in osteoblast cell cultures have shown that irradiation promotes a DNA double-strand break and cell cycle arrest that leads to transforming growth factor (TGFβ) expression, which in turn modulates osteoblast differentiation and mineralization (Lau et al. 2010). Xu et al. (2012) demonstrated in vitro differentiation and mineralization of murine osteoblasts and no alteration in osteoblast proliferation, at low doses of radiation. An osteoblastic cell culture study, which applied periapical irradiation, showed that a reduction in ROS production after low dose radiation did not change cell viability, cellular apoptosis, or proliferation. However, an impaired osteoblastic proliferation with increased ROS production was observed with a high dose of radiation without any changes in cell viability or cellular apoptosis (Pramojanee et al. 2012).

The difference in the origin, development, bone turnover, extracellular matrix, and osteoclastic nature makes the jawbone a structure very different from the long bones. Normally, the bone mesenchymal stem cells in the jawbone have a higher osteogenic potential and are more responsive to osteogenic factors (Omi and Mishina 2020). An irradiated comparative jawbone and long bone animal model study revealed accelerated pathological cellular changes and mechanistic changes of increased hypoxia, hypocellularity, and oxidative stress in the jawbone. The changes in the long bone remained subclinical much longer than those in the jawbone but enhanced adipocyte infiltration was observed in long bones when compared to the jawbone (Damek-Poprawa et al. 2013).

Osteoclasts are the key players in radiation-induced bone loss. In its early stages, irradiation promotes (1) a differentiation of osteoclast precursors, (2) an increase in the osteoclast number, and 3 an upregulation of the expression of osteoclast marker genes in marrow tissues. In the late stages, irradiation has a positive impact on bone hemostasis as osteoclasts are inhibited in their viability and function (Alwood et al. 2015; Donaubauer et al. 2020). Many authors have proposed that osteoclastogenesis occurs at a high dose through radiation-initiated inflammation associated with the secretion of pro-inflammatory cytokines such as tumor necrosis factor (TNF) and interleukins (IL), which directly stimulate the expression of RANKL (Barcellos-Hoff 1998; Lorimore et al. 2001; Zhai et al. 2019). At low doses of radiation, the viability and function of osteoclasts were inhibited. A periapical dental irradiation study conducted by Pramojanee et al. (2012) demonstrated that a low dose of radiation was able to reduce oxidative stress. Since oxidative stress can be promptly generated by irradiation, the reduction of cellular oxidative stress by a low dose of radiation might inhibit osteoclastogenesis. In contrast, an in vitro study indicated that osteoclasts responded in a dual way to irradiation. First, osteoclastogenesis was enhanced at a low dose of radiation, and second, osteoclast differentiation was decreased due to the high radiosensitivity of these cells (Zhang et al. 2018).

Decreased osteocyte density in the cortical bone was reported following the exposure to high doses of radiation. In several animal models, osteocytes seem to be relatively radioresistant and appear to remain viable for several months after a single dose of radiation although they are not fully functional (Jacobsson et al. 1985; Nishiyama et al. 1992; Rabelo et al. 2010; Chandra et al. 2014, 2015). This indicates that the increase in osteoclastic resorption activity and the decrease in osteoblastic activity is either due to the decrease in osteoprogenitor cell populations or to altered feedback and signaling between bone cells due to the decreased numbers of osteocyte in the lacuno-canalicular network. Osteocyte death is eventually followed by matrix resorption. The bone lacuno-canalicular system has also been studied in long bones and jawbone as it provides an ideal environment for the transfer of exogenous and endogenous signaling pathways leading to the release of secondary messengers, transcription factors, and the gene expression required for normal bone hemostasis (Rohrer et al. 1979; Tate et al. 2004; Willey et al. 2008; Domazetovic et al. 2017). Heinonen et al. (2018) conducted an experimental study in a canine model to assess alveolar bone remodeling after tooth extraction in an irradiated mandible. It was observed that radiation disrupted the osteocytes and their dendritic processes beneath the periosteum and lowered the connectivity between osteocytes of the surrounding bone tissue and the bone surface, which resulted in inadequate nutrition of the irradiated bone. Overall irradiation effects on bone and bone cells are presented in Table 2.

A high dose of radiation exposure causes a bone marrow failure and eventually death whereas a sub-lethal dose evokes bone marrow suppression. Hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) produce osteoclasts and osteoblasts respectively, both critical to the bone

Table 2. Effect of radiation on bone and bone cells.

Radiation dose	Osteoclast	Osteoblast	Osteocyte	Overall effect on bone health
High dose (>2Gy/fraction)	Increase in number Increase in surface area Increase in bone resorption Increase in differentiation	Decrease in number Damage to osteoblast precursors DNA strand breaks and cell cycle arrest Decrease in bone matrix production	Decrease in number Increased apoptosis rate of osteocytes Change in osteocyte lacunae Increase in the number of osteocytic lacunae Irradiation-induced bone loss	Decrease in bone mineralization Prone to fracture Imbalances in bone hemostasis
Low dose (<2Gy/fraction)	Decrease in differentiation Decrease in bone resorption Decrease in viability Decrease in function	Increase in differentiation Increase in bone mineralization Increase in proliferation Increase in activity	Morphological changes Affects viability	Promotes bone healing Disturbs the balances involved in bone hemostasis

Donaubauer et al. 2020; Willey et al. 2011; Ergun and Howland 1980; Hopewell 2003; Alwood et al. 2015; Pramojanee et al. 2012; Chandra et al. 2014; Chandra et al. 2015; Willey et al. 2008; Rohrer et al. 1979; Domazetovic et al. 2017.

turnover process. It has been shown that the deficit of the stem cell population in bone marrow following radiation can compromise bone architecture and structural integrity (Bonyadi et al. 2003; Green and Rubin 2014). In vivo studies demonstrated that both high and low irradiation doses deteriorate trabecular bone and there is a progressive conversion of hematopoietic marrow into adipocyte-rich areas. These changes represented a shift in differentiation favoring adipogenesis over osteogenesis in response to alterations in the radiation-induced bone marrow microenvironment (Green et al. 2012; Bolan et al. 2013). The activation of fibroblasts has a prominent role in the progression of ORN of the jaw through the remodeling of extracellular matrix as well as the secretion of growth factors and cytokines. The exosomes released from the human gingival fibroblasts during radiation therapy inhibit osteogenic differentiation of human bone MSC, which could serve as an alternative modality for the prevention and treatment of ORN of the jawbone (Zhuang and Zhou 2020).

Irradiation and changes in the bone's vasculature

Bone hemostasis is also influenced by the vascular system. The bone's vasculature transports nutrients and oxygen and enables the communication between endothelial cells and osteoclasts (Villars et al. 2002). After irradiation, the bone reacts by displaying either circulatory or metabolic effects (King et al. 1979). First, the circulatory hyperemic effect dominates followed by significant metabolic changes later; this concept has been supported by other authors who have studied the jawbone (Aitasalo 1986; Jegoux et al. 2010).

Radiation affecting the vasculature of bone induces changes in the Haversian canal and sclerosis of the connective tissue in the marrow, a phenomenon which was first reported by Ewing in 1926 (Ewing 1926). Investigations with long bone, and some also in jawbone, have shown that irradiation has many detrimental effects on the vasculature supplying osteogenic cells (1) reducing the blood flow by damaging vascular endothelial cells, (2) evoking a constriction and obliteration of blood vessels in the bone-forming area, and (3) diminishing the perfusion of osteogenic cells (Curi et al. 2007; Poort et al. 2017, Huang et al. 2018; Soares et al. 2020). In vitro studies revealed a reduced expression of vascular endothelial growth factor (VEGF), essential for blood vessel development, with doses less than 8 Gy. The decreased VEGF production appears to be due to the incapability of decreased number of cells to produce this growth factor (Pepper 1997; Dudziak et al. 2000).

The swelling and vacuolization of endothelial cells within the vascular channels of the osteons represent the early loss of vascularization followed by the formation of sclerotic connective tissue within the marrow cavity (Ergun and Howland 1980; Hopewell 2003; Willey et al. 2011). The consequences of late injuries are fibrosis in the subintima layer, hyaline-like material in the tunica media layer of blood vessels, and finally constriction of the vessel's lumen. Jawbones are especially at risk for vascular injury owing to the paucity of their vasculature and their superficial location (Williams and Davies 2006). Radiation-induced hypervascularity with the reduction in vessel volume fraction and vessel number as a late response in an irradiated human mandible. Small vessels are affected more by radiation than larger vessels contributing to the hypovascular situation in the post-radiation phase (Dekker et al. 2018).

Irradiation affects bone collagen

With the negative effect of radiation on the tissues, it is possible that it affects the collagen arrangement in bone and decreases the mineralization process. Some investigators have claimed that the plastic deformation of the bone is increased with radiation by the release of free radicals, the degradation of collagen molecules, and disturbances in the fibrillary sliding mechanism. This prevents the development of the proper molecular arrangement for the normal biomineralization process (Nguyen et al. 2007; Barth et al. 2011). A Raman microspectroscopic study of the human mandible after irradiation showed compositional changes in collagen structure and collagen cross-linking. Irradiation resulted in alterations of lamellar structure and deformation in collagen structure (Singh et al. 2015).

Radiation-induced structural changes in collagen revealed disturbances in parallel packing, a reduction in their diameter, breaks in collagen molecules, and abrupt intermolecular cross-links. Maslennikova et al. (2015) validated two structural changes in collagen within 24 hours following radiation (2 Gy) (1) disorganization of molecular structure and (2) formation of cross-links that improved the stability of

collagen. The process of reorganization and stabilization occurred randomly throughout the bulk of the tissue following 10 Gy radiation (Bailey et al. 1964; Fathima et al. 2004; Balli et al. 2009; Limirio et al. 2019). The fragmentation of collagen after irradiation was the primary mechanism causing a reduction in mechanical properties in bones (Pendleton et al. 2019). These changes and the disruption in the cross-linked profile of collagen showed more immature cross-links. This increase could disrupt the mature cross-link integrity or modify the interaction or binding between the organic matrix and hydroxyapatite mineral, leading to a premature mechanical failure of the bone (Barth et al. 2011; Bala et al. 2012; Abraham et al. 2016; Limirio et al. 2019).

Type I collagen synthesis is necessary for the osteoid formation and remodeling of bone. It has been demonstrated transforming growth factor (TGFβ) exerts a stimulatory effect on collagen production in bone (Chang et al. 1998). The activation of the type I collagen gene by TGFβ resulted in increased levels of type I collagen, probably following multiple transcriptional, posttranscriptional, and posttranslational events (Centrella et al. 1991). Irradiated osteoblasts have shown altered TGFβ receptor expression (Centrella et al. 1995). Aquino (2012) reported both direct and indirect effects of radiation on collagen's properties. As a direct effect, he showed that the scission of the polypeptide chain predominated when collagen was irradiated in a dry state (in the absence of water or oxygen), and this in turn, significantly increased collagen solubility in vitro and elevated the resorption of the bone matrix in vivo. A crosslinking reaction appeared as an indirect effect during the irradiation of collagen in the presence of water, probably due to the action of highly reactive, short-lived hydroxyl radicals, resulting from water radiolysis.

Radiation metabolomics of bone

Metabolomics is a promising discipline to detect and quantify tiny molecules (<1kDa) that are downstream of genomic, transcriptomic, and proteomic processes (Menon et al. 2016). Radiation can affect the biomolecules present in cells either by direct damage or indirect damage. The direct damage refers to breaks in specific bonds whereas indirect damage causes water radiolysis and the formation of reactive oxygen species (ROS) under stressful conditions which can damage DNA and other cellular components. The damage to the protein content of the cell is mainly caused by ROS. Similarly, lipid components of the cell membrane are highly vulnerable to radiation damage resulting in increased membrane permeability, changes in ion gradients, radical generation, changes in signaling, and ultimately cell death (Vit and Rosselli 2003; Fritz and Petersen 2011).

An in vitro study using metabolites as radiation markers detected significant differences in many metabolites like glutathione, NAD^+ (nicotinamide adenine dinucleotide) and spermine, because of an oxidative response and DNA damage (Patterson et al. 2008). Flow cytometric and mass spectrometry techniques revealed up-regulation of the levels of arginine, glutamine, creatine, and proline (Lee and Britz-McKibbin 2010). An impaired metabolic activity of immune T cells following radiation led to a reduction in glucose uptake, glycolysis, and energy metabolism, which is required for effective activation of cells (Li et al. 2015). Various other animal studies targeted metabolites by checking urinary markers, serum markers, and DNA damage biomarkers (Tyburski et al. 2008; Liu et al. 2013; Manna et al. 2013). Salivary metabolomic studies during radiation therapy for head and neck cancers have revealed changes in the metabolomic profile and salivary gene markers; these preliminary findings will need further validation before this approach can be used as an accurate biodosimetric tool. The radiation response in cultured head and neck squamous cell carcinoma cell lines demonstrated that the radioresistant cells had changed their metabolism to control the redox status, DNA repair as well as DNA methylation (Jonsson et al. 2019).

Irradiation induces inflammation

Inflammation is another response to radiation exposure characterized by enhanced expression of pro-inflammatory transcription factors and cytokines, such as TNF and ILs (Dudziak et al. 2000). High radiation doses augmented the osteoclastic activity driven by pro-inflammatory cytokines (Huang et al. 2018) whereas low doses exerted anti-inflammatory effects by reducing osteoclastic activity and functionality (Deloch et al. 2018). Oxidative stress is known to cause an imbalance between osteoblast and osteoclast activity and it also activates the differentiation of pre-osteoclast to osteoclast to accelerate bone resorption. By leading to processes favoring osteoclastogenesis, ROS induce apoptosis of osteoblast and osteocytes in the bone matrix through various signaling pathways such as mitogen-activated protein kinase (MAPKs), extracellular signal-regulated kinases (ERK1/2), c-Jun-N terminal kinase (JNK), and p38 MAPK (Henriksen et al. 2009; Marathe et al. 2012; Fontani et al. 2015). ERK can activate NF-kB (Nuclear factor kappa B ligand) to induce antiapoptosis and JNK and p38 promote apoptosis. Hence, an imbalance is formed between anti- and proapoptotic responses induced by inflammatory cytokines at different doses of radiation.

Pro-inflammatory cytokines such as TNF- α and ILs, regulate the activity of osteoclasts, as has been revealed in various inflammatory diseases that affect the bone (Smith et al. 2005; Walsh et al. 2005). Apart from directly affecting osteoclasts, these cytokines also induce the production of osteoblasts (Kimble et al. 1996). Hence, it has been suggested that radiation initiates an inflammatory cascade within bone and the environment around bone, either accelerating bone resorption or inhibiting bone formation. In one study, the elevated serum TNF-α levels and the subsequent increase in osteoclast precursor cell pool in the peripheral blood mononuclear cell population are correlated with the bone resorption (Ritchlin et al. 2003). RANKL plays an important role in controlling osteoclastogenesis and has major pathophysiological importance in the destruction of bone (Figure 2).

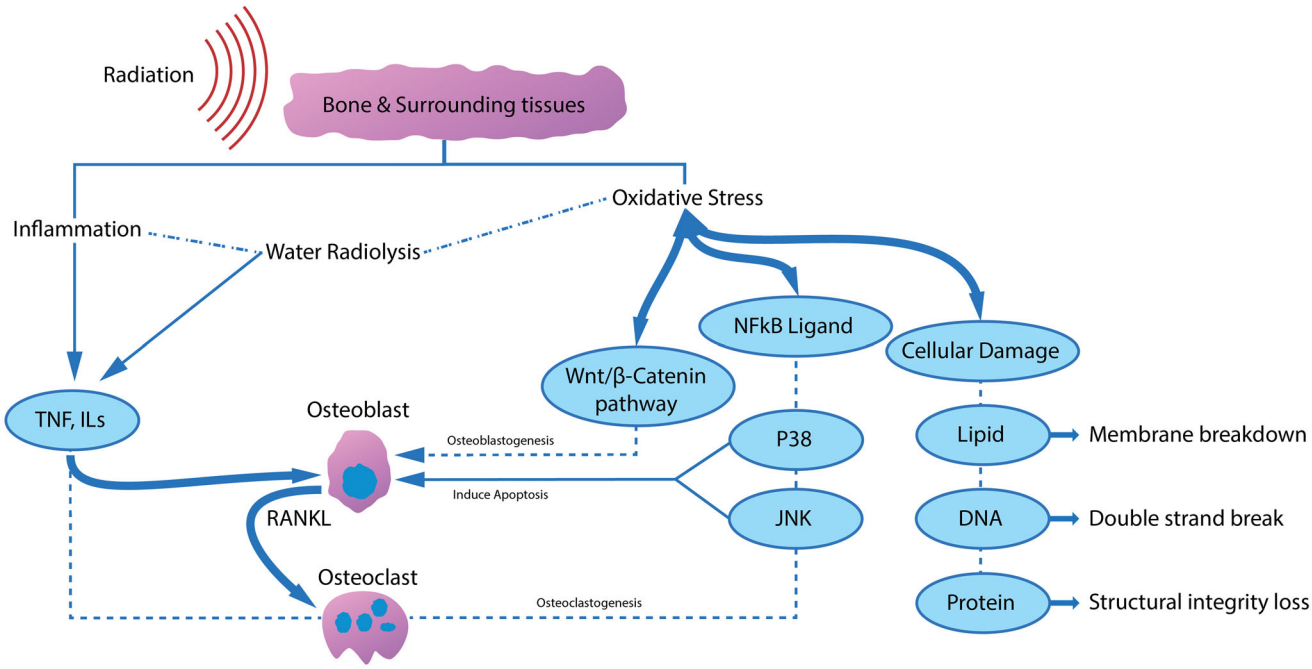

Figure 2. Radiation-induced inflammation and oxidative stress changes in bone cells and cellular structure. [TNF (Tumor necrosis factor), ILs (Interleukins), RANKL (Receptor-activated nuclear kappa-β ligand), Wnt (Wingless related Integration site), NFkB (Nuclear factor kappa B ligand), JNK (c-Jun-N terminal kinase), P38 MAPK (mitogen-activated protein kinase)].

The side effects of radiotherapy with higher radiation dose result in inflammation of the jawbone due to radiation caries attributable to a shift in oral flora. Bacterial invasion into the jawbone evokes periradicular inflammation of the jawbone with increased osteoclastic activity causing localized bone destruction. It was reported that in irradiated bone that the reaction of the bone tissue to the bacterial products had become altered toward the formation of free radicals, endothelial dysfunction, inflammation, microvascular thrombosis, fibrosis and remodeling, and finally bone and tissue necrosis (Hommez et al. 2012).

Conclusion

In this review, the effects of radiation on the structure, cells, and biomolecules of bone are discussed with an emphasis on the jawbone. Most of the studies on radiation damage to the bone have been performed in animal models and only a few studies have examined human jawbone tissues. A single fractionated dose of 2 Gy seems to be a critical threshold; above that, the effects of radiation are more detrimental. A high dose of radiation causes a rapid bone loss due to the increased osteoclastic activity and decreased bone formation whereas a low and fractionated dose of radiation has caused a downregulation of osteoclast function and a stimulation of osteoblasts. Radiation exposure will lead to a drastic decrease in bone marrow cells which are needed to prevent the destruction of the bone's architectural integrity. Inflammation and oxidative stress occur in the response to radiation that stimulates many molecular mechanisms such as triggering an early activation of osteoclast-mediated bone loss. Radiation also affects the surrounding soft tissues causing vascular damage and fibrotic changes which may further contribute to bone alterations or disturbances in bone healing processes. Hence, the complex interaction of altered cellular, vascular, and metabolic components of bone and in the adjacent tissues following radiotherapy, promotes structural changes in the bone. As a deleterious consequence of radiation, most jaw osteoradionecrosis occurs within months or even years after the treatment. The intensity of the reaction is often variable and may depend on various parameters related to the form of radiation delivery. Therefore if we are to devise effective countermeasures to radiation-induced bone loss, it is mandatory that we clarify the precise molecular and cellular drivers behind this phenomenon. We propose further research directions should utilize human jaw tissues in order to understand the early and late dose-dependent changes evoked by radiation as ways to enhance bone health after radiotherapy.

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