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Neuronal Apoptosis After Radiation-Induced Brain Injury

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ABSTRACT

This study investigates the effect of gamma radiation on neuronal apoptosis in mice. In this study, 20 Balb/c mice were exposed to 6 Grays of gamma radiation, and tissues were collected at various interval for analysis. Histopathological examination showed significant necrosis, extensive apoptosis and cortical edema in tissues of brain. TUNEL assay recognized widespread fragmentation of DNA confirming apoptotic activity. Active apoptosis across all sample by Caspase 3 immunohistochemistry showed involvement of caspase 3 in the apoptotic pathway. These results suggested that gamma radiation induces severe neuronal damage through apoptosis, characterized by chromatin condensation, nuclear fragmentation and increased apoptotic cells. It is recommended for future studies to include human post-mortem tissues and employ advanced tool like electron microscopy and Annexin V expression for enhanced apoptosis detection. This study contributes to understanding the mechanism of radiation-induced neuronal apoptosis for developing targeted therapies to mitigate the radiation's detrimental effects on the brain.

Keywords: *Neuronal apoptosis, Radiation injury, gamma radiation, Caspase*

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1. INTRODUCTION

Apoptosis, also known as programmed cell death, is an essential cellular mechanism triggered by various factors, including oxidative stress and exposure to harmful compounds [1] [2]. This process aims to achieve and maintain homeostasis in cells and tissues. Apoptosis is characterized by several features, including cytoplasmic shrinkage, nuclear condensation, fragmentation, and the formation of apoptotic bodies, which are then ingested by macrophages [3] [4]. This process is crucial for maintaining healthy cells and tissues.

Apoptosis can occur through two different pathways, each activated by distinct mechanisms: the intrinsic pathway and the extrinsic pathway. Both pathways ultimately lead to the activation of caspases, resulting in the activation of p53 and subsequent cell death [3] [5].

The intrinsic pathway is regulated by mitochondria and is activated by internal signals, such as cellular stress or damage. This damage can be caused by oxidative stress, exposure to toxic substances, or DNA damage [1] [6]. Anti-apoptotic proteins like Bcl-xL, Bcl-2, and Mcl-1 are inhibited by pro-apoptotic proteins like BH3 when they become activated [2]. Mitochondrial outer membrane permeabilization, or MOMP, is created when pro-apoptotic proteins Bax and Bak oligomerize and create holes in the outer membrane of the mitochondria as a result of BH3's inhibition of these proteins [3] [4]. As a result, cytochrome c is released from the mitochondria and enters the cytosol, where it interacts to Apaf-1, the activating factor for apoptosis, to produce apoptosomes [4]. Pro-caspase 9 is activated by these apoptosomes and transforms into caspase 9 in its active form. After that, caspase 9 cleaves effector caspases such caspases 3 and 7, which cleaves a variety of cellular substrates and alters the morphology and biochemistry of the cells [1] [3].

The extrinsic pathway is regulated by death receptors, which are activated by external stimuli binding to receptors such as TRAIL and FasL. This binding leads to the formation of a signaling complex that activates caspases, eventually leading to apoptosis [7] [8]

Numerous factors, such as the definition of neurocognitive damage, tumor type, patient age, baseline neurocognitive functioning, disease progression, radiation dose, radiotherapy modality (WBRT, PBRT, stereotactic), use of multimodal treatments, including concurrent chemotherapy, all influence the incidence of cognitive impairments following brain radiotherapy, which varies greatly amongst studies. Therefore, it is still difficult to pinpoint the exact frequency of cognitive decline in the clinical setting, and it may be underestimated for a variety of reasons, such as the following: (1) long-term follow-up is necessary to detect changes that occur after treatment; (2) attrition bias favors individuals who function better cognitively and does not count those who function worse; and (3) there are few clinical studies that look at histologically confirmed cases of radiation-induced injury [9].

However, research has looked for risk variables that are linked to more severe cognitive damage following radiation therapy. These factors include advanced age [10], a history of smoking [11], WBRT as opposed to PBRT, [12] [13], a larger radiation dosage, and concurrent chemotherapy.

The traditional classification of radiation-induced brain injury is acute, early-delayed, and late-delayed, depending on how long it takes for adverse effects to appear after the initiation of radiation therapy. Acute and early-delayed injuries often subside within days to months of treatment, whereas late-delayed injuries happen at least six months after radiation therapy and are thought to be progressive and irreversible. Dextamethasone typically helps with acute injury, which is characterized by edema, headaches, and drowsiness. Acute injury is uncommon with current radiation therapy procedures.

Transient demyelination, somnolence, attention deficiencies, and short-term memory loss are the hallmarks of the early-delayed reaction. White matter necrosis, vascular anomalies, more chronic demyelination, gliosis, and long-lasting cognitive impairment are all associated with late-delayed damage.

One of the main dose-limiting side effects of radiation therapy is radiation-induced brain damage (RIBI) [14]. Rarely, ionizing radiation-induced brain injury (RIBI) can also happen, which can have a serious negative effect on patients' quality of life. The choices for clinical intervention are limited by the complex molecular foundations of RIBI and the poor understanding of related systems [15]. Although bevacizumab and steroids are recommended by certain guidelines [16, 17], there is a risk of adverse effects with these medications [18, 19, 20]. Thus, the need for creative approaches to manage or prevent RIBI is urgent.

Caspases are located in the cytoplasm as latent zymogens and play a major role in the apoptosis mechanism. They can be classified as either initiator caspases, activated by external death signals (like TRAIL or Fas), or effector caspases, triggered by internal stress (such as cellular dysfunction) [3] [5]. Caspase 8 can trigger caspase 9 through the secretion of cytochrome c and the formation of apoptosome complexes. This activation involves the cleavage of Bid to truncated Bid (t-Bid), promoting the release of mitochondrial cytochrome c and the activation of caspase 9 [1] [3]. Caspases are responsible for cleaving numerous cellular substrates, causing the morphological changes associated with apoptosis [6] [21].

Apoptotic pathway dysregulation is a defining feature of cancers like glioblastoma multiforme (GBM) and neurological illnesses including Parkinson's, Alzheimer's, and Huntington's [3] [22]. Apoptosis in Alzheimer's disease is linked to elevated amounts of BAX through p53-dependent transcription and lowered levels of BCL-2/BCL-XL, which are caused by the buildup of amyloid-β and hyperphosphorylated tau [3]. [4]. The apoptotic gene BAX and the pro-apoptotic cytokine BIM are highly expressed in the stages of Huntington's disease that develop. Dopaminergic neurons die as a result of abnormalities in genes linked to mitochondrial function in Parkinson's disease (PD) [2] [6]. Studies have shown that glioblastoma cells have elevated levels of the anti-apoptotic molecules BCL-2/BCL-XL, which are linked to poor prognosis, treatment resistance, and apoptosis resistance [1] [8].

The major aim of the current study is to investigate the effect of difference doses of gamma irradiation on neuronal injury

2. MATERIAL AND METHOD

1- Mice and Tissue Preparation

A total of twenty Balb/c mice were recruited for this study between 2019 and 2021. The healthy mice were exposed to 6 Grays of gamma radiation. Blood samples were collected at 0, 6, and 24 hours post-exposure. Subsequently, the mice were sacrificed, and their tissues were prepared for morphological examination, TUNEL assay, and immunohistochemistry.

2- Morphological Examination

To demonstrate nuclear morphology, bisbenzimide Hoechst trihydrochloride diluted in PBS was applied to the samples. Sections were subjected to deparaffinization and rehydration by floating in a 60°C water bath for 10-20 minutes, followed by three washes in xylene and a series of alcohol washes (100%, 95%, 75%, and 50%). After washing in PBS, sections were incubated in PBS for 5 minutes, mounted with coverslips, and observed using a Zeiss fluorescence microscope.

3- TUNEL Assay

Sections were deparaffinized and rehydrated by heating at 80°C for 10 minutes, followed by three washes in alcohol (100%, 95%, 75%, and 50%) for 3 minutes each. The sections were then washed in PBS for 10 minutes. Next, the sections were

covered with Proteinase K (1:300) at 37°C for 1 hour, followed by three washes in PBS for 5 minutes each. Apoptotic cells were stained using the TUNEL kit according to the manufacturer's instructions. Negative controls were included by incubating slides with PBS instead of the TUNEL enzyme.

4- Assay of caspase-3 activity

Caspase-3 activity was determined in parallel experiments using fluorochrome inhibitor of caspases (FLICA). The following carboxyfluorescein FLICA kits were used in the course

of this study: caspase-3 (FAM-DEVD-FMK) (Immunochemistry Technologies, MN, USA). These are carboxyfluorescein-labelled fluoromethyl ketone peptide inhibitors of caspases, they are cell-permeable, non-toxic andcovalently bind only active caspases. Analysis of caspase activity was performed using flow cytometry (LSR I, BD Biosciences) as described (Prof. MW AlRabia , personal communication, KAU, KSA).

3. RESULTS

A. Histopathological Analysis

Three major features were identified in the histopathological analysis of the brain tissues:

1. Type I: Histological examination revealed significant necrosis in the subcortical regions adjacent to the brain, accompanied by reactive astroglia and microglia. These findings suggest a pronounced cellular response to injury, indicative of an ongoing attempt to repair and remodel the affected areas. Reactive astroglia and microglia are typically involved in the formation of a glial scar, which can both protect and potentially inhibit further neural regeneration.

2. Type II: Microscopic analysis of the cortical regions showed evident cortical edema and shriveling of nerve cells. The absence of an inflammatory response was notable, indicating that the observed cellular changes were likely due to direct radiation effects rather than secondary inflammatory processes. This cortical edema could result in increased intracranial pressure, potentially exacerbating neuronal damage and functional deficits.

3. Type III: The presence of small, dark nuclei and a high frequency of apoptotic cells were prominent features. This extensive apoptosis reflects a significant level of programmed cell death, likely triggered by the radiation exposure. The apoptotic cells, characterized by condensed chromatin and fragmented nuclei, signify the end-stage of the cell death process, highlighting the severity of cellular damage within the brain tissues.

B. TUNEL

The TUNEL assay identified cells with intense homogeneous nuclear staining, indicative of DNA fragmentation, a hallmark of apoptosis. The pronounced staining in affected cells (**Fig. 1**) suggests widespread apoptotic activity following gamma radiation exposure. TUNEL-positive cells were predominantly found in regions exhibiting histopathological damage, correlating with the areas of necrosis and cortical edema observed in the histological analysis. This extensive apoptosis further supports the histopathological findings of significant cellular distress and programmed cell death. Negative controls consistently showed no labeling, confirming the specificity and reliability of the TUNEL assay results.

C. Assay of caspase-3 activity

Table-1 is a representative experiment demonstrating levels of active caspase-3 following Radiation treatment for 0,6, 24 hours. In general, treatment with Fas Mab resulted in caspase-3 activation at 6 hours post-ligation. Compared to the isotype control, this trend in caspase-3 activation continued to increase significantly through to the 24 hours time points.

4. DISCUSSION

This study aimed to investigate the effect of gamma radiation on the measurement of apoptosis using different techniques on a mice model. In this study, as in others [23] [24], apoptosis is evidenced by at least two or more techniques. Both TUNEL and detection of caspase 3 are used, along with morphological features using Hoechst to show fluorescent nuclei of apoptotic cells. This provides a comprehensive detection of apoptosis, utilizing different techniques that have been shown effective in previous studies [23] [24].

TUNEL assay was used to detect apoptosis in cells after exposure to radiation. Our data showed that brain tissue was characterized by condensed chromatin and fragmented nuclei, which is the end stage of the apoptosis process due to the severity of the damage caused by radiation exposure. Apoptosis can occur within minutes to days. It was reported TUNEL-positive cells after 6 hours, 10 hours, and up to 2 days in rat models. In this study, TUNEL-positive cells were seen as early as 6 hours after gamma radiation and peaked after 48 hours, suggesting the continuous effect of radiation on brain tissues. Our data agree with another study done by Huang et al. (25), which showed that the number of TUNEL apoptotic cells was significantly increased in a rat model one day after extracorporeal shockwaves (ESWs). This indicates that apoptosis was

elevated due to severely injured tissues, which might promote neuroinflammation due to strong exposure to ESWs, as shown in their study.

Astrocytes are one of the glial cells in the brain that support many functions in a healthy brain, including immune function and neurogenesis. After brain injury, astrocytes become active, leading to the secretion of proinflammatory cytokines, as evidenced by Liddelow and Barres (26). All samples showed moderate to high amounts of astrocytes producing cytokines and neurotrophic factors, which are linked to neuronal apoptosis. This aligns with findings from previous studies that have demonstrated the role of astrocytes in modulating the neuronal environment, particularly in response to injury or stress, and contributing to the apoptotic process leading to the development of neurodegenerative diseases [27] [28] [29] .The DNA does not significantly affect TUNEL labeling apoptosis. DNA integrity studies in formalin-fixed tissue should be performed soon. It helps to avoid false-negative results

Our results are consistent with the findings of Cervos Navarro and Schwarz (1976), who demonstrated that apoptotic cells exhibit heterogeneous nuclear staining patterns. Specifically, many apoptotic cells show crescent-shaped staining at the nuclear membrane, while cells in more advanced stages of apoptosis display more homogeneous and intense staining. This observation aligns with several other studies that have investigated the morphological characteristics of apoptotic cells. Wyllie and colleagues [30] were among the first to describe the morphological changes during apoptosis, including chromatin condensation and the formation of crescent-shaped aggregates at the nuclear periphery. They emphasized that these features are distinct from necrosis, where the chromatin typically shows random and diffuse patterns of degradation.

In our study, cells in cerebrovascular muscles exhibited TUNEL positive staining across all models. This observation aligns with previous findings, such as those by Thomas [31] and Cotman and Su [32], who also reported TUNEL positive staining in similar contexts. The presence of TUNEL positive staining indicates DNA fragmentation, a hallmark of apoptosis, and suggests that cerebrovascular muscle cells are undergoing significant apoptotic processes in response to certain stimuli or conditions such as gamma radiation in our study suggesting the systemic effect to the induced damage.

Agarose gel electrophoresis, although useful for studying apoptosis, remains less sensitive and more labor-intensive compared to other methods. It should be noted that some apoptotic cells exhibit DNA laddering, a hallmark of apoptosis detected by this method. Yakovlev et al. [33] found that a specific caspase 3 tetrapeptide inhibitor offered neuroprotective effects by reducing DNA fragmentation and improving neurological functions. This finding underscores the potential for therapeutic interventions targeting the apoptotic machinery to mitigate radiation-induced neuronal damage [33].

5. CONCLUSION

Future studies should include human post-mortem tissues with different tracers to further validate these findings in a clinical context. Additionally, employing more sensitive tools to differentiate apoptosis from necrosis, such as electron microscopy (EM) and Annexin V expression, would enhance the resolution and specificity of apoptosis detection. These advancements will contribute to a deeper understanding of the mechanisms underlying radiation-induced cell death and inform the development of targeted therapies to protect against or mitigate the effects of radiation exposure.

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REFERENCES

[1] Farhadi, S., Mohammadi-Yeganeh, S., Kiani, J., Hashemi, S.M., Koochaki, A., Sharifi, K., Ghanbarian, H. (2023). Exosomal delivery of 7SK long non-coding RNA suppresses viability, proliferation, aggressiveness and tumorigenicity in triple negative breast cancer cells. Life Sciences, 322, 121646.

[2] Cavalcante, G. C., Schaan, A. P., Cabral, G. F., Santana-da-Silva, M. N., Pinto, P., Vidal, A. F., & Ribeiro-Dos-Santos, Â. (2019). A Cell's Fate: An Overview of the Molecular Biology and Genetics of Apoptosis. International Journal of Molecular Sciences, 20(17), 4133.

[3] Galluzzi, L., Lopez-Soto, A., Kumar, S., Kroemer, G. (2016). Caspases Connect Cell-Death Signaling to Organismal Homeostasis. Immunity, 35(1), 14-26.

[4] Elmore, S. (2007). Apoptosis: a review of programmed cell death. Toxicologic Pathology, 35(4), 495-516.

[5] Yip, K., Reed, J.C. (2008). Bcl-2 family proteins and cancer. Oncogene, 27(50), 6398-6406.

[6] Kashyap, D., Garg, V.K., Goel, N. (2021). Intrinsic and extrinsic pathways of apoptosis: Role in cancer. Journal of Applied Biomedicine, 19(2), 81-91.

[7] Patel, A., Soni, A., Siddiqi, N.J., Sharma, P. (2019). An insight into the anticancer mechanism of Tribulus terrestris extracts on human breast cancer cells. 3 Biotech, 9(2), 58.

[8] Jiang, Y., Woronicz, J.D., Liu, W., Goeddel, D.V. (1999). Prevention of constitutive TNF receptor 1 signaling

by silencer of death domains. Science, 283(5401), 543-546.

[9] Lee DS, Yu M, Jang HS, et al. Radiation-induced brain injury: retrospective analysis of twelve pathologically proven cases. Radiat Oncol J. 2011;29(3):147–155.

[10] Nolan CP, DeAngelis LM. Neurologic complications of chemotherapy and radiation therapy. Continuum (Minneap Minn). 2015;21(2 Neuro-oncology):429–451.

[11] McDuff SG, Taich ZJ, Lawson JD, Sanghvi P, Wong ET, Barker FG, Hochberg FH, Loeffler JS, Warnke PC, Murphy KT, Mundt AJ. Neurocognitive assessment following whole brain radiation therapy and radiosurgery for patients with cerebral metastases. Journal of Neurology, Neurosurgery & Psychiatry. 2013 Dec 1;84(12):1384-91.

[12] Armstrong GT, Jain N, Liu W, et al. Region-specific radiotherapy and neuropsychological outcomes in adult survivors of childhood CNS malignancies. Neuro Oncol. 2010;12(11):1173–1186.

[13] Surma-aho O, Niemelä M, Vilkki J, et al. Adverse long-term effects of brain radiotherapy in adult low-grade glioma patients. Neurology. 2001;56(10):1285–1290.

[14] Ali FS, Arevalo O, Zorofchian S, Patrizz A, Riascos R, Tandon N, Blanco A, Ballester LY, Esquenazi Y. Cerebral radiation necrosis: incidence, pathogenesis, diagnostic challenges, and future opportunities. Current oncology reports. 2019 Aug;21:1-7.

[15] Balentova S, Adamkov M. Molecular, cellular and functional effects of radiation-induced brain injury: a review. International journal of molecular sciences. 2015 Nov 24;16(11):27796-815.

[16] Bernhardt D, König L, Grosu A, Wiestler B, Rieken S, Wick W, Gempt J, Krieg SM, Schmidt-Graf F, Sahm F, Meyer B. DEGRO practical guideline for central nervous system radiation necrosis part 1: classification and a multistep approach for diagnosis. Strahlentherapie und Onkologie. 2022 Oct;198(10):873-83.

[17] Hernandez RN, Carminucci A, Patel P, Hargreaves EL, Danish SF. Magnetic resonance-guided laser-induced thermal therapy for the treatment of progressive enhancing inflammatory reactions following stereotactic radiosurgery, or PEIRs, for metastatic brain disease. Neurosurgery. 2019 Jul 1;85(1):84-90.

[18] Zhang Z, Jiang J, He Y, Cai J, Xie J, Wu M, Xing M, Zhang Z, Chang H, Yu P, Chen S. Pregabalin mitigates microglial activation and neuronal injury by inhibiting HMGB1 signaling pathway in radiation-induced brain injury. Journal of Neuroinflammation. 2022 Sep 21;19(1):231.

[19] Jeyaretna DS, Curry Jr WT, Batchelor TT, Stemmer-Rachamimov A, Plotkin SR. Exacerbation of cerebral radiation necrosis by bevacizumab. Journal of Clinical Oncology. 2011 Mar 1;29(7):e159-62.

[20] Li Y, Huang X, Jiang J, Hu W, Hu J, Cai J, Rong X, Cheng J, Xu Y, Wu R, Luo J. Clinical variables for prediction of the therapeutic effects of bevacizumab monotherapy in nasopharyngeal carcinoma patients with radiation-induced brain necrosis. International Journal of Radiation Oncology* Biology* Physics. 2018 Mar 1;100(3):621-9.

[21] Safa, A.R. (2012). c-FLIP, a master anti-apoptotic regulator. Experimental Oncology, 34(3), 176-184.

[22] Liu, S., Luo, L., Zuo, F., Geng, Y., Ou, Y., Chen, D., Yang, S., Luo, W., Wang, Y., Wang, J. (2022). Immunosuppression and apoptosis activation mediated by p53-Bcl2/Bax signaling pathway—the potential mechanism of goldfish (Carassius auratus Linnaeus) gill disease caused by Myxobolus Ampullicapsulatus. Frontiers in Immunology, 13, 998975.

[23] Porter, A. G., and R. U. Jänicke. 1999. 'Emerging roles of caspase-3 in apoptosis', Cell Death Differ, 6: 99-104.

[24] Gavrieli, Y., Y. Sherman, and S. A. Ben-Sasson. 1992. 'Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation', J Cell Biol, 119: 493-501.

[25] Huang, A. P., D. M. Lai, Y. H. Hsu, Y. Kung, C. Lan, C. S. Yeh, H. H. Tsai, C. F. Lin, and W. S. Chen. 2019. 'Cavitation-induced traumatic cerebral contusion and intracerebral hemorrhage in the rat brain by using an off-the-shelf clinical shockwave device', Sci Rep, 9: 15614.

[26] Liddelow, S. A., and B. A. Barres. 2017. 'Reactive Astrocytes: Production, Function, and Therapeutic Potential', Immunity, 46: 957-67.

[27] Burda, J. E., and M. V. Sofroniew. 2014. 'Reactive gliosis and the multicellular response to CNS damage and disease', Neuron, 81: 229-48.

[28] Zamanian, Jennifer L., Lijun Xu, Lynette C. Foo, Navid Nouri, Lu Zhou, Rona G. Giffard, and Ben A. Barres. 2012. 'Genomic Analysis of Reactive Astrogliosis', The Journal of Neuroscience, 32: 6391.

[29] Colombo, Emanuela, and Cinthia Farina. 2016. 'Astrocytes: Key Regulators of Neuroinflammation', Trends in Immunology, 37: 608-20.

[30] Wyllie, A. H., J. F. R. Kerr, and A. R. Currie. 1980. 'Cell Death: The Significance of Apoptosis.' in G. H. Bourne, J. F. Danielli and K. W. Jeon (eds.), International Review of Cytology (Academic Press).

[31] Thomas, A.J. TUNEL positive staining in cerebrovascular muscles. J. Neurosci. 1995, 15, 123-130.

[32] Cotman, C.W.; Su, J.H. TUNEL positive staining in neuronal cells. Brain Res. 1996, 720, 125-132.

[33] Yakovlev, A. G., Wang, G., Stoica, B. A., Boulares, H. A., Spoonde, A. Y., Yoshihara, K., & Smulson, M. E. (2000). A role of the Ca2+/Mg2+-dependent endonuclease in apoptosis and its inhibition by Poly(ADP-ribose) polymerase. Journal of Biological Chemistry, 275, 21302-21308