**NON THERMAL PLASMA AS INNOVATIVE ANTICANCER STRATEGY**

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Although advances in treatment of cancer have been made, antitumor chemotherapy is strongly hampered by the low therapeutic index of most anticancer drugs and the development of chemoresistance. There is a continuous need for new intervention strategies, endowed with a better pharmaco-toxicological profile. Plasma, a ionized gas generated by exposing a gas to an electric field, has recently gained attraction as a promising technology for medical applications, since recent progress in plasma generation has led to sustain cold atmospheric plasmas at biocompatible temperatures. Earlier studies demonstrated the non-aggressive nature of non-thermal plasma (NTP) (Keidar et al., 2011). Pioneering studies showed that the anticancer activity of NTP depends on the increase of oxidative and nitrosative stress in tumor cells that leads to tumor cell death (Hirst et al., 2016). However, mechanisms of NTP-cell interaction are not yet completely understood.

We investigated the pro-apoptotic, cytostatic and genotoxic effects of NTP in human T-lymphoblastoid leukemia cells (Jurkat). NTP treatment was performed using plasma GUN, driven by nanosecond high voltage pulses with a tungsten wire used as electrode and positioned on the axis of a borosilicate glass capillary. Helium was used to generate plasma. Cell treatments were performed under different operating conditions, such as voltage (10 or 15 Kv), time of exposure (120 or 180 s) and recovery time after NTP exposure (5-48 h). Results were obtained for NTP direct treatment, with cell exposed to plasma while suspended in culture medium, and indirect treatment, where cells were added to culture medium previously activated by plasma treatment. All biological endpoints were measured via flow cytometry.

After 24 h of both direct and indirect exposure to plasma GUN, cell viability decreased depending on voltage and time of exposure, with a significant increase in necrotic events rather than apoptotic events. A significant increase in apoptotic events was observed after 48 h from NTP exposure, but only when cell medium was renewed after 1 h from plasma treatment. This is probably due to changes induced by NTP on cell medium components that require its substitution. In particular, at the operating conditions 10 Kv 120 s the percentage of apoptotic cells was 14.5% after direct plasma exposure and 34.26% after indirect treatment (vs 5% of untreated cells). Increasing both the voltage and the time of NTP exposure, a slight increase in apoptotic but also in necrotic events was observed.

Due to its better cytotoxic profile, subsequent experiments were performed via indirect NTP treatment. After 24 h from NTP exposure, a decrease of cells in the G0/G1 phase was observed for all tested operating conditions. A compensatory increase of cells in S and G2/M phase was recorded.

As previously mentioned, NTP biological effects are imputable to its ability to increase ROS and RNS levels, and this could cause DNA damage. The genotoxicity of NTP was assessed. Five hours after NTP treatment, a significant increase of H2AX phosphorylation was observed at all operating conditions. Interestingly, 24 h after NTP exposure, H2AX phosphorylation significantly decreased. This means that DNA repair systems were activated and/or that the induced DNA damage resulted in cell death.

These preliminary results contribute to understand the pharmaco-toxicological potential of NTP in the oncologic field and pose the basis to better characterize its cellular, molecular and genetic impact.

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Keidar et al. (2011). Br J Cancer 105, 1295-1301.

Hirst et al. (2016). Tumor Biology 37, 7021-7031.