Potential of plasma based soft and/or combined cancer treatments

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Abstract: The antitumoreffect of NTP is now well established while clearly far from being understood. It is particularly striking to notice that this effect is observed almost whatever the plasma used and the treatment conditions. This led us to adopt a new strategy, using immuno-competent murine model, based on treatment under very soft conditions, combined or not with other treatments. This work underlines the potential role of the modification of the tumor microenvironment and the possible changes of the biological responses while questioning the role of the generated pulsed electric field.

Keywords: Plasma medicine, cancer treatment, soft treatment, combined therapy, electric field

1. Introduction

The antitumor effect of Non Thermal Plasmas (NTP) has been clearly shown in vivo on murine models with various cancer types (bladder, colon, glioblastoma, melanoma, ovary, pancreas). Although the mechanism is far from being fully understood, the therapeutic effect is now totally admitted. To date, the literature reports mainly induction of apoptosis, effect which is not restricted to cancer cells and probably mask other aspects of the NTP for cancer therapy. In case of plasma jet experiments, the observed effect are most of the time attributed to the very rich chemistry generated by the interaction of the rare gas plasma plume with the surrounding environment constituted either from the ambient air, or this last one in complex interaction with liquids at the interface with the targeted organ. Our recent experiments performed on tissue oxygenation [1] lead to the conclusion that probably the involved chemistry couldn’t, alone, completely allow to describe the observed phenomena [2]. In this context, there is still an unknown role of the electric field associated with the ionization front or generated in the environment of the plasma plume tip. Taking into consideration the recent vessel normalization based-cancer treatment, the NTP effect should be further investigated in view of blood vessels structure and function (blood flow) as well as tumor hypoxia compensation to confirm a possible NTP-based adjuvant approach for cancer treatments.

2. Antitumor effect of NTP

As underline above, the antitumor effect of NTP has been already shown in vivo in a large variety of cancers on murine models. In addition, many experiments demonstrated action on tumor cell lines in vitro [3][4]. The list is too long to be shown, but it can be said that the effect has been proven on more than 50 cell lines of most types of cancers. Beside the fact that this antitumor effect cannot be questioned, it must be stressed that those results, both in vitro and, especially, in vivo, have been obtained with a very large variety of experimental devices (DBDs, plasmas jets of all types) using extremely wide ranges of parameters including voltage, pulse duration, frequency, buffer gas and gas mixtures, distance to the target, time of exposure and protocols of application (number of fractions, combination or not with chemotherapy, heterotopic or orthotopic tumors, directly on targeted organ or through the skin). Typical examples of NTP antitumor effect in strongly different conditions are given in figure 1 and 2, showing almost the same efficacy. Figure 1 shows the results obtained in the treatment of colon cancer (HCT 116 cell line) on immuno-deficient Swiss nude mice using a DBD directly applied on the organ (the tumor activity is followed using bioluminescence). At day 22, in average, the tumor activity is reduced by more than 80 % in the treated group compare to the control group. These results have been obtained after a single treatment of 8 mn at 200Hz at a distance of 2 mm in ambient air, DBD surface covering the whole tumor surface.

Fig. 1. Colon Carcinoma – Evolution of the tumor bioluminescence for two mouse groups: CTRL, control; NTP, treated with DBD in air on day 7.
PaCa2-luc cell line) using a Plasma Gun (described in section 3) fed with neon gas, instead of the commonly used helium. Experiments were performed on externalized pancreas at 200 Hz, 0.2 l/mn at a distance of 5 mm of the tumor with three fractions of 10 mn delivered ten days apart. As in the precedent case, the data show a very strong effect with a reduction of tumor activity of the same order than the DBD treatment, this time the plasma spot not covering the entire tumor.

These results together with different ones previously published by the team or by others groups, see for examples [3], clearly show that whatever the NTP produced, the plasma is triggering some process chain, where the ROS and RNS seem to play an important role, that lead to a strong reduction of the tumor activity. It must be stressed that in all the above mentioned cases, experiments have been performed on immunodeficient mice which might in a way promote the obtained results, but, nevertheless, keeping in mind that some of the used cell lines are strongly chemo or radio resistant which still support the strong interest in plasma cancer treatment. It is then obvious that experiments have to be carried out on immunocompetent mice, experiments that are presented in the following section.

3. Plasma Gun treatment of breast carcinoma on immuno-competent Balb/C mice

Immuno-competent Balb/C mice were used to study the proliferation of orthotopically implanted 4T1 breast carcinoma (Mice were double grafted. One tumor was used to apply a specific treatment condition whereas the second tumor of the same mouse was used as control tumor (untreated)).

Fig. 3. Schematic of the Plasma Gun reactor.

These treatments were done with a Plasma Gun (PG) developed at GREMI. PG is a plasma jet (Fig.3) based on coaxial DBD discharge equipped with a glass capillary tube that allow to applied the created plasma plume at long distances from the DBD reactor. In this experiment, the capillary tube was 10 centimeter long and 4 mm in diameter. 6kV, microsecond duration high voltage pulses were applied to the reactor at pulse repetition rate of 200 Hz and 2kHz. In this study, focus was done on “soft” application under various conditions (connected or not to the ground, treated through H2O impregnated compress or directly, 2 kHz vs 200 Hz). Results including all conditions are shown in figure 3. As can be seen, data show a drastic reduction of tumor proliferation regardless the variations among the treatment conditions applied. This observation is in favor of a triggering effect, evidenced for the first time, of the plasma fraction which similarly affects the tumor proliferation (treated group figure 3) as compared to untreated tumor (contra-laterally grafted on each mice). This comparable reduction of tumor proliferation obtained whatever the plasma soft treatment conditions, with a significant reduction of standard deviations in all cases, clearly indicates a tumor growth regulation.
This, especially under very soft treatment conditions realized on immune-competent mice, is suggesting possible modifications in the microenvironment of tissue and tumors beside what have already been observed.

4. Tumor microenvironment under treatment

Accompanying the production of rich chemistry in the surrounding of the treated area, the application of a NTP can induce more global modifications in the tissues and probably in the tumors themselves. As we have already shown [1], Plasma Gun, and more generally plasma jets, treatments, can change the local characteristics of what concerns tissue oxygenation: oxygen partial pressure and blood flow. The application of the plasma plume to a living tissue leads to a sudden increase of tissue oxygen partial pressure which can reach up to 60 mmHg and last for tens of minutes after plasma application, and also an almost immediate increase in the blood flow (in average of 500 BPU) but that last, in this case, only during the plasma application.

Such important drastic changes, locally produced, which are still under study, are of primary importance when considering the role that can play tumor hypoxia in the production of aggressive cancer stem cells and cancer dissemination. The lack of oxygen is a major reason for the resistance of tumor cells to treatments, then improvement of oxygenation and perfusion open new opportunities for tumor treatments in combination with, for example, radiotherapy, and for tumor blood vessel normalization based strategies such as the one recently proven at CBM using allosteric effectors of haemoglobin to deliver more oxygen in hypoxic sites as found in the tumor (see figures 5 and 6) [5].
This effect of vessel normalization is highly significant in the case of solid tumor development. Among the various strategies that are currently developed to treat cancer, one of the most challenging is to address to angiogenesis regulation. The previously described strategies devoted to antiangiogenesis are no longer used except in the purpose of adapting the protocols to reach doses and time to normalize vessels rather than destroying them. It is the deleterious result of antiangiogenic strategies that lead to vessel destruction and total anoxia which produce strongly resistant cancer stem like cells.

The challenge then is to alleviate hypoxia by normalizing vessels [6]. This means to restore a certain level oxygen, sufficient enough to moderate HIF/VEGF production. This strategy reveals two main benefits: the vessel functionality allows synergy with anticancer drugs, the elevated levels of O2 increases the radiotherapeutic effects, finally by maintaining vessels normalization one can reach the stabilization of the pO2 thus stabilization of normalization.

These hypoxia alleviating strategies are approached from distinct angles and show in all cases [5, 7, 8] the beneficial effect on the tumor. Mainly changing the tumor microenvironment both humoral and cellular, the result of alleviating tumor hypoxia by vessel normalization is influencing the tumor immune response. This measures the huge potential of such means to treat cancer as an adjuvant potentiating the chemotherapy and more specifically the immunotherapy.

In case of plasma jets treatments alone or combined treatments, all the processes induced must be considered in possible combination with, or are potentially due to, the strong electric field present at the tip of the plasma plume and in the surroundings. Fields of more than 10 kVcm⁻¹ are proven, sufficient to induce electric effects such as electroporation, so probably affecting cell permeability. This can have some direct effect on cell viability [9] but also may help chemical penetration [10] emphasizing the study of combined treatment using both plasma and blood vessel normalisation strategy. It is then of importance to measure the electric field in conditions of treatment near and through the targeted tissues.

Recent development of a new non metallic non perturbative electric field probe allowed us to estimate the electric field generated by the plasma plume both above and in biological targets. In this work, we measured the electric field in real conditions of Plasma Gun applications on various biological samples and targets (tissues and liquids), using this new device (bi-component electro-optic EOP P2R05 BS010Kapteos probe) that allows time (ns) and space (1 mm³) resolved measurements in a two radial and longitudinal directions. Results indicate that the electric field generated under targeted tissues can be still significant quite deep contrary to what was expected. For example, in case of chicken skin, depending on the type, electric field between 0.1 and 1kV/cm can be achieved at depth between 1.5 to 3 mm. These significant values can be reached deeper in case of organs like liver, and this in presence or not of a liquid layer at the surface of the target. Such values of electric field produced on time duration directly related to the existence of the NTP plume (μs duration in the reported case) are sufficient to induce cell electroporation in much wider volume than initially though in tissue or directly treated tumors. These results clearly indicate that, beside the chemistry directly induce by the generated NTP, the potential role of the electric field must be taken into account.
5. Conclusion
The paper shows that clearly the plasma action on tumors must be somewhat revisited at the light of the different concepts evoked. The direct action of ROS and RNS shown before, while still valid, must considered in conjunction with effects of microenvironment modifications (oxygenation, blood flow, electro-poration, cell permeability) potentially driven by the associated applied pulsed electric field, together with induced systemic immune response as suggested by the results on breast cancer, but also underlined by Mir et al [10]. These results suggest new ways, especially new combined therapies, to consider the plasma and its therapeutic delivery in NTP-based tumor therapy.

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6. References