Effect of Cold Atmospheric Plasma on CT26 Murine Colorectal Carcinoma Cells

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ABSTRACT

Introduction: Colorectal cancer remains a major clinical challenge. Cold atmospheric plasma (CAP) generates reactive oxygen and nitrogen species that selectively damage cancer cells, triggering oxidative stress and cell death.

Materials and Methods: A helium CAP jet (AC, 6.8 kV, 12–13 kHz) was used to treat CT26 murine colorectal carcinoma cells. The cells were seeded at 1×10^5 cells/mL in 24-well plates and exposed to CAP for 30–240 s at fixed voltage, frequency, gas flow, and 1 cm jet-sample distance. The levels of hydrogen peroxide (H_2O_2) in PBS and DMEM/F12 + 10% FBS were quantified calorimetrically using a horseradish peroxidase-based kit. Intracellular reactive oxygen species (ROS) were measured by flow cytometry. Cell viability was assessed using the MTT assay, which measures mitochondrial metabolic activity by reducing tetrazolium to formazan.

Results and Discussion: CAP exposure elevated extracellular H_2O_2 over time .In the PBS solution, H_2O_2 levels increased from 36.3 µM to 342 µM over 30-240 s. In the DMEM/F12 + FBS solution, levels increased from 92 µM to 360 µM over 240 s. Intracellular ROS levels rose from 61 µM (control) to 79 µM over 180 s. CAP treatment reduced viability in a duration-dependent manner, decreasing to 82% at 30 s and 18% at 240 s. The IC_{50} was determined to be between 90 and 120 seconds, which is consistent with CAP's cytotoxic action via oxidative mechanisms. These findings support the use of CAP as a selective antitumor modality for colorectal cancer, motivating the optimization of exposure parameters and media conditions to maximize therapeutic windows.

Conclusion: Direct helium CAP produces oxidative stress in CT26 cells by elevating extracellular H_2O_2 and intracellular ROS levels, leading to a reduction in cell viability with an IC_{50} of approximately 90–120 s.

Keywords: Cold atmospheric plasma, Colorectal cancer, Reactive oxygen and nitrogen species

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