

First achievements and opportunities for cancer treatment approach using non thermal plasma

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Résumé

This abstract summarizes the experimental results obtained and plasma delivery strategy developed in GREMI for the evaluation of antitumor action of dielectric barrier discharge and plasma gun for cancer treatment. Detailed analysis of biological effects following cold plasma application for both in vitro and in vivo experiments reveals the role of ROS, DNA damage, cell cycle modification and apoptosis induction. Recent characterization of plasma splitting and mixing in capillary geometry, using the plasma gun, together with preliminary tolerance study dealing with lung and colon treatment indicate that endoscopic plasma delivery may be a new and valuable therapy in cancerology.

This work deals with the development of two non thermal plasma sources and their application in cancerology. A floating electrode dielectric barrier discharge device (FE-DBD) and a plasma jet generator, labelled plasma gun [1], are used for both in vitro and in vivo assessment of cold atmospheric pressure plasma as a new therapy for cancer treatment. The first experiments were performed using the FE-DBD as an external plasma applicator on mice subcutaneously grafted with a tumor. The operation of the FE-DBD at low electrical power, a few watt, and correspondingly moderate plasma flux on the mouse skin surface allow repetitive plasma delivering for a few minutes during a few consecutive days. Following this plasma application protocol neither systemic behaviour, cardiac and pulmonary rhythm alterations nor severe skin burns were measured during tolerance studies. A five day DBD plasma treatment was shown to induce a significant delay and slowing down of the growth of U87 glioma cancer, in comparison with non treated control group [2]. This first demonstration of in vivo plasma therapeutic effect on a resistant cancer target was at the origin of three main experimental investigations: the study of the plasma delivery protocol, in vitro studies on different cell lines and the development of a new plasma source likely to allow endoscopic treatments. In vitro studies concern U87 (brain tumor) and HCT116 (colon tumor) cell lines for which both FE-DBD and plasma gun were used. The two plasma sources were shown to be efficient to kill the cell after plasma application over periods of a few tens of seconds. A more detailed analysis of FE-DBD action mechanism in in vitro cancerous cell treatment has been performed. The crucial role of ROS was proven by the comparable effect of a direct DBD plasma exposure and an indirect treatment protocol for which the culture medium was exposed to plasma and then transferred in culture wells containing cancerous cells. The use of ROS scavenger confirms that these species are, in vitro, the main agent in the cell destruction. DNA damage characterization, apoptosis quantification, and cell cycle analysis have then been performed. Briefly, formation of DNA double strand break damages and an accumulation of cells in the S phase were measured. Significant enhancement of the portion of cells in an apoptotic stage was also measured following in vitro plasma treatment. Dealing with the in vitro effect of plasma, the analogy of our results with plasma exposure with conventional cancer treatment strategies such as radio and chemo therapies, leads to suggest the following scenario to explain the cancerous cell destruction under plasma action. The key element is associated with in situ ROS generation leading successively to DNA damage, cell cycle arrest, and apoptosis induction. A very promising but questioning results concern the effect of plasma treatment during in vivo application. Apoptosis increasing and cell cycle alteration leading to an accumulation of cells in the S phase, were also measured by analyzing in vivo plasma treated tumors with the same detection techniques. The figure 1 presents the cell cycle measurements for in vitro and in vivo studies. Comparable G0/G1 fraction decrease, S phase accumulation and G2/M level are detected for in vivo and in vitro treated cells in comparison with the control group distribution.

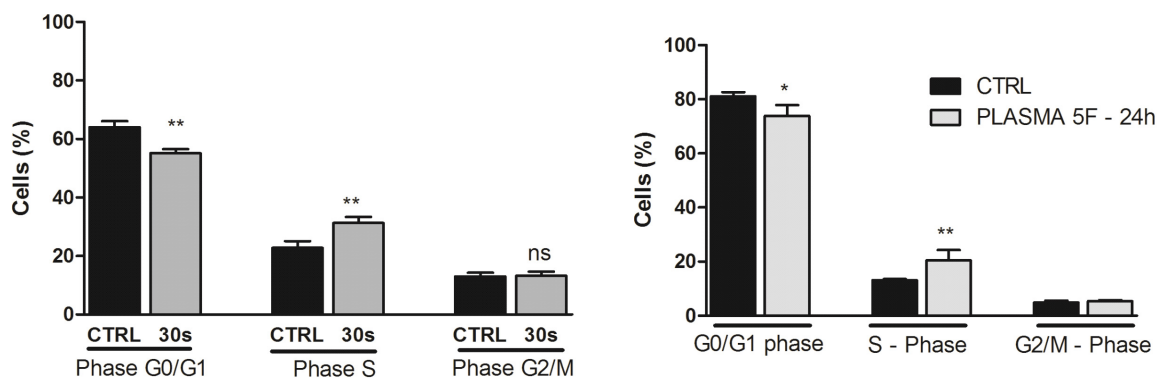


Fig. 1: Cell cycle distribution in control and plasma treated groups for in vitro (left) and in vivo (right) U87 tumor.

The role of ROS, reported to be of greater influence for in vitro plasma action, during in vivo treatment remains unclear, the main issue lying in the potential diffusion of these active species through the mouse skin to the tumor volume. In vivo plasma effects may also be correlated with plasma triggered in situ, i.e. in tumor environment, ROS release through local surface modification of charge density, temperature, or pH value. Such local pH modification was for instance observed on the skin surface where acidification occurs while subcutaneously a slight increase of the pH value was measured. Such chemical modification may induce in situ ROS release which may then interact with the tumor cells eventually through similar pathways as those encountered during in vitro trials. If this assumption sounds, the tumor treatment will probably be greatly optimized by delivering chemically active plasma in the close vicinity of the targeted cells. To check the efficiency of this mode of plasma application, there exists a need for a plasma source likely to allow endoscopic treatment. The main targets of our study concern colorectal and lung cancers for which access requires a specific care. The plasma gun set up is based on a pulsed nanosecond DBD reactor coupled with high aspect ratio flexible capillaries through which ionization wave sustained plasma propagation occurs. The possibility to use the plasma gun in multi branched volume, including branch splitting and connection at different locations, was recently experienced with success. Thus the plasma propagation was shown to be possible at large distances from the primary DBD plasma up to a few tens of centimetres through capillary structure exhibiting both splitting and connection of different branches. This unique specificity of the plasma gun among all other various plasma jet sources appears especially relevant for in vivo plasma application. The possibility to perform plasma delivery through micrometric catheter suitable for mice treatment by coloscopy or tracheotomy and flushed with low rare gas flow was recently achieved together with preliminary evidence for a reasonable tolerance of both colorectal and lung tissues under plasma exposure of a few minutes. The feasibility of such plasma application in combination with a proper matching of the plasma gun characteristics allows in situ in vivo study and optimization of antitumor action of cold plasma. Recent studies on the plasma jet action [3] on colorectal cell lines confirms cell growth arrest [4] and report a selective apoptosis induction for different tumoral and normal cells [5].

References

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