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Differential Effects of 5-Fluorouracil on Human Skin Melanocytes and Malignant Melanoma Cells in vitro

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We have observed differential effects of the cytotoxicity of 5-fluorouracil (5-FU) on human skin melanocytes and malignant melanoma cells in vitro. In the absence of 5-FU the melanoma cells multiplied much more rapidly than the melanocytes. With a 7-day exposure of 5-FU (1.92×10^{-5} to 3.84×10^{-4} M) all the melanoma cells died by 5 W, while even two times longer exposure (a 14-day exposure) of 5-FU the melanocytes survived till 6 W and increased at the lower concentration. In the presence of 5-FU there was a different compensatory increase in [^{3}H] thymidine incorporation between the two cell types. That is, compared with the large increase observed in the incorporation by the melanoma cells, a small increase in the incorporation by the melanocytes was observed. The reason for these differential effects of 5-FU may be the difference in the cell cycle and the post-injury cell renewal of the two cell types. *Key words: Growth curve and DNA synthesis.* (Received February 10, 1986.)

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5-fluorouracil (5-FU) is one of the antimetabolites which inhibits DNA synthesis and the primary mechanism for this effect is the thymidylate synthetase inhibition. It has been reported that 5-FU is more toxic to proliferating than to non-proliferating cells (1) and that it is more toxic to rapidly dividing, exponential phase cells than to the stationary phase cells (2). These findings are important not only for the use of the drug in chemotherapy, but also for the application of the drug to some technical or diagnostic procedures.

In previous studies we observed a different effect of cytotoxicity of 5-FU on normal keratinocytes and melanocytes (3). We have used this observation to isolate pure populations of melanocytes in primary cultures without the necessity for additional separation methods. In the present study we compared the sensitivity of human skin melanocytes and melanoma cells with 5-FU and found that there was a differential sensitivity between the two cell types.

The purpose of this paper is to show the difference and to discuss some possible reasons for it and the possibility of applicating this observation to the diagnosis of borderline malignant melanomas.

MATERIALS AND METHODS

Cell culture

Culture of human melanocytes. Split-thickness sections of human skin were made with a Castroviejo keratotome set to cut at 0.2 mm, as previously described(4). The tissue was incubated in 0.25% trypsin in phosphate-buffered saline for 30 min at 37°C and washed with 150 mM NaCl to remove excess trypsin. The epidermis was separated from the papillary dermis, and keratinocytes and melanocytes were released into the medium by gentle agitation and plated onto 35 mm plain plastic Petri dishes imprinted with a 1 mm^2 grid on the bottom surface. For culture of melanocytes the cells

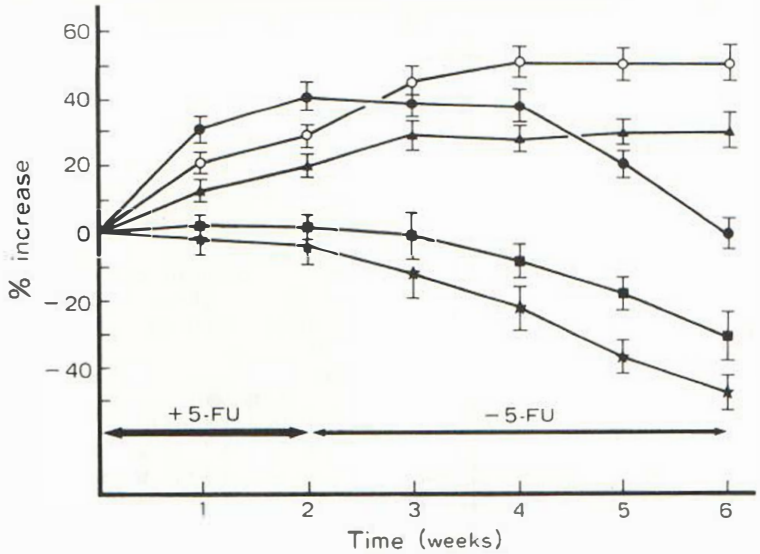

Fig. 1. A growth or survival curve of melanocytes after addition of various concentrations of 5-FU and that of non-5-FU medium as control.
Control (●—●);
1.92×10^{-5} M 5-FU (○—○);
3.84×10^{-5} M 5-FU (▲—▲);
1.92×10^{-4} M 5-FU (■—■);
3.84×10^{-4} M 5-FU (★—★).

were maintained in McCoy's 5A medium with 10% newborn calf serum with CAMP-elevating agents ($(1\times10^{-9}$ M cholera enterotoxin (CT) and 3.3×10^{-5} M isobutyl methylxanthine (IMX)) and anti-biotics (100 units/ml of PC and 100 μg/ml of SM). They were fed twice weekly and maintained at 37°C in an atmosphere of 94% air and 6% CO_2.

Culture of human melanoma cells. Human melanoma cell lines (A3) were purchased. They were detached by 0.25% trypsin, plated onto 35-mm plain plastic Petri dishes imprinted with a 1 mm^2 grid on the bottom surface and fed with MEM supplemented with 10% fetal calf serum, 3.3×10^{-5} M IMX, 1.0×10^{-9} M CT and anti-biotics (100 units/ml of PC and 100 μg/ml of SM). They were fed twice weekly and maintained at 37°C in an atmosphere of 94% air and 6% CO_2.

Study of the effect of 5-FU on melanocytes and melanoma cells

One week after plating 5-FU (1.92×10^{-4} to 3.84×10^{-5} M) was added to the dishes once or twice (once a week, a 1-week or 2-week continuous exposure, respectively). Control dishes received 150 mM NaCl. Comparative growth curves were obtained by direct counting of melanocytes and melanoma cells on Petri dishes having 1 mm^2 grids. Five individual grids were counted, the cell numbers averaged, and growth expressed as the percent increase or decrease from day 0.

Thymidine incorporation study

After one week of the first subculture (S_1), duplicated incubations containing either no drug or 3.84×10^{-5} M 5-FU were prepared. After 1 h the medium was changed to new one and [^{3}H] thymidine (2 μci/ml in McCoy's 5A and antibiotics) were added. Incubation flasks were removed after 23 h. The medium was removed and rinsed with 0.9% NaCl 3 times. The cells were harvested with 0.25% trypsin, and 1% EDTA solution in Ca^{2+}, Mg^{2+} free phosphate buffer saline, pH 7.3. The volume of cell suspension remaining after removal of aliquots for counting of cell number was measured (about 0.9 ml), and the cell suspension chilled on ice. Two ml of cold carrier RNA-DNA solution (0.5 mg/ml RNA, DNA) was added followed by an equal volume of cold 10% TCA. To determine the incorporation of [^{3}H] thymidine the resulting precipitates (after 12 h at 4°C) were collected on Whatman GF/C glass fiber papers (W & R Balston, Ltd) and washed extensively with cold 5% TCA. The papers were placed in a scintillation vial, dried at room temperature overnight, and the incorporation of isotopes measured in Beckman liquid scintillation counter with a counting efficiency of 36.7%. Duplicated dishes were used for each determination and for cell counts.

RESULTS

Growth curve

The melanocyte in controls showed 40% increase at peak at 2 W, and then gradually decreased. The melanocytes in the presence of 5-FU (a 2-week continuous exposure)

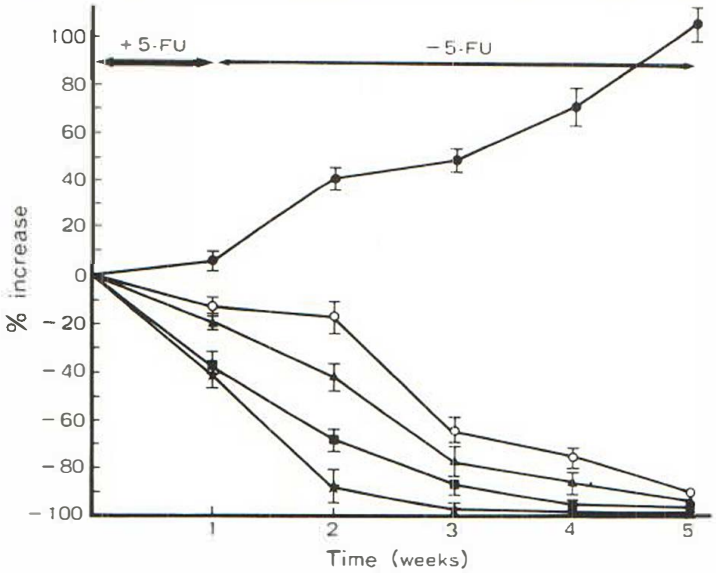

Fig. 2. A growth or survival curve of melanoma cells after addition of various concentrations of 5-FU and that of non-5-FU medium as control.
Control (●—●);
1.92×10^{-5} M 5-FU (○—○);
3.84×10^{-5} M 5-FU (▲—▲);
1.92×10^{-4} M 5-FU (■—■);
3.84×10^{-4} M 5-FU (★—★).

showed different growth curves depending on the concentration: at 1.92×10^{-5} and 3.84×10^{-5} M 5-FU, they showed 53% and 28% increase at 6 W, respectively, while at 1.92×10^{-4} and 3.84×10^{-4} M 5-FU, they showed 30% and 42% decrease at 6 W, respectively (Fig. 1). The melanoma cells in controls showed rapid increase more than 100% at 5 W. On the other hand, the melanoma cells in the presence of 5-FU (a 1-week continuous exposure) showed rapid decrease and all of them died by 5 W (Fig. 2).

Thymidine incorporation

The incorporation of [^{3}H] thymidine into DNA by human melanocytes and melanoma cells, in the absence and presence of 5-FU, is shown in Table I. In the absence of the drug, the incorporation by the melanocytes was 16.65 ± 0.75 CPM/cell$\times10^6$ and by the melanoma cells 20.85 ± 0.85 CPM/cell$\times10^6$. In the presence of the drug, the incorporation by the melanocytes was 86.10 ± 6.20 CPM/cell$\times10^6$ and by the melanoma cells 877.50 ± 111.80 CPM/cell$\times10^6$. Thus, in the absence of 5-FU the incorporation of [^{3}H] thymidine by the two types of cells was small, and showed little difference between them. In the presence of the drug, on the other hand, it became about 40 times higher in the melanoma cells and about 5 times in the melanocytes. When compared with the incorporation by the melanoma cells, the incorporation by the melanocytes was much lower, about one-tenth.

DISCUSSION

The present study revealed that melanoma cells were rapidly killed by 5-FU, while melanocytes were not killed but even increased. The most possible reason for this may be

Table I. *Effect of 5-FU on [^{3}H] thymidine incorporation into human melanocytes and melanoma cells DNA*

Melanocytes	without 5-FU	16.65 ± 0.75 CPM/cell$\times10^6$
	with 5-FU	86.10 ± 6.20 CPM/cell$\times10^6$
Melanoma cells	without 5-FU	20.85 ± 0.85 CPM/cell$\times10^6$
	with 5-FU	877.50 ± 111.80 CPM/cell$\times10^6$

that 5-FU is selectively toxic to proliferating cell and that melanoma cells multiply more rapidly than the melanocytes in vitro. It has been shown that, in studies on the effect of a brief exposure to 5-FU on the viability of mouse L cells, the rapidly dividing, exponential phase cells are three times as sensitive as the stationary phase cells (2). It has also been shown that a 24-hour exposure of mice to 5-FU is more toxic to lymphoma cells than to the normal cells of the bone marrow (1). This differential sensitivity reflects the higher growth fraction of the lymphoma cell population, which suggests that the drug would be equally toxic to both tumor cells and normal cells if their proliferative status were the same. Thus 5-FU is cycle specific in that sense that it is selectively toxic to proliferating cells. Other factors such as a difference in the ability of cells to convert FU to FdUMP (5) and a major difference in pyrimidine nucleotide metabolism (6) might also be of importance for the differential sensitivity, although these factors were not investigated.

The present study also revealed that at 23 h after a one-hour exposure of 5-FU there was a much higher increase in [^{3}H] thymidine incorporation in the melanoma cells than in the melanocytes. The increase in [^{3}H] thymidine incorporation by both cell types after 5-FU exposure might be explained as follows: 5-FU inhibits DNA synthesis at an early event. Following this early inhibition phase, a state of recovery and actual overactivity of damaged cells occurs. In fact, similar responses have been noted in hairless mouse epidermis in vivo (7) and cultured mammalian cells (8, 9) after ultraviolet radiation. Since the melanoma cells were more damaged than the melanocytes by 5-FU, it is reasonable that there is a higher increase in [^{3}H] thymidine incorporation by the melanoma cells than by the melanocytes in a state of recovery. However, the increase of [^{3}H] thymidine incorporation might not necessarily indicate a higher proliferation of the cells, but also changes in the utilization of [^{3}H] thymidine (10).

A diagnosis of the early stage of borderline malignant melanomas particularly occurring from nevocellular nevus and dysplastic nevus syndrome is important and often difficult histologically. The different effect of 5-FU on melanocytes and melanoma cells disclosed in the present study will make it easier to answer the question, if the cells are melanoma cells or melanocytes, by comparing the growth curve of the [^{3}H] thymidine incorporation by the cells in the absence with that in the presence of 5-FU in vitro. This may be proved in a future study.

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