Structural Changes in Cell Membranes After Ionizing Electromagnetic Field Exposure

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Invited Paper

***Abstract*—Ionizing radiation damages chemical structures in biological tissues mainly through the generation of reactive oxygen intermediates (ROI). Consequences on the cell's transcriptional mechanisms are widely investigated but cannot account for the rapid lethal effects in victims of accidental high-dose radiation exposure. ROI-mediated structural damage of cell membranes through lipid peroxidation can lead to increased ion permeability followed by the loss of ion-homeostasis and cell death. We monitored radiation-induced changes in the morphology of isolated skeletal muscle cells. During the first two hours after gamma irradiation large blebs were formed on the surface of muscle cells. These blebs are the initiation point for a successive total collapse of the cells over a time period of about 15 min similar to Ca^{2+}-influx mediated cell contraction. Acute radiation-induced death as well as possible relations to lipid peroxidation, cell membrane permeability increases, irradiation-induced apoptosis are discussed. Our ongoing research on ionizing electromagnetic field effects on cell membranes and the induced structural phenomena are closely linked to pertinent membrane damage by some nonionizing electromagnetic fields and ionized gases.**

***Index Terms*—Blebbing, ionizing radiation, lipid peroxidation, membrane structure.**

I. Introduction

RADIATING high-energy electromagnetic pulses (>124 eV, >30 PHz) produce reactive oxygen intermediates (ROI) in tissues through ionization of water. Associated with the water ionization is an energy dissipation typically in the range of about 33 eV, enough to break a strong chemical bond. Thus ionizing radiation primarily damages chemical structures in biological tissue by a direct mechanism as well as through follow-up chemical reactions of the free-radical ions [1]. Past research on ionizing radiation damage in living cells has mainly focused on two areas: 1) DNA damage resulting in transcriptional and replicative dysfunction in the cells resulting in cell death and 2) the characterization of lipid peroxidation in the cell membrane and, if rapid enough associated membrane permeability changes. Our research focuses on the structural changes induced in the cell membranes and the subsequent loss of barrier function as a consequence of lipid peroxidation by high-energy irradiation.

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The described structural changes in the cell membrane and discussed repair methods are not limited to ionizing irradiation effects but are also applicable to nonionizing electromagnetic fields, e.g., electroporation by large electric field pulses, and membrane permeabilization through microwave heating. In addition, it might stimulate the discussion on the mechanisms of sterilization via plasma bursts and ionized gases.

II. Radiation Injury and Dose Response

The acute sequelae of high-energy radiation exposure have been generally described [2], [3]. They vary in severity depending on several parameters including the type of radiation, the total dose, dose rate, the energy of the radiation, and the exposure field size. By now it is commonly accepted, that the 50% lethal dose after 60 days ($LD_{50/60}$) of a human total body irradiation is about 4 Gy (1 Gray (Gy) = 100 Rad = 1 Joule absorbed per kilogram) [4]. In whole body doses of 3–8 Gy, the marrow stem cells are damaged and death secondary to infection from neutropenia, impairment of immune function, and bleeding thrombocytopenia can occur in ~30 days. Therapy for most patients involves guarding against overwhelming infection and fluid status maintenance. Although bone marrow transplant is considered a treatment for the hematopoietic syndrome, only one case has been reported after the Chernobyl accident in which the transplant saved a life [3]. In whole body doses of ~10 Gy, death can occur from depopulation of the epithelial lining of the GI tract, leading to ulceration, infection, hemorrhage, and massive fluid loss becoming fatal in 3–10 days. Therapy for these victims is only supportive in nature—such as maintaining fluid status. Much higher doses (of ~100 Gy) can result in death within minutes following severe nausea, vomiting, loss of coordination, respiratory distress, seizures, and coma. This is referred to as the neurological syndrome. Although the mechanisms are unknown, they may involve acute cell membrane permeabilization associated with direct neuronal damage or capillary damage leading to increased intercranial pressure.

III. Radiation Injury Mechanisms

The mechanisms of radiation-induced biomolecular damage involve the generation of ROI followed by their attack on proteins, lipids, and carbohydrates. As ROI are by-products in cel-

lular metabolism [5] the rate of radiation-induced ROI generation must exceed the normal ROI handling capacity of cells to cause significant cell damage.

Molecular alterations of the DNA/RNA affect cell replicative and transcriptional mechanisms [6]. Disruption of covalent bonds in nuclear DNA, directly through the energy deposition of the high-energy electromagnetic pulses in the tissue or through secondary ROI activity, leads to chromosomal damage and cell death during cell growth and repair [7]–[9]. Cell death caused by nucleic acid alteration is usually not immediate, rather delayed for days or weeks.

Since many acute sequelae after high dose irradiation appear too soon to result from interference in cell replication, rapid cell death within hours may be to some extent caused by membrane related mechanisms. These mechanisms are probably more relevant to the interphase death of postmitotic cells such as neurons, but have also been implicated in the rapid cell death of lymphocytes and acute vascular changes due to capillary epithelium dysfunction [10], [11]. Although other molecules are replaced faster than DNA/RNA and therefore changes in their structures are less important, all cells strongly depend on a properly functioning cell membrane. The main constituent of cell membranes are lipids which self-aggregate to a bilayer separating a cell's interior from its exterior. In particular, lipids that contain unsaturated fatty acid chains, as do most lipids in mammalian cell membranes, are extremely vulnerable to lipid peroxidation through ROI.

IV. Lipid Peroxidation

Lipid peroxidation has been shown to occur after irradiation of various membranes, mainly using pure lipid vesicles, erythrocyte ghosts, or intact erythrocytes [12], [13]. Peroxidation of the fatty acid residues of membrane phospholipids results in proton desaturation, which is followed by the alteration of bond angles in the carbon backbone. The excluded volume of peroxidized cell membrane fatty acids is thus increased, leading to perturbations in the structural organization of the lipid bilayer. In addition, the accumulation of polar products (lipid hydroperoxides, aldehydes, etc.) gives rise to an increase of the dielectric constant of the membrane interior which facilitates the movement of charged particles across the membrane [1]. Potential cross-linking of lipid molecules could influence the microviscosity of the membrane interior.

There is also evidence that irradiation induced alterations in membrane proteins involve reduction in lectin binding [14], damage to ionophores such as gramicidin A and nonactin [15], and decreased capping in lymphocytes [16]. Observed chemical alterations of irradiated RBC membranes include oxidation of –SH groups [17], [18] as well as lipid peroxidation [17], [19], [20].

The relative contribution of protein versus lipid damage to radiation-induced structural cell membrane changes remains to be defined and is perhaps cell-type specific.

V. Membrane Permeability

The most essential function of the cell membrane is to provide a diffusion barrier against ionic transport. The energy required for moving solvated ions across a planar phospholipid bilayer in an aqueous environment approaches 100 kT [21], indicating the strong impediment to passive ion-diffusion across the bilayer. Because most of the calories used by mammalian cells ultimately are invested in maintaining the ionic gradient across the cell membrane [22], the importance of the structural integrity of the lipid bilayer is clear.

Some investigators have postulated that membrane lipid peroxidation is the basic mechanism of radiation-induced changes in membrane permeability and that the increased ionic transport across the membrane could lead rapidly to cell death [12], [23]. The altered structure and associated changes in the function of the membrane disrupt the vital electrochemical control mechanisms of the membrane necessary for cell survival [1], [13]. When the membrane is permeabilized, mutual diffusion of ions across the membrane increases and ATP-fueled protein ionic pumps cannot keep pace. Under these circumstances, the metabolic energy of the cell will be quickly exhausted, and the cell will progress to biochemical arrest and eventually to necrosis. This cascade of events might constitute the principal mechanism of radiation necrosis in nongrowing cells.

Permeabilization of plasma membranes by ionizing radiation has been extensively documented as well. Lymphocytes exposed to as little as 0.3 Gy have been shown to lose nearly 50% of their cytoplasmic potassium ions [24]. Higher doses (0.5–100 Gy) produced alterations in Na^+-active membrane transport as well as altered amino acid transmembrane transport [25]. The majority of previous investigations focused on irradiation induced changes in ion transport across the membranes of erythrocytes [24], [26]–[28]. In addition, the role of irradiation in structural alterations of RBC membranes was monitored by changes in membrane fluidity and osmotic fragility [19], [29], [30].

It is worthwhile to point out that cell membrane permeabilization is a common cause for tissue necrosis in a variety of other physicochemical tissue injuries which often cause disability or death in young people. A few are: 1) ischemia-reperfusion injuries such as myocardial infarction, cerebrovascular stroke, cerebral palsy from difficult childbirth, and testicular torsion (ROI mediated as well), 2) electrical injuries (electric field mediated pore formation in the bilayer), and 3) burns and frost-bite (physicochemical effects disrupting the sensitive self-assembly balance of the lipids). These diseases represent the most common cause of death in young people.

VI. Experimental Results

If the mechanisms of radiation-induced interphase cell death are most likely related to the structural changes in the cell membranes, then these changes have to be characterized in greater detail. Cell surface "blebbing" is known as an early consequence of hypoxic and toxic injury to cells [31]. A rise in cytosolic free Ca^{2+} had been suggested as a stimulus for bleb formation [32], [33] and the final common pathway to irreversible cell damage [34], [35]. Lemasters *et al.* could not verify the correlation between cytosolic free Ca^{2+} and bleb formation but found a correlation between bleb formation and ATP depletion in a chemical hypoxia model on hepatocytes [36]. Since hypoxia is known to produce cell damage via ROI similar to ionizing radiation we

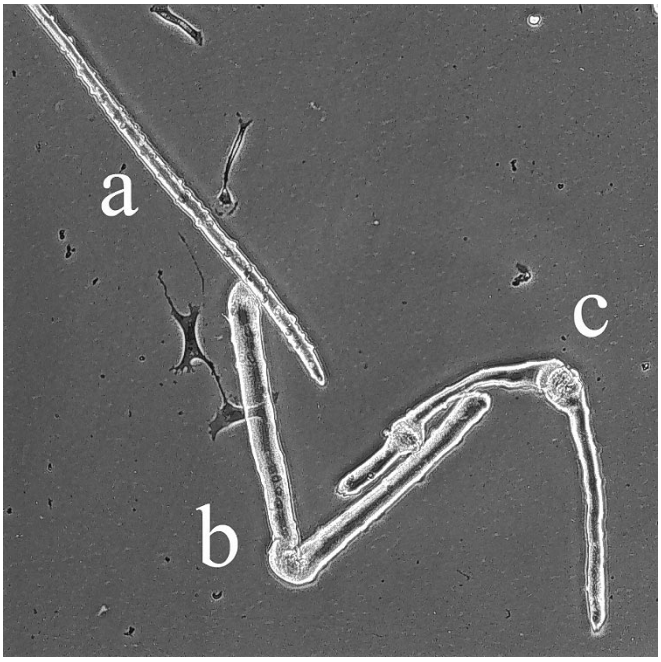

Fig. 1. Phase-contrast photomicrographs of skeletal muscle cells after irradiation with 40 Gy. Shown are (a) a healthy cell and (b),(c) two cells showing initial bleb formations causing the cells to kink and ultimately collapse. The onset of these events has been noted about 1 ± 0.5 h postirradiation (scale bar is 100 μm).

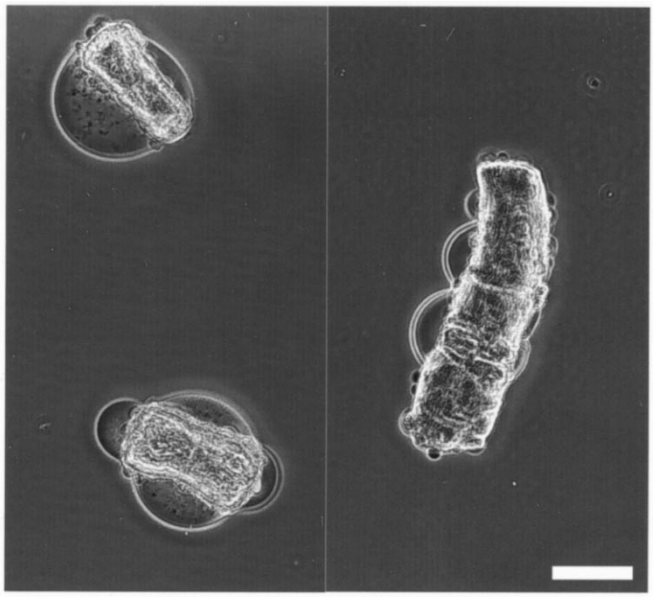

Fig. 2. Phase-contrast photomicrographs of skeletal muscle cells after irradiation with 40 Gy. Fully contracted, dead cells show large remaining blebs with presumably quite altered lipid composition. Time from the onset of the first bleb formation on a cell to its total collapse was about 15 min (scale bar is 50 μm).

tested the hypothesis that bleb formation is an intermediate step in radiation induced cell membrane changes as well.

We chose skeletal muscle cells as their size of several hundred microns in length and about 50 μm diameter deemed a very suitable object to microscopically observe irradiation-induced structural changes *in vitro.*

A. Material and Methods

The care and protocol for use of laboratory animals were in accord with all applicable guidelines and approved by the Institutional Animal Care and Use Committee. Individual postmitotic skeletal muscle cells were isolated from Sprague Dawley rat *flexor digitorum brevis* muscles. After allowing them to rest from the mechanical stress of the trituration for three days, cells in culture medium were transferred from the culture dish onto microscope cover slips, exposed to irradiation and immediately monitored by phase-contrast microphotography for 4 h. Irradiation was performed using a ^{60}Co source (1.24 MeV, 300 EHz, Gammacell 220, AECL, Chalk River, Ontario) at a level of 40 Gy and a dose rate of about 1.5 Gy/s. During the exposure and following observation period cell medium temperature was kept at 37 $^{\circ}$C through either a warming blanket or a heated microscope stage, respectively. Cell death of contracted cells was confirmed by vital fluorescent dye uptake.

B. Results

During the first 1–2 h postirradiation the majority of the exposed cells developed blebs. Generally, one bleb was formed initially on a cell and this bleb manifested the point from which the muscle cells started to collapse (see Fig. 1). The time period from the first appearance of a bleb until the full contraction (cell death) was about 15 min. Large blebs remained on the cell surfaces, frequently appearing as one large bubble surrounding the cell (see Fig. 2).

VII. Discussion

These results demonstrate that ionizing radiation damage induces bleb formation on the skeletal muscle cell surface in vitro similar to hypoxia-induced ROI-mediated cell membrane damage. In general, lipid peroxidation has been classified as one of many toxic processes related to oxidative stress. The question remains whether the increased influx of Ca^{2+} and other ions due to lipid-peroxidation needs to be potentiated by the accompanying destruction of transport proteins [37] or whether cell death can be caused by simply shifting the balance too far toward free ion influx. The morphological observations presented here certainly indicate that ROI-induced lipid peroxidation drastically changes the structure of muscle cell membranes. Other data from our laboratory demonstrate associated irradiation-induced significant membrane permeability increases and successive cell death in skeletal muscle cells *in vitro* [38]–[40]. Our results on irradiation-induced efflux increases from fluorescent dye-loaded skeletal muscle cells [39] are currently being confirmed using menadione in control experiments. Menadione (Vitamin K3) blocks cellular free radical buffering and induces lipid peroxidation through superoxide anion-free radical production.

Most recently, irradiation-induced apoptosis has been linked to a membrane-originated increase of the second messenger ceramide in the cytoplasma immediately after irradiation [41], [42]. Ashwell *et al.* [43] observed that oxygen-radicals produced by ionizing radiation damaged the phospholipid layer of the cell membrane and triggered apoptosis in B-lymphocytes. The mechanisms linking the irradiation-induced cell membrane damage to postmitotic cell death are not yet understood.

VIII. Surfactant Treatment

In addition to the experiments described above, we investigated the consequences of the morphological changes with respect to membrane permeability and subsequent cell death. We also tested the potential of surfactants to restore the cell membrane integrity of irradiated cells. The fluorescent dye leakage rates and survival percent of isolated primary rat skeletal muscle cells as well as hemoglobin leakage from isolated erythrocytes was determined after ionizing radiation exposure. In each of these models, a beneficial treatment effect of poloxamer surfactants from the class of tri-block copolymers on the experimental outcome was found [44]. Irradiation-induced fluorescent dye leakage from calcein-loaded skeletal muscle cells was totally prevented when Poloxamer 188 (P188) was added at a concentration of 0.1 mM at 10 min postirradiation (160 Gy) [45], [46]. Hemoglobin leakage from irradiated erythrocytes was significantly reduced when the buffer was supplemented with Poloxamine 1107 (P1107) at ten min postirradiation (600 Gy). The magnitude of the protective effect on the permeabilized membrane was surfactant concentration dependent in the range from 0.25 to 1.0 mM [47]. Irradiation-induced skeletal muscle cell necrosis, as determined by a double fluorescent staining assay, was almost completely prevented at 4 h and still significantly reduced at 18 h postirradiation (40 Gy) when P188 (1 mM) was present in the media compared to the nonsurfactant treated, irradiated control cells [48].

Further studies to better understand the mechanisms of surfactant-induced membrane sealing after radiopermeabilization have to be supported by an additional knowledge of the irradiation-induced structural changes in the membranes. Although in each of our radiopermeabilization experiments the membrane integrity could be improved or restored by a particular copolymer surfactant, it is not yet clear why P188 is more effective than P1107 in restoring membranes of skeletal muscle cells than those of erythrocytes and vice versa. Certainly, a mechanistic characterization of the structural changes leading to the presented postirradiation membrane morphology will promote the development of surfactant sealing therapies.

To fully understand the cascade of events leading from lipid peroxidation to cell death and the beneficial effect of surfactants various alternative mechanisms have to be investigated. Preliminary results from our lab suggest that media supplementation with high-energy metabolites and anti-oxidants in addition to P188 treatment further enhances the survival of postmitotic cells after irradiation. In addition, the effects of surfactant treatment postirradiation on calcium activated pathways, phosphorylation cascades and apoptotic markers need to be explored.

IX. Conclusion

Today, there is no generally established strategy to prolong human survival after exposure to radiation levels (>30 Gy) capable of inducing rapid death within hours. In the aftermath of the radiation accidents in Chernobyl, Ukraine, and Goiania, Brazil, concern about the medical risks of radiation and nuclear energy is substantial. With more and more countries developing nuclear power technologies, both for energy production and military purposes, the risk of accidental radiation exposure may increase. With the continuing development and growing use of radiation assisted diagnostic and therapeutic methods in health care, the probability of unintentional over-exposure increases as well, despite technical safe guards. In addition, although radiation injury is not as common today as thermal or electrical burns, in the future, space travel and habitation will bring humans into more frequent exposure to massive irradiation from turbulent solar activity.

Thus, ionizing irradiation-induced structural changes in cell membranes as presented here require to be studied more intensively to understand the underlying mechanisms of cell membrane-initiated cell death and to develop surfactant-based or other therapies to its prevention.

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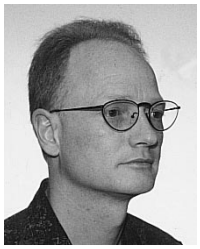

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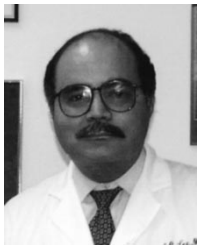

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Dr. Lee is a MacArthur Fellow (1981), and Searle Scholar (1985). He is Chairman of the Surgical Research Committee and the Director of the Electrical Injury Research Program. He has received more than 25 professional awards including being designated distinguished alumni of both engineering and medical alma maters. Research awards include the 1988 James Barrett Brown Award, American Association of Plastic Surgions, for "advancing knowledge in the field of plastic surgery"; the 1995 and 1996 American Burn Association Lindberg Awards for scientific contributions; the American Electrical Power Association Award in 1997 for "advancing electrical safety and health." He has served as President for both clinical and scientific professional societies, Associate Editor of two journals, and on the board of international corporations. He was recently elected to the American Institute of Medical and Biological Engineers.