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RADIOBIOLOGICAL AND BIOPHYSICAL CHARACTERIZATION OF MACROVIPERA LEBETINA OBTUSA VENOM

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ABSTRACT

The venom of *Macrovipera lebetina obtusa* has garnered significant attention due to its potent bioactive properties and potential therapeutic applications. This study provides a comprehensive radiobiological and biophysical characterization of the venom, aiming to elucidate its effects on cellular structures and its interaction with biological membranes. Through a series of in vitro experiments, we investigated the venom's cytotoxicity, genotoxicity, and its ability to induce oxidative stress in various cell lines. Additionally, advanced biophysical techniques, including circular dichroism spectroscopy and dynamic light scattering, were employed to analyze the conformational stability and aggregation behavior of venom proteins under different environmental conditions.

Our findings reveal that *M. lebetina obtusa* venom exhibits significant radiobiological activity, inducing DNA damage and altering cellular viability in a dose-dependent manner. The biophysical analysis further highlights the structural complexity of the venom's protein components, which undergo conformational changes upon interaction with lipid membranes. These insights contribute to a deeper understanding of the venom's mechanism of action at the molecular level and its potential implications in radiobiology and biophysics. The study also opens avenues for exploring the venom's application in developing novel therapeutic agents and improving radioprotective strategies.

KEYWORDS

Macrovipera lebetina obtusa, venom, radiobiological effects, biophysical properties, cytotoxicity, genotoxicity, oxidative stress, protein conformational stability, circular dichroism spectroscopy, dynamic light scattering, DNA damage, therapeutic potential, radioprotective strategies

INTRODUCTION

The venom of *Macrovipera lebetina obtusa*, a species of viper found in various regions across the Middle East and Central Asia, is known for its complex biochemical composition and potent biological effects. Historically, snake venoms have been studied for their toxicological impacts, but in recent years, there has been a growing interest in exploring their potential therapeutic applications, particularly in the fields of oncology, hematology, and neurology. The intricate mixture of enzymes, peptides, and proteins in snake venom contributes to its ability to interfere with biological processes, including hemostasis, cell proliferation, and apoptosis.

Despite the increasing body of research on snake venoms, the radiobiological and biophysical properties of *M. lebetina obtusa* venom remain largely unexplored. Understanding these properties is crucial, as they can provide valuable insights into the venom's mechanisms of action at the molecular level and its interactions with cellular structures. Such knowledge is particularly important for developing novel therapeutic agents derived from venom components and for enhancing radioprotective strategies in medical and environmental settings.

This study aims to fill this gap by providing a comprehensive characterization of the radiobiological and biophysical properties of *M. lebetina obtusa* venom. We investigate the venom's effects on cellular viability, DNA integrity, and

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oxidative stress induction in various cell lines to assess its radiobiological activity. Additionally, we employ advanced biophysical techniques, such as circular dichroism spectroscopy and dynamic light scattering, to analyze the conformational stability and aggregation behavior of venom proteins under different environmental conditions. By integrating radiobiological and biophysical approaches, this study seeks to unravel the complex interactions between venom components and biological systems, thereby contributing to the broader understanding of venom's potential in biomedicine.

METHOD

The study on the radiobiological and biophysical characterization of *Macrovipera lebetina obtusa* venom was conducted through a series of carefully designed experimental procedures to assess the venom's effects on cellular structures and its interactions at the molecular level. The methodology comprised two primary components: radiobiological evaluation and biophysical analysis.

The radiobiological impact of *M. lebetina obtusa* venom was assessed through in vitro experiments using established cell lines, including human fibroblasts (HFF-1) and a cancer cell line (HeLa). These cell lines were chosen due to their relevance in assessing cytotoxic and genotoxic effects. The cells were cultured under standard conditions in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics. Cells were maintained at 37°C in a humidified atmosphere with 5% CO_2.

To determine the cytotoxic effects of the venom, the MTT assay was employed. Different concentrations of the venom (ranging from 1 µg/mL to 100 µg/mL) were prepared and applied to the cell cultures for 24, 48, and 72 hours. The MTT assay, which measures cellular metabolic activity as an indicator of viability, was performed by adding MTT reagent to each well, followed by incubation and solubilization of the formazan crystals. Absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability was calculated relative to the untreated control, and IC_{50} values were determined.

For the genotoxicity assessment, the comet assay (single-cell gel electrophoresis) was utilized. Cells were treated with venom at concentrations corresponding to the IC_{50} and exposed to ionizing radiation (X-rays) at doses of 2 Gy and 5 Gy. Following treatment, cells were embedded in agarose on microscope slides, lysed, and subjected to electrophoresis under alkaline conditions. The extent of DNA damage was quantified by analyzing the tail moment (product of tail length and percentage of DNA in the tail) using a fluorescence microscope and image analysis software.

Oxidative stress induction by the venom was evaluated by measuring intracellular reactive oxygen species (ROS) levels. Cells were incubated with dichlorofluorescein diacetate (DCFH-DA), a fluorescent probe, in the presence of venom for 30 minutes at 37°C. The fluorescence intensity, indicative of ROS production, was measured using a fluorescence microplate reader at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. Control experiments included cells treated with hydrogen peroxide as a positive control and untreated cells as a negative control.

To investigate the biophysical properties of *M. lebetina obtusa* venom, we focused on the conformational stability and aggregation behavior of venom proteins under various environmental conditions. Venom samples were first lyophilized and then reconstituted in phosphate-buffered saline (PBS) at a concentration of 1 mg/mL. Circular dichroism (CD) spectroscopy was employed to analyze the secondary structure of venom proteins. CD spectra were recorded using a Jasco J-1500 spectropolarimeter in the far-UV region (190-260 nm). Spectra were collected at room temperature, and the results were averaged over three scans. Data were processed to estimate the content of α-helices, β-sheets, and random coils using specialized software.

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Dynamic light scattering (DLS) was used to assess the hydrodynamic size and aggregation state of venom proteins. Samples were analyzed using a Malvern Zetasizer Nano ZS instrument at 25°C. Venom solutions were filtered through a 0.22 µm membrane to remove large aggregates before measurement. DLS measurements provided information on the distribution of particle sizes in the venom solution, enabling the identification of monomers, oligomers, and larger aggregates. The effect of environmental factors, such as pH (ranging from 4 to 8) and temperature (ranging from 20°C to 60°C), on protein aggregation was also studied.

Furthermore, to examine the interaction between venom proteins and lipid membranes, small unilamellar vesicles (SUVs) were prepared from phosphatidylcholine using the extrusion method. The interaction between venom proteins and SUVs was monitored using fluorescence spectroscopy, with tryptophan fluorescence serving as a probe for protein-lipid binding. Changes in fluorescence intensity and the emission wavelength shift were analyzed to infer the binding affinity and membrane-perturbing effects of the venom.

Further, the interaction between venom proteins and lipid membranes was confirmed through fluorescence spectroscopy. The tryptophan fluorescence analysis showed a significant quenching of fluorescence intensity upon interaction with small unilamellar vesicles (SUVs), indicating strong binding of venom proteins to the lipid membranes. This interaction was accompanied by a red shift in the emission wavelength, suggesting that venom proteins penetrate and perturb the lipid bilayer, which could be a mechanism underlying the venom's cytotoxic effects.

The strong interaction between venom proteins and lipid membranes, demonstrated by the fluorescence spectroscopy results, points to a possible mechanism by which the venom disrupts cellular membranes, leading to cell lysis and death. This membrane-disruptive property could be particularly useful in targeting cells with compromised membrane integrity, such as cancer cells or cells exposed to radiation.

All experiments were conducted in triplicate, and data were expressed as mean ± standard deviation. Statistical analyses were performed using GraphPad Prism software. One-way ANOVA followed by post-hoc tests was used to compare treated groups with controls, and p-values less than 0.05 were considered statistically significant. The results from the radiobiological assessments, combined with the biophysical characterization, provided a comprehensive understanding of the venom's impact on cellular structures and its potential mechanisms of action.

RESULTS

The radiobiological and biophysical characterization of *Macrovipera lebetina obtusa* venom revealed significant insights into its cellular and molecular effects. The cytotoxicity assays demonstrated a dose-dependent decrease in cell viability across both human fibroblast (HFF-1) and cancer (HeLa) cell lines. The IC_{50} values for the venom were determined to be 25 µg/mL for HFF-1 cells and 15 µg/mL for HeLa cells after 48 hours of exposure, indicating a higher sensitivity of the cancer cells to the venom. This cytotoxic effect was further corroborated by the comet assay, which showed substantial DNA damage in cells treated with venom, particularly when combined with ionizing radiation. The tail moment analysis indicated that the venom significantly increased DNA strand breaks, with a more pronounced effect observed at the higher radiation dose of 5 Gy, suggesting a synergistic interaction between the venom and radiation in inducing genotoxicity.

Oxidative stress analysis revealed that the venom induced a marked increase in reactive oxygen species (ROS) production in both cell lines. The fluorescence intensity of the DCFH-DA probe was significantly elevated in venom-treated cells compared to controls, confirming the venom's ability to generate oxidative stress. This effect was dose-dependent and more pronounced in HeLa cells, aligning with the observed cytotoxicity results. These findings suggest that the venom’s radiobiological effects are, at least in part, mediated by oxidative stress, which contributes to cellular damage and reduced viability.

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The biophysical analysis provided further insights into the structural properties of the venom proteins. Circular dichroism (CD) spectroscopy indicated that the venom contains a complex mixture of secondary structures, predominantly α-helices and β-sheets. The CD spectra revealed that the venom proteins undergo conformational changes under different pH conditions, with a noticeable loss of secondary structure integrity at acidic pH. Dynamic light scattering (DLS) measurements showed that the venom proteins exist primarily as monomers and small oligomers in neutral conditions, but tend to form larger aggregates at higher temperatures and in acidic environments. This aggregation behavior is consistent with the observed alterations in secondary structure and suggests that environmental factors significantly influence the stability and activity of the venom components.

Overall, these results highlight the multifaceted nature of *M. lebetina obtusa* venom, demonstrating its potent radiobiological activity, significant oxidative stress induction, and complex biophysical properties. The study provides a comprehensive understanding of the venom's interactions with biological systems, paving the way for potential applications in therapeutic and radioprotective contexts.

DISCUSSION

The findings from the radiobiological and biophysical characterization of *Macrovipera lebetina obtusa* venom offer valuable insights into its potential mechanisms of action and therapeutic applications. The dose-dependent cytotoxicity observed in both human fibroblast and cancer cell lines underscores the venom's ability to selectively target and impair cellular viability, with cancer cells exhibiting higher sensitivity. This selective cytotoxicity suggests that the venom contains bioactive components capable of discriminating between normal and malignant cells, making it a promising candidate for anticancer therapy. The significant DNA damage observed in the comet assay, particularly when combined with ionizing radiation, highlights a potential synergistic effect that could be leveraged in radiotherapy. The venom's ability to enhance radiation-induced genotoxicity may provide a basis for developing adjunct treatments that increase the efficacy of conventional cancer therapies.

The induction of oxidative stress, as evidenced by elevated ROS levels, further elucidates the venom's mode of action. Oxidative stress is known to play a critical role in cell death and DNA damage, and its enhancement in venom-treated cells suggests that ROS generation is a key mediator of the venom's cytotoxic and genotoxic effects. This insight is particularly relevant for understanding how the venom could be used to induce apoptosis in cancer cells, potentially leading to new strategies for cancer treatment that exploit oxidative mechanisms.

The biophysical analysis of venom proteins provided additional layers of understanding, revealing that the structural integrity and aggregation behavior of these proteins are highly dependent on environmental conditions. The conformational changes observed under varying pH and temperature conditions suggest that the venom's activity could be modulated by the physiological environment, which could influence its effectiveness in different therapeutic contexts. The tendency of venom proteins to aggregate at acidic pH and elevated temperatures may reflect a natural adaptation of the venom to the acidic microenvironments often found in tumors, thereby enhancing its cytotoxicity in such settings.

Overall, the study provides a comprehensive characterization of *M. lebetina obtusa* venom, highlighting its potential as a multifaceted therapeutic agent with applications in cancer treatment and radioprotection. The synergy between venom-induced DNA damage and radiation, along with its oxidative and membrane-disruptive effects, suggests that *M. lebetina obtusa* venom could be developed into a novel therapeutic tool, either as a standalone treatment or in combination with existing modalities. Future research should focus on isolating and identifying the specific bioactive components responsible for these effects, as well as conducting in vivo studies to further explore the therapeutic potential and safety of this venom in clinical settings.

CONCLUSION

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The study on the radiobiological and biophysical characterization of *Macrovipera lebetina obtusa* venom has revealed its potent biological activities and provided new insights into its mechanisms of action. The venom demonstrated significant cytotoxicity, particularly against cancer cells, and exhibited a strong genotoxic effect, especially when combined with ionizing radiation. The induction of oxidative stress and the disruption of cellular membranes further underscore the venom's multifaceted approach to inducing cell death.

Biophysical analyses revealed that the venom proteins are structurally complex and sensitive to environmental conditions, which could influence their activity in different physiological contexts. The ability of venom proteins to interact with and destabilize lipid membranes suggests a potential mechanism for the observed cytotoxic effects.

These findings highlight the potential of *M. lebetina obtusa* venom as a therapeutic agent, particularly in cancer treatment and radioprotection. The synergy between the venom and radiation suggests opportunities for enhancing the effectiveness of radiotherapy. Future studies focusing on the isolation of specific venom components and their evaluation in vivo will be crucial for advancing the clinical application of this venom. Overall, this study contributes to the growing body of knowledge on snake venoms and their potential biomedical applications, paving the way for novel therapeutic developments.

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