THE INCREASE IN THE PHOTODYNAMIC POTENTIAL OF DACARBAZINE AS A RESULT OF PH DEPENDENCE

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Abstract

Photostimulated chemotherapy (PSChT) is a relatively new therapeutic method whose principle is based on increasing the therapeutic index of certain cytostatics as a result of their interaction with optical radiation. For a particular chemotherapeutic agent to be effective in PSChT-type applications, it must fulfill several conditions, the most important of which is to have a positive photodynamic potential that consists in the resonant transfer of energy between the irradiation source and its molecule. In this study, Dacarbazine (DTIC) was used as the photosensitizing agent, and a high-pressure mercury lamp was used as the irradiating agent. Preclinical studies in BL / 6 inbred mice carrying B16 murine melanoma and spectrophotometric determinations were performed to identify whether the DTIC-PSChT method enhances the efficacy of chemotherapy.

Key words: photostimulated chemotherapy (PSChT), dacarbazine, murine B16 melanoma, tautomerization

INTRODUCTION

At the beginning of the 21st century, cancer, as a biological phenomenon, is among the main morbid entities, as a degenerative disease, with a serious evolution. Due to its consequences on multiple levels: human, economic and social, the image of cancer persists in the darkest of all known diseases, being surrounded by a myth of incurability and suffering. And yet, the accumulated knowledge, both scientific and therapeutic, has begun to dispel this myth of helplessness and to outline possible ways to eliminate this disease.

One of the most aggressive forms of cancer is malignant melanoma, which has as its starting point at the melanocytic system. It is frequently found on the skin, but it can also be found in other tissues and organs that contain melanocytes.

This tumor is intensively studied due to its increased prevalence at relatively younger ages compared to other neoplasms, the increasing incidence, the metastatic potential, high resistance to currently available therapeutic protocols and high mortality (Diaconu I. et al., 1996; Ferlay J. et al., 2012; Forsea A.M. et al., 2012).

One of the active substances used in standard therapeutic protocols for metastatic malignant melanoma is dacarbazine (5- (3,3-dimethyl-1-triazeno) imidazole-4-carboxamide). Also known as imidazole carboxamide, it is a synthetic analogue of the natural purine precursor (5-amino-1H-imidazole-4-carboxamide) (Correa F.M. et al., 2019; Eggermont A.M.K. et al., 2004; https://go.drugbank.com/drugs/DB00851).

The various manifestations of the chemical structure of DTIC regarding its activity as a chemotherapeutic agent have been reported by Freeman and Hutchinson, the determinations being performed by using X-ray diffraction phenomena (Freeman H.C. et al, 1979). Recent studies show that DTIC has the ability to tautomerize depending on the pH of the solution in which it is reconstituted, finding a much more intense therapeutic activity, enhanced by the action of UV radiation to which the cancer patient is exposed. In this way it was determined that the DTIC activity depends on both the illumination and the pH value of the solution (Eggermont A.M.K. et al., 2004; Chis M. et al., 2016).

All these findings are particularly important in the use of DTIC as a photosensitizing chemotherapeutic agent in PSChT-type applications, because it is reflected by the "red shift" of the absorption spectrum of dacarbazine as shown by our study, this shift being beneficial for

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melanoma therapy (Fumarel R., 2003; Fumarel R. et al., 2000).

MATERIAL AND METHOD

1. Spectrophotodynamic determinations

Initially, the photodynamic potential of DTIC was determined by absorption spectroscopy. To accomplish this goal, we diluted the DTIC powder in an alkaline aqueous solution, buffered with sodium bicarbonate with a pH between 13-15 (fig. 1). This was measured using a Perkin Elmer LAMBDA 25 double-beam spectrophotometer.

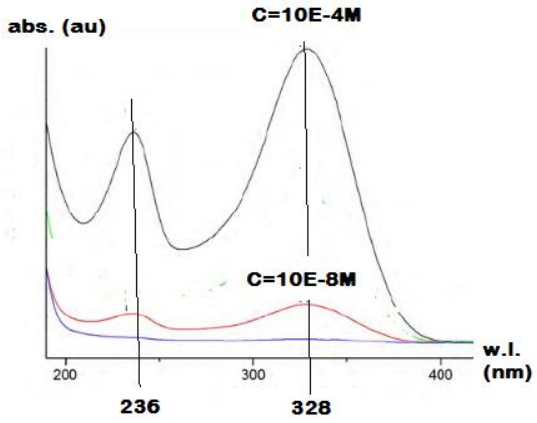

Figure 1. Absorption spectrum of DTIC at different concentrations

The concentration of DTIC in the solution was set at 10^{-2}M. Until the measurement, the samples were stored in a plastic container, wrapped in aluminum foil, at room temperature.

The DTIC powder was purchased from Sigma-Aldrid and used as such.

2. Photodynamic activation

This activation is achieved by irradiating B16 melanoma after administration of the intratumoral DTIC solution, using a specially designed equipment that has as source a high-pressure mercury vapor lamp whose spectrum can be seen in Figure 2.

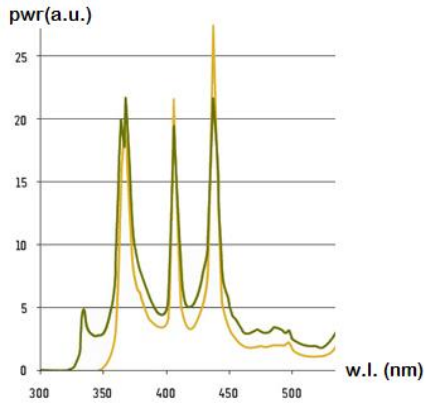

Figure 2. Emission spectrum of irradiation equipment

Irradiation was performed within 6-10 minutes, approximately one hour after administration of the alkaline cytostatic solution. This procedure must be carried out in compliance with the standard protection rules regarding ultraviolet radiation, respectively the use of UVA goggles.

3. The preclinical experimental model

The preclinical experimental model consisted in the subcutaneous transplantation of a solid melanic tumor - murine melanoma B16 on a number of 30 inbred BL / 6 mice divided into 3 equal groups, respectively: group I - control (mice with melanoma, untreated), group II - experimental (mice with melanoma treated with DTIC) and group III - experimental (mice with melanoma treated by the DTIC-PSChT method).

In order to determine the cytostatic effects, tumor fragments were collected from these animals, from which smears were performed, to highlight the cytomorphology of the cells and histological slides. The smears were stained by the May-Grunwald Giemsa method, and the pieces for histopathological examination were embedded in paraffin, sectioned at the microtome at 4-6 microns, and stained with trichrome Masson.

RESULTS AND DISCUSSIONS

1. Spectroscopic determinations:

The electronic absorption spectrum of DTIC, at a neutral pH value, is dictated by two main transitions, namely: (i) the allowed electronic transition between the fundamental level of the molecule and the first allowed excited state, respectively at 328 nm and (ii) the electronic transition allowed between the fundamental level and the second allowed electronic state, at 236 nm. These positions are invariant at the DTIC concentration in solution. On the other hand, UVA spectra were subsequently recorded at different pH values of the solution. In this case, major differences were observed that lead to a "red shift" of both electric bands associated with the two premature states of excitation.

In this way, the first band will move by about 26 nm at a pH difference of 7 and 13-15, respectively, and the second band of interest in increasing the photodynamic potential of the substance, under the same conditions for performing the measurements will have a movement of about 20 nm (Fig. 3).

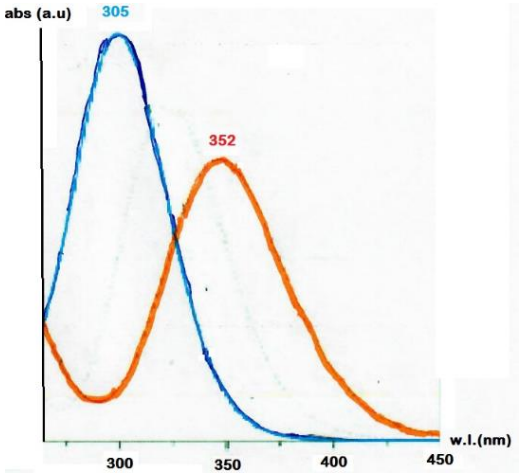

Figure 3. Displacement of the absorption bands depending on the pH

Thus, it is assumed that by increasing the pH of the DTIC solution, the process of resonant interaction will be significantly accentuated.

2. Preclinical experimental determinations:

When examining the smears and histological slides, performed from the tumor masses in the three groups inoculated with tumor tissue, we found special cytopathological aspects, depending on the applied therapeutic scheme. Thus, in the case of group I, the cytomorphological examination revealed the presence of melanocytic tumor cells, mainly of epithelioid type, but also of fusiform type. They had a high degree of anaplasia, with nuclei of various shapes and sizes, sometimes even monstrous, with coarsely arranged chromatin. The cytoplasm has different shades, from light blue - smoky, to dark blue, intensely basophilic (Fig. 4).

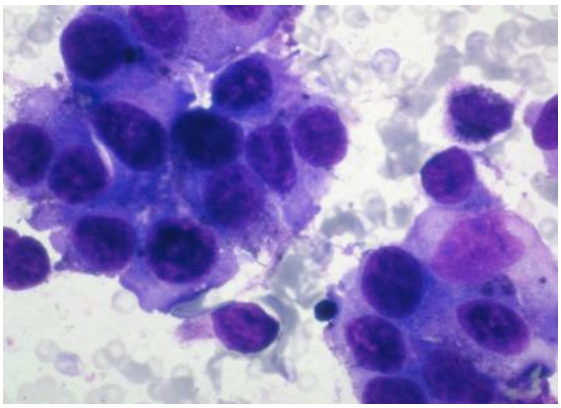

Figure 4. Murine B16 melanoma, cytomorphological aspect group I

Histopathologically, cell pleiomorphism, neovascularization and infiltrative character were found. In general, the mitotic index was high, the number of mitoses per microscopic field at target 10 being about 3-4 (Fig. 5).

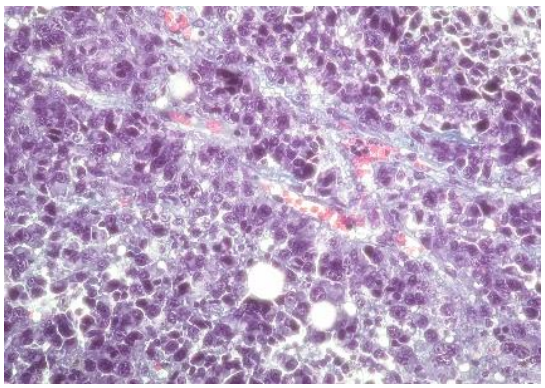

Figure 5. Murine B16 melanoma, histopathological aspect group I

In the case of group II, the cytomorphological examination showed significant differences compared to group I. Thus, the cellularity in the smear was much less represented, many tumor cells being necrotic (Fig. 6).

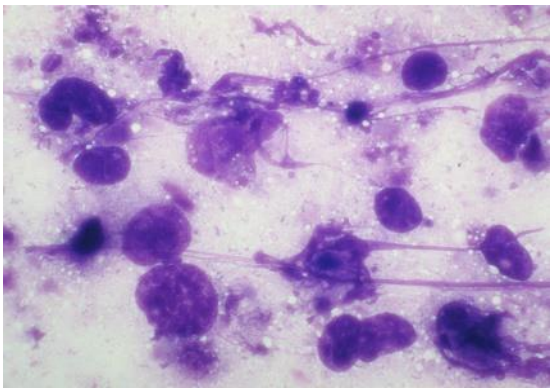

Figure 6. Murine B16 melanoma, cytomorphological aspect group II

Histopathologically, there are areas of cell and tissue necrosis, but also perivascular areas where clones of resistant tumor cells are present and thay have maintained their viability and have not responded to DTIC treatment (Fig. 7).

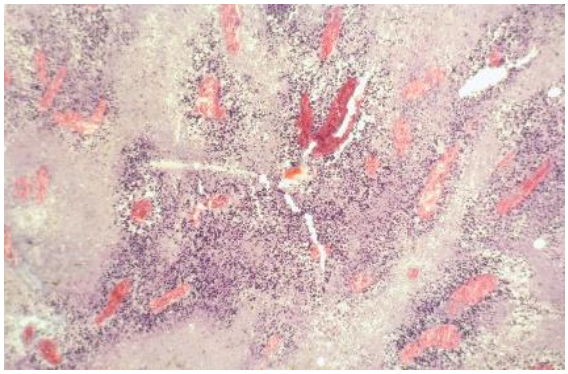

Figure 7. Murine B16 melanoma, histopathological aspect group II

Cytomorphological examination of the tumor, in group III, showed a poor cellular representation and a lot of necrotic detritus in the

smear (Fig. 8), and histopathological aspects showed large, structured areas of tissue necrosis, affecting blood vessels, hemolysis and the presence of hemosiderin which shows the superiority of the DTIC-PSChT method (Fig. 9).

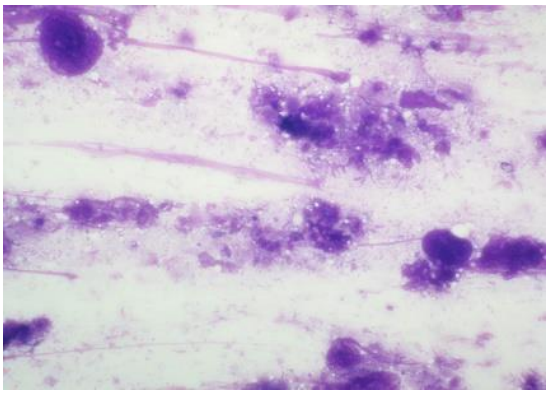

Figure 8. Murine B16 melanoma, cytomorphological group lot III

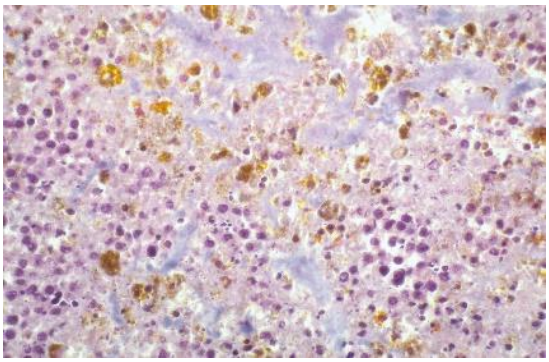

Figure 9. Murine B16 melanoma, histopathological aspect group III

CONCLUSIONS

Following the preliminary determinations performed, it is observed that, depending on the pH value of the administered solution, it has conformations and tautomers that substantially modify its photodynamic potential.

In order for the radiation emitted by the equipment to the cytostatic models to be as efficient as possible, it is necessary for the DTIC solution to have a pH as high as possible (pH = 13 - 15), to be as alkaline as possible.

The cytomorphological and histopathological results of murine B16 melanoma, in the three groups studied, attest to the fact that the use of the DTIC-PSChT method was significantly effective. Thus, it was found that a large part of the tumor was destroyed, the assessment of its destruction being made taking into account the presence of large areas of necrosis in the tumor mass as well as the destruction of neoplasm blood vessels.

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