# Atmospheric non-equilibrium plasma promotes cell death and cell-cycle arrest in a lymphoma cell line

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Although advances in cancer treatment have been made, antitumor chemotherapy is strongly hampered by the low therapeutic index of most anticancer drugs, development of chemoresistance and relapse (Arcangeli, Curr Med Chem 19:683, 2012). There is a cogent need of new anticancer strategies, endowed with a better pharmaco-toxicological profile, eventually in association with conventional chemotherapy. In this context, atmospheric non-equilibrium plasma (or cold plasma), providing a blend of chemical and physical components, is drawing interest as a promising anticancer strategy (Keidar, Br J Cancer 105:1295, 2011). Pioneering studies showed that the biological effect of plasma depends on its ability to produce reactive oxygen and nitrogen species that increase oxidative and nitrosative stress in tumor cells and leads to tumor cell death (Graves, Plasma Process Polym 11:1120, 2014). However, mechanisms of plasma-cell interaction is not completely understood, as well as the selectivity associated to the various plasma generated species. We investigated the effects of plasma treatment on the viability, proliferation, and cell-cycle progression of mouse lymphoma cells (L5178Y). Plasma treatment was performed by means of a wand electrode dielectric barrier discharge driven by nanosecond high voltage pulses. Two different sets of operating conditions have been considered: in the first case (T1), a 60 s treatment was performed keeping a 1.25 mm distance between the tip of the plasma source and the surface of the liquid medium (gap); in the second case (T2), a 120 s treatment was performed setting the gap at 2.50 mm. Cell viability, proliferation and cell-cycle analysis were assessed after 6, 24 or 48h from plasma exposure through flow cytometry. After 48 h from the exposure, cold plasma induced a dose-dependent decrease in cell viability at both T1 and T2 treatment conditions (57.3% and 43%, respectively *vs* 71.2% of untreated cells). Furthermore, plasma affected cell proliferation both after 24 and 48 h from the exposure. The highest effect was observed at the T2 condition (36.7% after 24 h and 30.0% after 48 h respectively *vs* 100% of untreated cells). After 24 h from treatment, plasma induced an accumulation of cells in the G2/M phase for both exposure conditions (65.3% at the T1 condition and 55.9% at the T2 condition *vs* 39.6% of untreated cells). After 48 h from the exposure, the accumulation of cells in the G2/M phase was statistically significant only for the T2 condition (52.2% *vs* 42.8% of untreated cells). On the basis of our results, the plasma-induced cell-cycle arrest is reversible for the T1 condition, but not for the T2 condition, where cells cannot rescue form the plasma-induced cell-cycle arrest. Taken together, these results demonstrate that the growth inhibition of lymphoma cells induced by plasma treatment is imputable to cell death and cell-cycle arrest in which G2 accumulation is a key event. The simultaneous appearance of G2 block and cell death suggests that cell death is a primary direct effect due to plasma treatment, and not a secondary effect due to the cells' inability to overcome growth arrest and proceed through the cell cycle. The results of this study will contribute to open up new pharmacological prospects for cancer therapy. Future applications of such understanding will provide a clear rationale for designing *in vivo* experiments and pave the way for future application of plasma in the oncological field.