

ESTIMATION OF DNA DAMAGE AND CYTOTOXICITY OF ANTHRACYCLINE ANALOGS IN HUMAN MELANOMA CELLS ON EARLY AND LATE PASSAGES

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Abstract: Human malignant melanoma is a major problem characterized by both rapid rising incidence and strong chemoresistance. The aim of present experiments was to estimate the effects on DNA of new anthracycline analogs in melanoma cells obtained from various patients, cultured on early (E) and late (L) passages. For determination of cytotoxic effect, MTT assay was used and comet assay was used for the detection of DNA damage. The discrepancy between the intensity of DNA damage processes and IC₅₀ values may indicate that there are some critical loci in the genome, responsible for cell death.

Keywords: human melanoma, anthracyclines, cytotoxicity, DNA damage

Melanoma is the most lethal form of skin cancer and the incidence and mortality rates are rapidly rising. Malignant melanoma is characterized by a strong chemoresistance and poor patient prognosis. The molecular mechanisms underlying its resistance to chemotherapy remain unclear, but are speculated to involve the dysregulation of apoptotic pathways or defective DNA repair processes (1, 2).

In our previous study on the new anthracycline analogs (annamycin, WP903) proposed as more effective against resistant tumors, a higher sensitivity of human melanoma cells, than in other cell line used, was observed (3,4).

For this reason, the aim of present experiments was to estimate the effects of new anthracycline analogs in melanoma cells obtained from various patients, cultured on early and late passages.

For determination of cytotoxic effect (IC₅₀) MTT assay was used. A technique of single cell gel electrophoresis (comet assay) was used for the detection of DNA damage.

All obtained data were compared with those for adriamycin, a member of the anthracycline family of compounds, chemotherapeutic agent widely used in treatment of cancers.

EXPERIMENTAL

Reagents

Adriamycin (ADR) was purchased from Fluka, Germany; new synthesized anthracycline deriva-

tives: WP903 and annamycin (ANN) were received from MD Anderson Cancer Center, Houston, USA; thiazolyl blue (MTT), SDS, agarose type II, agarose type VII-A, ethidium bromide, EDTA, Trizma, TritonX-100, antibiotic antimycotic were obtained from Sigma, USA. Fetal calf serum was from Bioproduct, Hungary and PBS from IITD, Poland.

Cell cultures

Four human melanoma cell lines: MEW 151, MEW 152, MEW 155 and MEW 164 on early (E) and late (L) passages were used. They were derived from melanoma cell line collection established in culture from melanoma metastases, surgically removed from patients in the Warsaw Cancer Center.

Cells were grown in a minimum Eagle's medium (MEM), supplemented with 10% fetal calf serum and antibiotics in Nunclon flasks and a humidified atmosphere at 37°C in 5% CO₂.

As reported by Kulik et al. (5), the cells used in this work were characterized by molecular markers.

MTT test

An MTT cytotoxicity assay was carried out by the method of Mosmann (6) and modified by Gruber and Anuszevska (7).

The IC₅₀ values were evaluated (IC₅₀ is the drug concentration required to decrease cell density to 50% of that in the untreated culture after incubation time). The L/E factor was counted as the ratio

of IC₅₀ cells cultured on late passages to IC₅₀ cells cultured on early passages.

Comet assay

The comet assay was performed according to Singh et al. (8) with minor modification (9). The comets were examined at 200× magnification using a fluorescence microscope (BX Olympus Optical) which was connected through CCD camera to an image analysis system (Lucia G/Comet Assay 3.5). Finally, the tail moment was chosen to characterize DNA damage induced by the anthracycline treatment.

Fifty comets per slide have been evaluated, with two replicates used for each sample and data were pooled in one sample.

RESULTS AND DISCUSSION

For the current study, human melanoma cells originated from four patients, cultured on early and late passages were chosen: MEW 151, MEW 152, MEW 155 and MEW 164 (Table 1).

Sensitivity of cells to anthracycline analogs: ADR, ANN and WP903, was defined on the basis of IC₅₀ values obtained for each cell line in MTT assay at the concentration range 0.05-15 µg mL. As shown in Table 2, anthracyclines were more cytotoxic on early passages than on the late ones for all used cells.

The L/E factor for ADR was in the range from 1.3 to 6.6. The biggest difference between values of IC₅₀ L and IC₅₀ E defined for ADR was obtained in the case of MEW 151 and MEW 152 cells. The most sensitive to ADR was MEW 151/E cell line and most resistant to ADR was MEW 152/L cell line.

The sensitivity to ANN or WP903 was similar in the case of all cell lines, only in the case of MEW 164 cells, ANN induced toxic effect significantly stronger in the cells on late passages than in the cells on early passages. The rate of IC₅₀L/IC₅₀E for ANN and WP903 was lower than 2 for both compounds.

Because the results obtained with ANN and WP903 were compared with those of ADR and the highest L/E factor was observed for MEW 151 and MEW 152 cells treated with ADR, these two cell lines were chosen for DNA damage analysis.

The comet assay since its invention in 1984 r. (10) as a technique for the direct visualization of DNA damage and repair in the individual cells, has proved to be a very useful method for studying genotoxicity in cells exposed *in vivo* and *in vitro* to a variety of physical and chemical agents (11, 12).

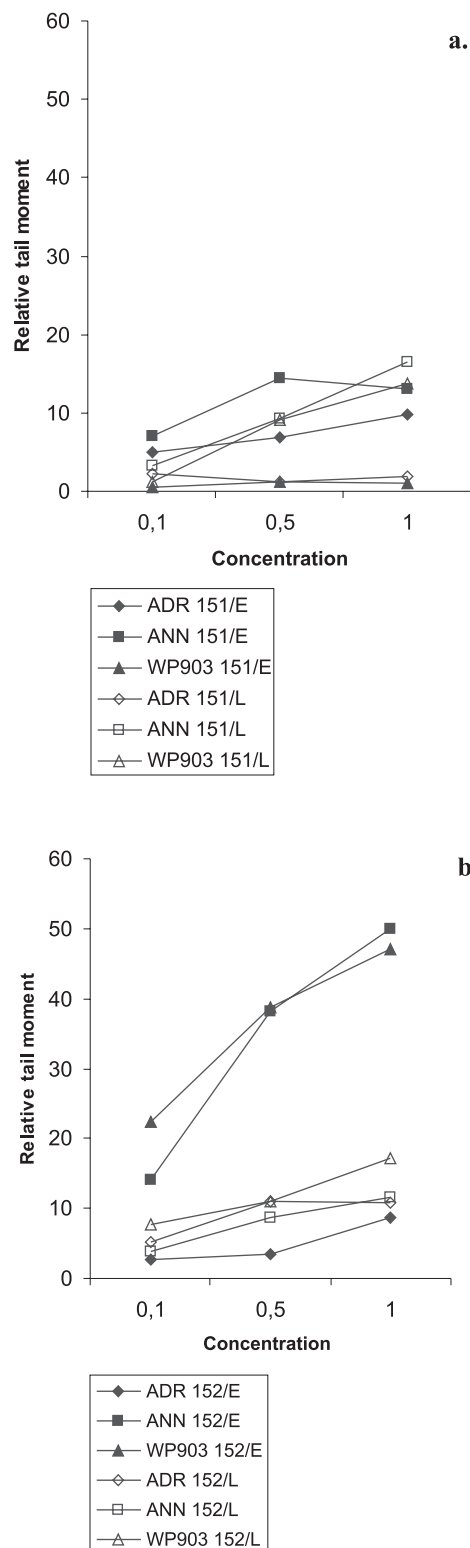


Figure 1. Influence of anthracycline derivatives on DNA damage in: a. MEW 151 and b. MEW 152 cells, measured as a relative tail moment in comet assay.

Table 1. List of chosen human melanoma cell lines.

Cell lines	Passages E*	Molecular markers ¹ stated on E passages	Passages L**	Molecular markers stated on L passages
MEW 151	20 – 31	Tyrosinase Muc – 18 Mage – 3 Mart – 1	136 – 141	NR ²
MEW 152	16 – 25	Tyrosinase	108 – 114	NR
MEW 155	26 – 38	Mart – 1	141 – 148	NR
MEW 164	20 – 31	Tyrosinase Mart – 1	130 – 141	NR

* E – cells on early passages

** L – cells on late passages

¹ All markers were assayed by a single PCR reaction as reported by Kulik et al. (5)

² NR – markers not revealed

Table 2. IC₅₀ values (µg/mL) for chosen anthracyclines in human melanoma cells. Values represent the mean ± SD from 3 independent determinations.

Cell lines	ADR	L*/E**	ANN	L/E	WP903	L/E
MEW 151/E	0.32 ± 0.1	6.6	2.17 ± 0.6	0.9	0.60 ± 0.1	1.8
MEW 151/L	2.10 ± 0.6		1.98 ± 0.6		1.09 ± 0.2	
MEW 152/E	5.64 ± 1.0	2.1	1.79 ± 0.4	1.6	1.74 ± 0.4	1.3
MEW 152/L	11.8 ± 2.2		2.89 ± 1.3		2.28 ± 0.9	
MEW 155/E	1.27 ± 0.1	1.3	1.37 ± 0.5	1.5	0.30 ± 0.1	1.2
MEW 155/L	1.60 ± 0.5		2.06 ± 0.8		0.35 ± 0.1	
MEW 164/E	2.55 ± 0.8	1.7	2.87 ± 0.9	0.16	0.67 ± 0.2	1.9
MEW 164/L	4.34 ± 0.9		0.45 ± 0.2		1.28 ± 0.5	

* L – cells on late passages

** E – cells on early passages

The results of the comet assay are presented in the form of histograms showing the number of cells as a function of some cell property, e.g. tail moment or % DNA in the tail. The tail moment was chosen to characterize DNA damage induced by anthracyclines.

The data obtained with human melanoma cells MEW 151 treated with anthracycline analogs pointed to the lack of any correlation between the number of passages, IC₅₀ value and DNA lesions (Figure 1a). As far as MEW 152 cells are concerned, there is the correlation between tail moment values, the number of passage and cytotoxicity (Figure 1b). Cells on early passages showed higher tail moment and lower IC₅₀ value as opposed to cells on late passages. This observation suggests that MEW 152 cells on early passages can be more susceptible to DNA damage which generates stronger toxic effect.

On the one hand, it should be stated that ageing cells lose the activity of some peptides and lack of these peptides could influence its susceptibility on treatment of cytotoxic agents, on the other hand, in the light of the results it can be assumed that the correlation between drug cytotoxicity and cell passage seems to be cell specific and it has not to be related to their molecular markers.

The discrepancy between the intensity of DNA damage processes and IC₅₀ values observed in MEW 151 cells may indicate that there are some critical loci in the genome, responsible for cell death and this effect may be not related to the level of DNA lesions (13).

The data obtained in this work also suggest that information of the age of tumor cells may help to anticipate therapeutic efficacy of the anticancer drugs.

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