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Acknowledgement—This study was supported by the 'Bundesministerium für Forschung und Technologie'.

Eur J Cancer, Vol. 26, No. 8, pp. 905–907, 1990.
Printed in Great Britain

0277–5379/90 $3.00 + 0.00

Anti-proliferative Effects and Phenotypic Alterations Induced by 8-hydroxyquinoline in Melanoma Cell Lines

Jardena Nordenberg, Abraham Novogrodsky, Einat Beery, Miriam Patia, Lina Wasserman and Abraham Warshawsky

The effect of the transition metal chelator, 8-hydroxyquinoline (8-HQ), was examined on the growth and phenotype expression of B16 mouse melanoma cells. Micromolar concentrations of 8-HQ inhibited the growth of B16 cells as well as human melanoma cell lines. Removal of 8-HQ from the culture medium restored normal cell growth. Growth inhibition by 8-HQ was accompanied by phenotypic alterations that included changes in cell morphology, increased production of melanin and enhanced activities of the enzymes γ-glutamyl transpeptidase and NADPH cytochrome *c* reductase. These changes might be associated with a better differentiated phenotype. *Eur J Cancer*, Vol. 26, No. 8, pp. 905–907, 1990.

INTRODUCTION

THE TRANSITION metal cations, iron, copper and zinc, are involved in regulation of metabolic pathways related to cell proliferation [1, 2]. Several groups of chelating agents interfere with the growth of various cell types, including melanoma cells [2–4]. 8-hydroxyquinoline (8-HQ) and its halogenated derivatives are fungitoxic and amoebicidic [5, 6]. Yamato *et al.* have prepared tropolone derivatives, incorporating 8-quinolinol side groups. These compounds inhibit KB human epidermoid carcinoma cells *in vitro* and are highly potent against P-388 leukaemia in vivo [7–9]. We have evaluated the effects of 8-HQ on melanoma cell growth and phenotypic expression.

MATERIALS AND METHODS

Cell growth

B16 F10 melanoma cells were plated at 4×10^4 cells per ml 'RPMI 1640', supplemented with 10% fetal calf serum and antibiotics in tissue culture dishes (3 cm). In selected experiments 5×10^4 SKMEL-28 or RPMI 7951 human melanoma cells, MCF-7 breast cancer cells or human fibroblasts derived from ascitic fluid of an ovarian cancer patient were incubated in 0.5 ml growth medium in multiwell plates (0.9 cm). Cultured neonatal cardiomyocytes were used as non-proliferating normal control cells. Cells were incubated in the absence or presence of 8-HQ (Fluka Chemical Corporation) at 37°C in a humidified atmosphere of 5% CO_2 in air for different times. Cell growth was measured by counting cells in a Coulter counter after detachment with EDTA (1 mmol/l).

Phenotypic alterations

Cell morphology was evaluated by light microscopy after fixation of the cells with formalin and staining with haematoxylin-eosin. For assessment of melanin content and enzymatic activity of NADPH cytochrome *c* reductase and γ-glutamyl transpeptidase, 5×10^5 cells were plated in 10 ml culture medium in the presence or absence of 8-HQ for 72 h. Melanin and enzymes were extracted and measured spectrophotometrically [10–12].

Statistical significance of the results was evaluated with paired or unpaired *t* tests.

RESULTS

The number of untreated B16 F10 melanoma cells increased 23 fold over 92 h of incubation (Fig. 1). 8-HQ inhibited cell growth in a concentration dependent manner, leading to complete growth inhibition at 5 μmol/l. Cells remained attached and 95% viable at concentrations up to 5 μmol/l. Higher concentrations were cytotoxic towards the cells. The results in Fig. 2 show that 8-HQ also inhibited the growth of SKMEL-28 and RPMI 7951 human melanoma cells at micromolar concentrations. MCF-7 human breast cancer cells were also found to be highly sensitive to growth inhibition by 8-HQ—a 70% decrease in cell number was obtained at 5 μmol/l. The growth of human fibroblasts was inhibited by 40% at 5 μmol/l 8-

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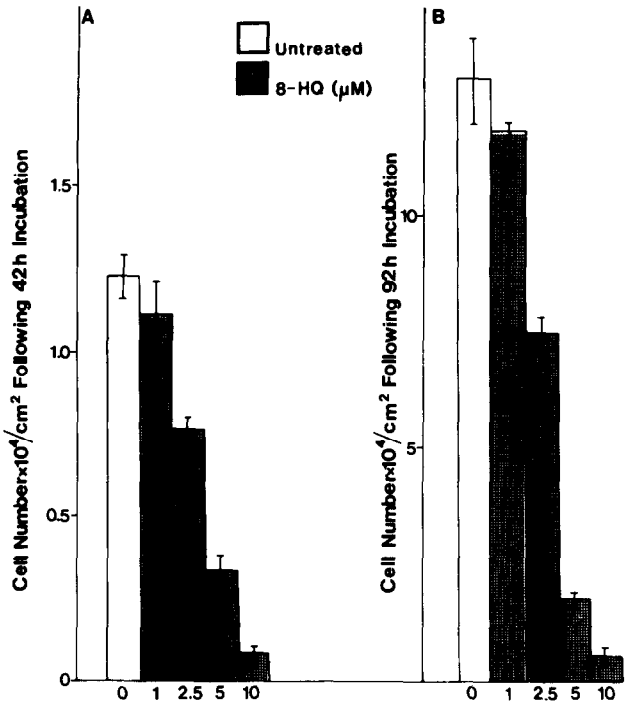

Fig. 1. Effect of 8-HQ on B16 F10 melanoma cell growth. Cells were incubated in presence or absence of 8-HQ for 42 (A) or 92 h (B). Values are means (S.E.) for six replicates done with different cell preparations. Untreated vs. 2.5 μmol/l 8-HQ, $P < 0.01$; untreated vs. 5, 10 μmol/l 8-HQ $P < 0.001$.

HQ and no decrease in viability was found up to 20 μmol/l. Incubation of normal cultured cardiomyocytes in the presence of 8-HQ 5–20 μmol/l for 72 h did not affect their density, viability, beat rate (mean 109 [S.D. 8]/min for untreated and 119 [7] for 8-HQ treated) or DNA content (8.3 μg per plate for untreated and 9.5 μg per plate for 8-HQ treated cells).

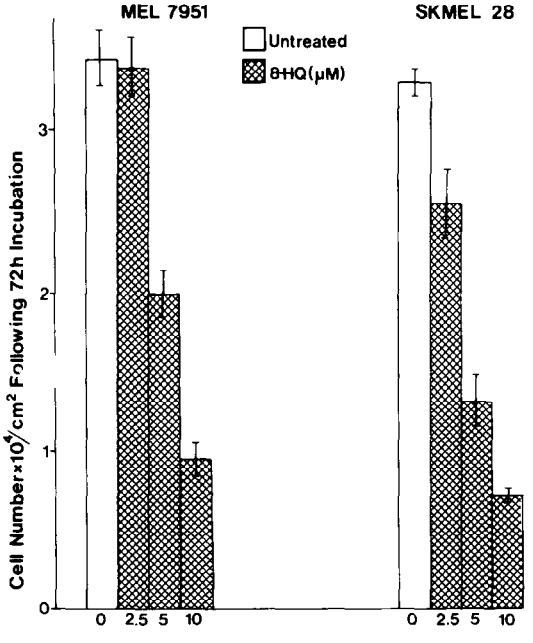

Fig. 2. Effect of 8-HQ on human melanoma cell growth. Cells were incubated for 72 h in the presence or absence of 8-HQ. Six replicates with two different cell preparations. RPMI 7951 untreated vs. 5 μmol/l 8-HQ, $P < 0.02$; untreated vs. 10 μmol/l 8-HQ, $P < 0.01$; SKMEL-28 untreated vs. 5 μmol/l 8-HQ $P < 0.01$; and untreated vs. 10 μmol/l 8-HQ, $P < 0.001$.

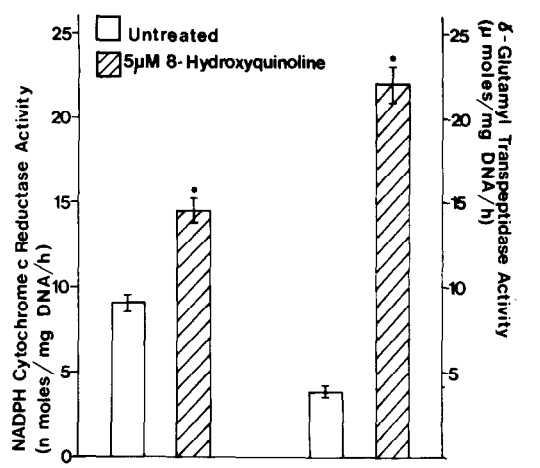

Fig. 3. Effect of 8-HQ on activities of NADPH cytochrome c reductase and γ-glutamyl transpeptidase in B16 F10 melanoma cells. Six to seven experiments done with various cell preparations. * = Treatment vs. no treatment, $P < 0.001$.

Removal of 8-HQ (5 μmol/l) from the growth medium led to a rapid reversion of its growth inhibitory effect. 4×10^4 cells were incubated in the absence or presence of 8-HQ 5 μmol/l. 96 h later the number of untreated cells increased by 18 fold (7.1×10^5), whereas the number of 8-HQ treated cells increased only by about 2 fold (7.6×10^4). Then, untreated or 8-HQ pretreated cells were replated at 7×10^4 cells in 1 ml culture medium and incubated in the absence of 8-HQ for 72 h. Both untreated and 8-HQ pretreated cells showed a similar increase in cell number: about 13 fold for untreated cells (8.8×10^5/ml) and about 11 fold for 8-HQ pretreated cells (7.6×10^5/ml).

The anti-proliferative effect of 8-HQ on B16 melanoma cells was accompanied by phenotypic alterations. 8-HQ induced a morphological change in the cells that was reflected by cell enlargement and development of long appendages. These morphological changes were reversible after removal of 8-HQ from the culture medium. The B16 F10 cells that were used in our experiments are low in melanin. Following incubation of the cells in the presence of 8-HQ 5 μmol/l for 72 h, melanin concentration increased from 0.84 (0.09) μg/10^6 cells to 1.90 (0.41) μg/10^6 cells ($P < 0.01$, for five experiments). 8-HQ treatment also resulted in enhancement of NADPH cytochrome *c* reductase activity and in a striking increase in the activity of γ-glutamyl transpeptidase (Fig. 3).

DISCUSSION

Micromolar concentrations of 8-HQ inhibited the growth of B16 F10 mouse melanoma cells and induced phenotypic alterations, including morphological changes, increased melanogenesis, an increase in the activity of the endoplasmic reticulum associated enzyme, NADPH cytochrome *c* reductase, and a striking increase in γ-glutamyl transpeptidase, an enzyme that participates in the synthesis of 5-S-cysteinyldopa (a precursor of pheomelanin [13]). These phenotypic alterations resembled those induced by millimolar concentrations of other chemical inducers of differentiation and might reflect a more differentiated phenotype [10–12, 14].

The anti-proliferative effect of 8-HQ was not selective for melanoma cell lines, since human breast cancer cells as well as proliferating fibroblasts were also inhibited by this compound. The resistance of cultured cardiomyoctes to 8-HQ may suggest that its effect is restricted to rapidly proliferating cells.

The mechanism of action of 8-HQ has not been explored. However, possible targets for its activity as a chelating agent might be mitochondrial cytochromes as well as ribonucleotide reductase. Our results with the *in vivo* anti-leukaemic activity found for 8-HQ derivatives [7], suggest that hyroxyquinoline derivatives should be further evaluated for their antitumour activities.

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Acknowledgements—This study was supported by a grant from the Israel Cancer Association to Zwi Dov Kessler. We thank Dr Gania Kessler Icekson of the Rogoff Research Institute for providing the cultured cardiomyocytes.

Eur J Cancer, Vol. 26, No. 8, pp. 907–911, 1990.
Printed in Great Britain

0277–5379/90 $3.00 + 0.00
Pergamon Press plc

Role of Hypoxanthine and Thymidine in Determining Methotrexate plus Dipyridamole Cytotoxicity

Thomas C.K. Chan and Stephen B. Howell

The nucleoside transport inhibitor dipyridamole can potentiate the cytotoxicity of methotrexate by a mechanism that was thought to be related to the inhibition of thymidine salvage. In human ovarian carcinoma cells thymidine only partly reversed the *in vitro* cytotoxicity of methotrexate plus dipyridamole at sub-millimolar concentrations, above which the cytotoxicity of thymidine itself became evident. Hypoxathine with thymidine, or hypoxanthine alone at a higher concentration, completely reversed methotrexate and methotrexate plus dipyridamole cytotoxicity. The effects of dipyridamole on cellular cyclic adenosine monophosphate (cAMP) levels and on ^{3}H-methotrexate efflux in 2008 cells were examined. At 10 μmol/l, dipyridamole did not alter cAMP content or methotrexate influx in ovarian carcinoma cells, but reduced the rate of efflux of ^{3}H-methotrexate by 25%. In Chinese hamster ovary cells and their folylpolyglutamyl synthase-deficient variant AUX B1, the reduced methotrexate efflux by dipyridamole was not due to increased polyglutamation, since increased retention was observed in both cell lines. The data support the hypothesis that dipyridamole potentiated the activity of methotrexate by inhibiting the salvage of hypoxanthine, and to a lesser extent, that of thymidine. The ability of dipyridamole to increase the cellular retention of methotrexate was probably a non-specific action of dipyridamole on the cell membrane, and may have a role in the observed synergy.
Eur J Cancer, Vol. 26, No. 8, pp. 907–911, 1990.

INTRODUCTION

THE ABILITY to salvage preformed metabolites by tumour cells may be an important determinant of tumour sensitivity to antimetabolite chemotherapy [1–4]. We and others have demonstrated that dipyridamole, a potent membrane nucleoside transport inhibitor, can potentiate the anti-tumour activity of both purine and pyrimidine antimetabolites [5–9]. Although the effects of anti-metabolite potentiation can be demonstrated easily in cytotoxicity assays and in animal models, the mechanisms by which dipyridamole acts are not clearly known. In addition to its ability to inhibit nucleoside transport across the plasma membrane, dipyridamole inhibits phosphodiesterase activity