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EFFECTS OF SHORT TIME EXPOSURE OF HTB140 MELANOMA CELLS TO FOTEMUSTINE AND DACARBAZINE

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Abstract

Different experimental set-ups were designed to study cytotoxic and cytoststic effects on HTB140 melanoma cells after 1 h treatment with fotemustine (FM) or dacarbazine (DTIC). FM induced dose dependent cell inactivation, boosted by its toxicity, particularly for higher doses. DTIC treatment for 1 h was insufficient to provoke almost any effect on melanoma cells. Good correlation between viability and proliferation assays applied was detected for both drugs.

Introduction

Melanoma cells become resistant to a variety of chemotherapeutic drugs very soon after initial use. Inherent radio-resistance, as well as drug resistance, reprogram cell survival pathways and affect cell proliferation after each individual treatment [1, 2, 3]. Such behaviour of melanoma cells disables a long term application of most drugs with only a few cytotoxic compounds showing activity against this tumour. Fotemustine (FM), a member of the chloroethylnitrosourea (CENU) class of alkylating agents has been proven active against disseminated melanoma and primary brain tumours with clinical application being limited by its toxicity [4]. The addition of an aminophosphate to the nitrosourea radical provided higher permeability of FM through cell membranes and blood – brain barrier during the treatment. Spontaneous decomposition of nitrosoureas generates electrophilic species, responsible for DNA alkylation, thus producing therapeutic effects. Production of isocyanates cause toxic side effect of FM through carbamoilation of proteins [5]. Another commonly used drug, approved and frequently used for melanoma treatment, is a monofunctional alkylating agent dacarbazine (DTIC) having relative responses in 15 to 20 % of cases, but with short duration [4, 6]. DTIC and its derivatives with higher membrane permeability somehow improved the response of melanoma brain metastasis.

Results and Discusion

Cytotoxic and cytostatic effects of alkylating agents FM (Ital farmaco S.p.A) or DTIC (Aventis Pharma S.p.A) were analysed on HTB140 human melanoma. Cells were exposed for 1 h to drug concentrations ranging from 0.05 to 2 mM.

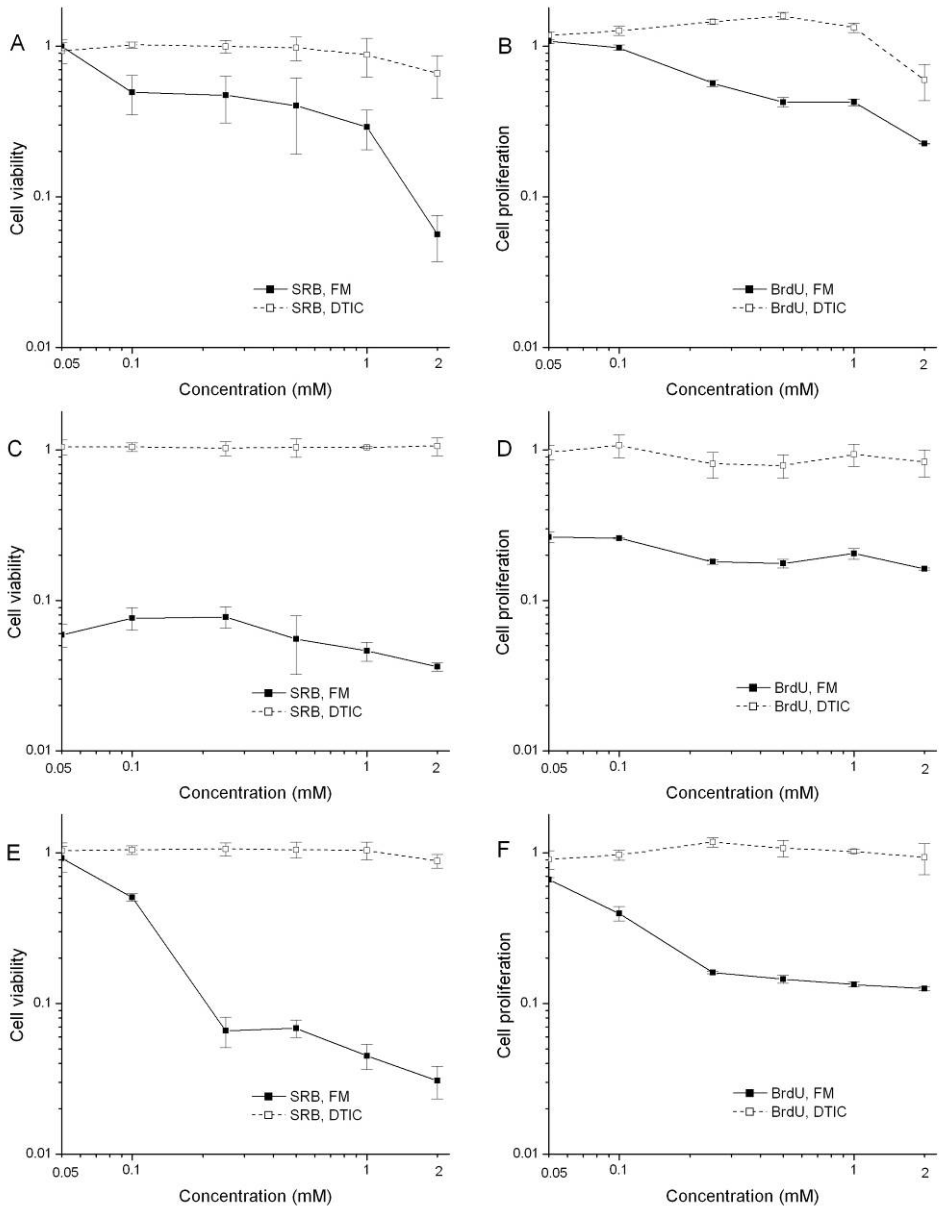

Fig. 1. Dose dependent cytotoxic (A, B) and cytostatic (C - F) effects of FM and DTIC 7 days after administration to HTB140 cells, estimated by SRB and BrdU.

Experimental conditions for evaluation of viability and cell proliferation capacity varied and were designed to fallow short term drug effects *in vitro*.

In the first experimental set-up, when screening drug cytotoxicity, 24 h after plating cells were treated with FM or DTIC for 1 h when drug-containing medium was replaced with fresh medium **(Figure 1A, B)**. When evaluating cytostatic effects of drugs, another two experimental set-ups were involved. After drug administration for 1 h, cells were either immediately replated **(Figure 1C, D)**, or were incubated for 16 h in fresh medium and than replated **(Figure 1E, F)**. In all experiments cells were incubated for 7 days, when cell viability and proliferation were assessed by SRB (MP Biomedicals, Inc) and BrdU (Roche Applied Science) assays respectively. The absorbance was measured using microplate reader (Victor, Wallac) at 450nm.

Obtained results implicated that in all three experimental set-ups, when HTB140 human melanoma cells were treated with DTIC, the drug administration for 1 h was not sufficient to provoke almost any effect on cell growth and proliferation **(Figure 1A-F)**. Contrarily, the use of FM revealed major dose dependent cell growth inhibition under all three experimental conditions. Cytotoxicity of FM pointed out moderate dose dependent decrease of viability and proliferation capacity **(Figure 1A, B)**. Replating of cells for evaluation of FM cytostatic power produced much stronger cell inactivation, except for the largest dose. Immediate replating **(Figure 1C, D)**, compared to replating 16 h later **(Figure 1E, F)**, does not allow cells to recover from the initial stress induced particularly by smaller FM doses, while higher doses made this replating time difference practically irrelevant.

It seams that FM treatment provoked certain viable cells to be more fragile and less adhesive to the support, thus lost during immediate repalting procedure. However, incubating cells for 16 h enabled some of them to restore their growth ability so that they were not lost during tripsinization. Still, this is valid only for smaller drug doses. Estimated cell viabilities evaluated through total protein content (SRB) were supported by proliferative capacity (BrdU) of cells within each experimental set-up.

Conclusion

Large discrapance in the level of cell elimination between DTIC and FM, when applied within a short time skale on HTB140 cells, was due not only to the different mechanisms triggered by each drug but also to the very high toxicity of FM.

Reference

[1] I. Petrović et al., Int. J. Radiat. Biol., 2006, **82**(4), 251-265.
[2] M. S. Soengas and S. W. Lowe, Oncogene, 2003, **22**, 3138 – 3151.
[3] I. Passagne et al., J. Pharmacol. Exp. Ther., 2003, **307**(2), 816-823.
[4] U. Jungnelius et al., Eur. J. Cancer, 1998, **34**(9), 1368-1374.
[5] R. A. Kroes et al., Proc. Natl. Acad. Sci., 1991, **88**, 4825-4829.
[6] D. Grossman and D. C. Altieri, Cancer Metast. Rev., 2001, **20**, 3-11.